1 Understanding summertime thermal refuge use by adult Atlantic salmon using remote sensing,

2 river temperature monitoring and acoustic telemetry

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24 **Abstract**

25 Adult Atlantic salmon return to natal rivers several months before spawning, and during summer,

26 can be subjected to temperatures that exceed their upper temperature tolerance limits. Salmon

27 use thermal refuges to minimize exposure to high temperatures, but little information exists

28 regarding behavioral thermoregulation by adult Atlantic salmon. We examined behavioral

29 thermoregulation by Atlantic salmon during summer in-river residence in a Quebec river with a

30 novel combination of thermal infrared (TIR) remote sensing, river temperature monitoring, and

31 acoustic telemetry. Adults engaged in behavioural thermoregulation at cooler ambient river

32 temperatures (17-19°C) than previously recorded for this species and maintained body

33 temperature within a narrow range (16-20°C) via use of cool and warm refuges. Adults used

34 large, stable, stratified pools as refuges, allowing multiple individuals to thermoregulate

35 simultaneously without leaving the pool. Low river discharge and high temperatures can be

36 physical barriers to salmon migration, preventing them from accessing suitable refuges (e.g.

37 pools). Identifying and maintaining connectivity to thermal refuges may be critical for

38 persistence of Atlantic salmon populations as climate changes and rivers warm.

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Introduction

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48   Salmonid species have narrow temperature tolerance ranges, making them highly

49 susceptible to environmental warming resulting from climate change (Brett 1956; Pörtner and

50 Farrell 2008; Jonsson and Jonsson 2009). Outside their range of optimal temperatures (where

51 aerobic scope is greatest and growth and metabolism are maximized), capacity for aerobic

52 activity decreases to the point where aerobic scope is zero and metabolism depends on anaerobic

53 processes (Pörtner and Farrell 2008; Jonsson and Jonsson 2009). Above the incipient lethal

54 temperature, fish become thermally stressed and long-term survival is not possible unless access

55 to cooler water is available (Elliott and Elliott 2010). Although the upper incipient lethal

56 temperature varies among species and populations of salmonids, it generally falls between 20°

57 and 28°C (Goinea et al. 2006; Jonsson and Jonsson 2009; Elliott and Elliott 2010).

58   Salmonids cope with high temperatures using behavioural thermoregulation, whereby

59 individuals actively seek out and use cold-water refuges, i.e. discrete patches of water that are

60 cooler than the surrounding ambient river temperature (Kaya 1977; Berman and Quinn 1991;

61 Torgersen et al. 1999). Such cold water patches may be formed by cool tributaries and their

62 associated confluence plumes, groundwater seeps, thermal stratification of deep pools or alcoves,

63 or hyporheic exchange (Bilby 1984; Ebersole et al. 2003; Torgersen et al. 2012). Behavioural

64 thermoregulation has been observed in multiple species of salmonids during all phases of the life

65 cycle (Gibson 1966; Berman and Quinn 1991; Tanaka 2000) and records of Atlantic salmon

66 (*Salmo salar*) using cool water refuges during high temperature events were described as early as

67 1939 (Huntsman 1942).

68   Atlantic salmon return to natal rivers during summer, many months before the onset of

69 spawning. While over-summering in rivers, Atlantic salmon can be subject to temperatures that

70 approach or surpass the upper incipient lethal level. Although the optimal and critical

71 temperatures are not explicitly known for adult Atlantic salmon, temperatures of $26°$ to $27°C$ have

72 been associated with mortality of migrating adult Atlantic salmon and there is consensus in the

73 literature that temperatures between $20°$ and $23°C$ are stressful (Shepard 1995; Wilke et al. 1995;

74 Breau 2013). High summer temperatures, therefore, may reduce the number of adult salmon that

75 survive to spawn during the autumn, with associated negative impacts on population

76 productivity. Because salmon metabolism increases with water temperature, high temperatures

77 also reduce the energy available for individuals to engage in costly reproductive efforts, e.g.

78 gamete production, mate selection, male-male competition, and nest construction by females

79 (Gilhausen 1980; Berman and Quinn 1991; Hasler et al. 2012). Such non-lethal effects of high

80 temperature on reproductive capacity can negatively impact productivity, even in the absence of

81 heat-induced mortality. In order to retain sufficient energy stores for spawning, therefore, some

82 adult salmon require access to cool thermal refuges during their long, in-river pre-spawning

83 residence (Berman and Quinn 1991; Newell and Quinn 2005; Hasler et al. 2012).

84   Given the importance of thermal refuges to persistence and management of salmon

85 populations, it is surprising that to date, there has been little research directed at understanding

86 the temperatures that trigger refuge use by adult Atlantic salmon or the specific refuge types

87 used. Studies of thermal refuge use by juvenile Atlantic salmon (e.g. Cunjak et al. 2005; Breau et

88 al. 2007; Dugdale et al. 2016) have been widely conducted, whereas records of refuge use by

89 adult salmonids largely have been restricted to species of Pacific salmon (e.g. Goniea et al. 2006;

90 Donaldson et al. 2009; Keefer et al. 2009).

91   Several tools are now available for monitoring river temperature and tracking fish

92 movements in rivers across a range of temporal and spatial scales. Remote sensing of river

93   temperature via aerial thermal infrared (TIR) imagery permits estimation of river temperature at

94   a single time point over large spatial scales, whereas monitoring via temperature loggers can

95   provide a long-term record of temperature at specific locations (Torgersen et al. 2001; Torgersen

96   et al. 2012; Dugdale 2016). Temperature sensors built into very high frequency (VHF) and

97   ultrasonic (acoustic) transmitters allow direct measurement of internal body temperature of

98   tagged fish (Brewitt and Danner 2014; Gotkowsky 2017). We used a unique combination of TIR

99   imagery, river temperature monitoring, and acoustic telemetry to 1) characterize the types of

100   thermal refuges used by adult Atlantic salmon during the pre-spawning in-river residence and 2)

101   identify the temperatures at which adults initiated thermal refuge use in a gravel bed river in

102   Quebec, Canada. We expected that adult Atlantic salmon would engage in behavioural

103   thermoregulation to remain within a narrow temperature range (Berman and Quinn 1991; Newell

104   and Quinn 2005; Gotkowsky et al. 2017). Specifically, we expected that adults would use cool

105   water patches when river temperatures reached the range thought to be stressful to adult Atlantic

106   salmon (i.e. 20° to 23°C, Shepard 1995; Wilke et al. 1995; Breau 2013). We further expected that

107   patterns in behavioural thermoregulation would follow the diurnal cycle of heating and cooling

108   patterns, with adults using cool refuges during the warmest hours of the day (Ebersole et al.

109   2001).

110   **Materials and methods**

111   *Study area*

112   The Rivière Sainte-Marguerite Nord-Est (hereafter, Nord-Est) is a salmon river in

113   Quebec, Canada, approximately 190 km northeast of the city of Quebec. The Nord-Est drains a

114   catchment of ~1000 km^2, and joins the Rivière Sainte-Marguerite 5 km upstream from its

115   confluence with the Saguenay River (Fig. 1). During summer, river temperature routinely

116 exceeds 22°C and during some years (2005, 2007, 2012, 2014), river temperatures greater than

117 26°C were recorded in the lower seven kilometres of the river (Boyer et al. 2016).

118   A natural waterfall (Chute Blanche) at river kilometre (rkm) 7 blocked upstream

119 migration of returning adult salmon until 1981, when a fish ladder was installed to allow adult

120 salmon to bypass the waterfall. The installation of the fish ladder opened approximately 18 km of

121 additional river habitat to salmon for spawning and juvenile rearing. All adult Atlantic salmon

122 that return to the fish ladder are captured in an entry cage for counting and measurement before

123 being allowed to pass into the main fish ladder. A pair of impassable waterfalls at rkm 33.7

124 (Chute du 16 Miles) and rkm 36.2 (Chute du 18 Miles) currently prevent further upstream

125 movement by returning adults.

126   In 2014, a translocation program was initiated to trap a subset of the adults returning to

127 the fish ladder at Chute Blanche for transport upstream of the pair of impassable falls.

128 Transported adults are able to access approximately 13 km of river habitat, before further

129 upstream passage is blocked by another natural barrier. As part of the evaluation of the success

130 of the translocation program, all salmon transported between 2014 and 2016 were tagged with

131 acoustic transmitters to assess post-transport movement patterns. Our study leveraged the

132 existing infrastructure and tagging program on the Nord-Est to assess thermal refuge use by adult

133 Atlantic salmon during summer 2016, by tagging a subset of transported salmon with

134 temperature sensing acoustic tags (V13T–H, Vemco, Halifax, NS). We conducted our study in

135 two river reaches; the river reach between Chute Blanche and Chute du 16 Miles (hereafter, the

136 downstream sector) and upstream of Chute du 18 Miles (hereafter, the upstream sector). Due to

137 challenging river access, we were unable to deploy acoustic receivers throughout the entirety of

138 the downstream sector, so we focused our efforts on the seven kilometres immediately

139 downstream of Chute du 16 Miles, which are accessible by canoe (Fig. 1).

140 *TIR imagery and acoustic telemetry array*

141   We used TIR imagery to locate cold patches at the riverscape scale, which we

142 subsequently used to inform placement of an acoustic receiver array designed to monitor

143 movements and body temperatures of tagged Atlantic salmon. Airborne thermal and optical

144 imagery (resolution of approximately 2.6 cm and 18.7 cm respectively from ~300 m AGL) was

145 obtained from an aerial survey of the Nord-Est conducted on 25 August 2014 using the same

146 method as Dugdale et al. (2013). We processed images and identified cool patches using the

147 custom MATLAB graphical user interface and methods described by Dugdale et al. (2013).

148 Briefly, thermal images were used to identify surface temperature anomalies, which were

149 verified against the corresponding optical images to distinguish true water temperature anomalies

150 from landscape features (e.g. shading on bedrock or vegetation). True surface anomalies that

151 were $\geq 0.5°C$ cooler than ambient river temperature were classified as cool thermal refuges

152 (Dugdale et al. 2013). Identified refuges were classified by type according to the definitions of

153 Ebersole et al. (2003) and Torgersen et al. (2012), which are described in detail by Dugdale et al.

154 (2013).

155   We deployed acoustic receivers in TIR-identified cool patches that were ≥ 0.5 m depth,

156 the minimum depth required for receiver deployment. This depth requirement was deemed

157 unlikely to bias the results of the present study because refuges shallower than this threshold are

158 unlikely to provide thermal habitat suitable for long-term use by adult salmonids (Torgersen et

159 al. 1999; Baird and Krueger 2003; Torgersen et al. 2012). Because only cool water patches that

160 intersect the river surface can be identified by TIR (Dugdale 2016), acoustic receivers were also

161 deployed in five deep pools known to be used by adult salmon for holding during in-river

162 residence (Frechette, unpublished data). Deployment of acoustic receivers in deep pools enabled

163 identification of subsurface cool refuges that otherwise would have been missed via evaluation

164 of TIR imagery. Receivers were deployed in late June before tagging commenced and were

165 recovered towards the end of the spawning period in late October. Raw telemetry data were

166 downloaded from receivers using VUE software (Vemco, Halifax, NS) and processed using the

167 VTrack package for R (Campbell et al. 2012).

168 *River temperature monitoring*

169 Water temperature within the Nord-Est was recorded at 15-min intervals using

170 temperature loggers (HOBO UA-002-64, Onset, Bourne, MA) installed at a number of sites (Fig.

171 1a). Before deployment, we cross-calibrated temperature loggers and temperature-sensing

172 transmitters in an ice-water bath to develop a correction factor. Temperature responses varied

173 among individual loggers ($0.010 - 0.678°C$, mean $= 0.2°C$) so logger-specific correction factors

174 were applied to each temperature logger prior to use in subsequent analyses. Temperature

175 loggers (N = 7) were housed in white PVC tubes to shield them from direct sunlight and shifting

176 river sediment, and were either attached to the anchoring blocks used to deploy acoustic

177 receivers or attached to rock-filled plastic sacks. Temperature loggers were deployed in well-

178 mixed areas of the river mainstem (one in the upstream sector at rkm 45 and one in the

179 downstream sector, at rkm 30), in the two confluence tributary plumes identified in the upstream

180 sector (U1 and U5), and in two deep pools known to be used by salmon during in river residence

181 (U8 in the upstream sector and D3 in the downstream sector). We suspected that the upstream

182 pool (U8) might be thermally stratified, so we deployed a second temperature logger c. 10 cm

183 below the surface float that was attached to the anchor for the acoustic receiver. The difference

184   between the temperatures measured 10 cm below the surface and 10 cm above the substrate

185   allowed us to test for the presence of thermal stratification in pool U8.

186   *Salmon capture and tagging*

187   Salmon included in this study were part of the translocation program detailed in the

188   'study area' section. Twenty individuals were captured at the fish ladder and transported to

189   upstream release sites (Fig. 1a) where they were tagged with acoustic transmitters equipped with

190   temperature sensors (Vemco V13T–H), allowed to recover in-river, and released. The

191   temperature-sensing transmitters allowed us to obtain measurements of instantaneous internal

192   fish temperature whenever tagged salmon were detected by an acoustic receiver. Sixteen salmon

193   were released in the upstream sector, c. 3 km upstream of Chute du 18 Miles (adjacent to pool

194   U3) and four salmon were released in the downstream sector, c. 3 km downstream of Chute du

195   16 Miles (adjacent to pool D4).

196   Fish were transported in a 1 600-L tank with a water recirculation system and supplied

197   with oxygen. No transport occurred when water temperatures exceeded 20°C and a maximum of

198   four fish were transported at the same time. Tagging was accomplished within one hour of

199   arrival at the release site. Before tagging, salmon were placed in an aerated anaesthetic bath

200   containing 30 mg L^{-1} acetyleugenol. Dosage time varied depending on water temperature. Once

201   the salmon was adequately anesthetized (determined by gill respiration rate and strength of the

202   reflex response), it was placed on a V-shaped surgery table. During the surgical procedure,

203   salmon received a maintenance dose of acetyleugenol (15 mg L^{-1}) and water administered over

204   the gills using a custom-designed reservoir. The acoustic tag was inserted into the abdominal

205   cavity anterior to the pelvic girdle through an incision using aseptic technique. The incision was

206   closed with three or four simple interrupted sutures. During handling, fish length (fork and total

207   length) and maxillary length were measured. Established regression relationships between

208   maxillary length and fork length permitted sex identification for individuals that had not yet

209   developed evident external sexual characteristics (Maisse et al. 1988; Prévost et al. 1991).

210   Following surgery, fish were placed in an aerated recovery tank to be weighed before transfer to

211   a water-filled stretcher for transport to the in-river recovery cage.

212   Salmon were held in the recovery cage and checked every 15 minutes until they regained

213   equilibrium and were swimming freely. The recovery cage in the upstream sector was equipped

214   with a door that was opened once salmon were swimming freely, allowing individuals to

215   volitionally exit the cage. If an individual remained in the cage one hour after regaining

216   equilibrium, it was released by hand. The recovery cage in the downstream sector did not have a

217   door so individuals were released by hand one hour after they regained equilibrium.

218   *Seasonality and location of refuge use*

219   We obtained consistent position and temperature recordings from 15 of the 20 tagged

220   salmon during July and August 2016 (Table 1). Four salmon tagged in the upstream sector and

221   one salmon tagged in the downstream sector left the study area within two days of tagging and

222   were not included in analyses. Two of the salmon tagged in the upstream sector (Fish D-01 and

223   Fish D-02) moved downstream over the impassable falls within six days of release (termed

224   fallback) and were detected in the downstream sector. As these fish were present in the

225   downstream sector for the majority of the study period, they were grouped with the individuals

226   released in the downstream sector for all analyses. Statistical analyses were conducted using R

227   version 3.3.0 (R Core Team 2014) within R Studio version 1.0.136 (R Studio Team 2016).

228   To identify when and where salmon used thermal refuges, we computed the difference

229   (ΔT) between salmon body temperature (T_B) recorded by acoustic receivers and mainstem river

230   temperature (T_R) measured by temperature loggers. Because fish temperature was logged

231   intermittently (only when a fish was in proximity of the receiver), while ambient temperature

232   was logged at 15-minute intervals, we used cubic spline interpolation to estimate mainstem river

233   temperature at the exact moment that fish temperature was measured and logged by the acoustic

234   receiver, which allowed us to calculate the instantaneous difference (ΔT_I) between interpolated

235   mainstem river temperature (T_{RI}) and fish temperature (T_B). We generated plots of ΔT_I by day

236   for each individual, and color-coded the location (receiver identity) where the detections were

237   recorded (Fig.2).

238   In the literature, cool refuges have been defined either using a specific temperature

239   differential between the mainstem and cool patches, e.g. 2°C (Torgersen et al. 2012) or 3°C

240   (Ebersole et al. 2001; 2003), or as simply water that is cooler than the mainstem (Baird and

241   Krueger 2003). Donaldson et al. (2009), however, found evidence of behavioural

242   thermoregulation in sockeye salmon (*Oncorhynchus nerka*) when the differential between body

243   and river temperatures was 1°C. Given the inconsistency in the literature regarding definitions of

244   thermal refuges and/or behavioural thermoregulation, we chose to use a threshold of 1°C to

245   identify the location and timing of refuge use. We classified fish as using cool refuges when ΔT_I

246    $\leq -1^{\circ}$C. Conversely, values of $\Delta T_I \geq 1^{\circ}$C indicated that fish were located in water that was

247   warmer than the ambient mainstem river temperature.

248   *Identification of behavioural thermoregulation*

249   We assessed trends in behavioural thermoregulation over the course of the summer using

250   the difference (ΔT_H) between mean hourly internal fish temperature (T_{BH}) and mean hourly

251   ambient mainstem river temperature (T_{RH}). We used mean hourly measurements because river

252   temperature did not change substantially within the hour. To determine whether salmon

253 exhibited behavioural thermoregulation, we regressed T_{BH} against T_{RH} for each individual

254 (Berman and Quinn 1991). If salmon did not behaviourally thermoregulate (i.e., fish temperature

255 depended on mainstem river temperature), we would expect a 1:1 relationship between T_{BH} and

256 T_{RH}. If a fish did behaviourally thermoregulate (i.e., salmon actively selected water that was

257 warmer or cooler than ambient mainstem river temperature), we would expect a significant

258 departure from the 1:1 relationship (Hillyard and Keeley 2012). We accounted for temporal

259 autocorrelation in the data by applying weighted stratified sampling to the time series of T_{BH} and

260 T_{RH}. Specifically, the observations of each individual salmon were divided into four strata, with

261 each stratum representing one week during the month of August (each stratum consisted of 8

262 days, except for the fourth stratum, which was 7 days). One half of the total observations for

263 each fish were randomly sampled, with the number of samples taken from each stratum weighted

264 by the proportion of observations in the stratum relative to total number of observations during

265 the month of August. The subsampled data were used in the linear regression model and a

266 bootstrapping routine (1000 replicates) was used to confirm that estimates of model coefficients

267 remained constant. Regression assumptions of normality and homogeneity were assessed by

268 visually examining residual plots and the assumption of independence was assessed using the

269 autocorrelation function (Zuur et al. 2009). We used a Student's t-test to test the null hypothesis

270 that the slope of the regression coefficient did not differ from unity.

271   To determine mainstem river temperatures at which salmon exhibited behavioural

272 thermoregulation, we subset the mainstem river temperature into 1°C bins for the range of

273 ambient mainstem temperatures observed within each river sector. We then plotted the frequency

274 histogram for values of ΔT_H within each 1°C river temperature bin. We computed the median

275 value of ΔT_H within each 1°C river temperature bin and generated 95% confidence intervals (CI)

276 around the estimate of median ΔT_H via bias corrected and accelerated (BCa) bootstrap

277 resampling, which adjusts for bias and skewness using the 'boot' package in R (Efron and

278 Tibshirani 1993; Davison and Hinkley 1997; Canty and Ripley 2017). If the 95% CI did not

279 overlap zero, then median ΔT_H (hereafter ΔT_{Hmed}) was considered to differ significantly from

280 zero. A negative value of ΔT_{Hmed} indicated that at a given river temperature, salmon were

281 predominantly detected in cool patches (i.e. they used patches of water that were cooler than

282 ambient mainstem river temperature), whereas a positive ΔT_{Hmed} indicated that salmon were

283 predominantly detected in warm patches. No significant difference between ΔT_{Hmed} and zero

284 indicated that there was no preferential use of cool or warm patches at the given temperature.

285 To examine diurnal patterns in refuge use, we merged records of ΔT_H for all fish, and for

286 each hour of the day, summed the amount of time that fish occupied a) cool patches [$\Delta T_H \leq -$

287 $1°C$], b) the main stem [$-1°C > \Delta T_H > 1°C$] and c) warm patches [$\Delta T_H \geq 1°C$]. We used Chi-

288 square contingency table analysis to test the null hypothesis that the water type occupied (cool

289 patch, main stem, or warm patch) was independent of the hour of the day, and graphically

290 examined diurnal patterns in refuge use.

291 *Within-pool behavioural thermoregulation*

292 We hypothesized that salmon using large pools containing cool water patches would

293 behaviourally thermoregulate within the pool to occupy cool patches, ambient pool temperature,

294 or warm patches as needed to maintain a narrow range of body temperatures (e.g. Nielsen et al.

295 1994; Newell and Quinn 2005; Gutowsky et al. 2017). We tested this hypothesis using

296 temperature data from the loggers attached to the VR2 anchors in pools. The temperature

297 difference between internal fish temperature and ambient temperature measured in the pool

298   allowed us to assign fish to patch type (ambient pool temperature, cool patch, or warm patch) for

299   all detections of individuals that occurred in the pool.

300   We estimated instantaneous ambient pool temperature (T_{AI}) for each measurement of fish

301   temperature (T_B) that was logged by the acoustic receiver using the cubic spline interpolation, as

302   described previously. We then calculated instantaneous pool-specific ΔT ($\Delta T_{IP} = T_B - T_{AI}$),

303   which we used to assign patch state (cool, ambient, or warm patch) to all detections of each

304   individual in the pool. We again used $1°C$ as a threshold to distinguish between the use of cool

305   patches ($\Delta T_{IP} \leq -1°C$), ambient river temperature ($-1°C > \Delta T_{IP} > 1°C$), or warm patches ($\Delta T_{IP} \geq$

306   $1°C$).

307   We employed empirical cumulative density functions to determine the temperatures at

308   which salmon moved into cool or warm patches. To ensure that a new detection of an individual

309   in a patch truly represented the initiation of patch use, and not an individual that was simply on

310   the edge of a cool or warm patch (in which case it might appear to sporadically move into and

311   out of the patch), we set a threshold of 15 min to define a patch residence. That is, for a fish to be

312   considered resident in a warm or cool patch, it must have remained in the patch for a minimum

313   of 15 min. The threshold of 15 min was chosen because this was the sampling resolution of our

314   in-river temperature loggers.

315   **Results**

316   *TIR imagery and acoustic telemetry array*

317   Although we classified thermal refuges throughout the Nord-Est (Fig. 1b), here we

318   present counts of only those cool refuges located in the accessible study reaches. We identified

319   18 cool patches in the downstream sector and 36 in the upstream sector. Three classes of cool

320   patch were identified downstream: lateral seep (n=12), confluence plume (n=5), and cold alcove

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321   (n=1). As in the downstream sector, lateral seeps (n=16) were the most prevalent cool patch type

322   identified in the upstream sector. The other classes identified upstream, in order of prevalence,

323   were: cool side channel (n=6), confluence plume (n=5), hyporheic upwelling (n=5), springbrook

324   (n=3), and cold alcove (n=1). Although we did not directly measure the size of most cool patches

325   identified using TIR imagery, we used the optical images coupled with knowledge of the system

326   to determine that the majority had a surface extent of less than 1 m^2 and were in water less than

327   0.5 m depth, making them too shallow for receiver deployment.

328   We deployed 13 acoustic receivers during summer 2016 (Fig. 1a). The only cool patches

329   identified from TIR imagery that were deep enough for receiver deployment ($\geq$ 0.5 m) were

330   either seeps located in deep pools or confluence plumes (Fig. 1a). Maximum water depth within

331   pools was measured on 25 July 2016 at a river discharge of 26 m^{3}s^{-1} using a single beam echo

332   sounder (Hydroball, CIDCO, Rimouski, QC). Confluence plume depth was measured with a

333   meter stick during receiver deployment. In the downstream sector, we deployed receivers in two

334   pools containing lateral seeps (D1 = 2.1 m deep and D2 = 5.8 m deep). In the upstream sector,

335   lateral seeps were identified in three large pools (U3, U4, and U7), which had maximum depths

336   ranging between 3 and 4 m. A small patch of cool hyporheic upwelling was identified in a fourth

337   large pool (U8), which was the deepest pool in both study sectors (maximum depth = 6.2 m).

338   Three of five confluence plumes were deep enough for receiver deployment (1 – 1.5 m at a river

339   discharge of 21 m^{3}s^{-1}). We were only able to deploy receivers in two of these sites (U1 and U5)

340   because a strong counter-current prevented receiver deployment at the third confluence plume.

341   We deployed receivers in all remaining accessible large pools (maximum depth: 2.7 – 5.3 m) in

342   the upstream (n=3) and downstream (n=2) sectors.

343   Our analyses focused on data from 15 individuals that were consistently detected during

344   July and August (Table 1). Internal body temperature was logged whenever salmon were in

345   proximity of a receiver, i.e., when they were in confluence plumes or pools containing receivers.

346   Temperature records were not obtained when salmon were transiting between pools or in habitat

347   not monitored by a receiver. Range testing conducted during the summer of 2014 indicated that

348   under calm conditions, the detection efficiency of V13 acoustic tags by VR2W receivers

349   deployed in pools in the Nord-Est was 95% at a distance of 40 m and 80% at 200 m (water depth

350   ≥ 0.5 m). Reduced receiver detection range was only observed when river discharge exceeded 60

351   m^3s^{-1} or during discrete heavy rainfall events (Frechette, unpublished data). During the study

352   period, median river discharge was 18.7 m^3s^{-1} (range = 8.8 – 39.4 m^3s^{-1}) and only two rain events

353   occurred during summer 2016 (both during July) that could have interfered with tag detection

354   over a period of less than 12 hours. In cases when multiple salmon were present within a pool,

355   there was some evidence of tag collision, which prevented temperature records from being

356   registered by a receiver despite the salmon being within range of the receiver. Given the high

357   temporal frequency at which data was transmitted to the receivers (i.e. every 30 to 90 sec), data

358   lost because of tag collisions represents only a tiny fraction of the total number of detections

359   recorded from each individual (Table 1). Consequently, we do not expect that the loss of tag

360   detections from rainfall or tag collisions biased our analysis of behavioural thermoregulation.

361   *Seasonality and location of refuge use*

362   All observed cool patch use occurred during August (Fig. 2), which was the only month

363   when measured mainstem river temperature remained within the range considered to be stressful

364   to adult Atlantic salmon (20-23°C, Breau 2013) for more than a few hours at a time (Fig. S1).

365   Three high temperature events, defined as periods during which mainstem river temperature

366   exceeded 22°C in the upstream sector and approached or exceeded 20°C in the downstream

367   sector, occurred during August 2016. The initial onset of behavioural thermoregulation was

368   linked to the high temperature event that occurred 4-6 August (day 217-219), when mainstem

369   river temperature approached 23°C and remained greater than 20°C for three days. Before this

370   event, river temperature was rarely greater than 19°C (Fig. S1) and there was no evidence of cool

371   patch use (Fig. 2). During or immediately following the first high temperature event, all salmon

372   moved into pools containing thermal refuges.

373   Cool refuge use ($\Delta T_I \leq -1^\circ$C) occurred in six locations (Fig. 2): D3 (downstream sector)

374   and U2, U3, U6, U7, and U8 (upstream sector). Of the pools where cool patch use occurred, only

375   three had cool patches that were also identified from TIR images (U3, U7 and U8). Data from

376   temperature loggers installed at the bed and surface of U8 (cross-validated with spot

377   measurements from a temperature probe) indicated that U8 was thermally stratified during

378   August 2016 (Fig. S2). Temperature recorded 10 cm below the surface ranged from 0.77°C to

379   1.7°C warmer than the temperature recorded 10 cm above the substrate. This temperature

380   differential is greater than the thresholds used to define thermal stratification used by Matthews

381   et al. (1994; 0.5°C) and Gendron (2013; 0.1°C), despite the fact that the bottom logger was not

382   placed in the coolest area of the pool. No cool patch use was identified in either of the

383   confluence plumes (U1 and U5), nor in other pools containing lateral seeps (U4 and D1) that

384   were identified via thermal imagery. Fish used cool patches for extended periods of time (≥ 2

385   weeks), in pools U8 (upstream sector) and D3 (downstream sector), which were the only pools

386   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

387   to -5.03°C in U8. Aside from brief forays into other pools, all tagged fish remained in D3 or U8

388   during the warmest part of August, with up to 90% of available individuals in the upstream

389   sector (8 of 9) detected in pool U8 and 100% of available individuals in the downstream sector

390   (5 of 5) detected on a given day.

391   *Identification of behavioural thermoregulation*

392   Although all tagged salmon engaged in behavioural thermal regulation, individuals that

393   were in the downstream sector during August exhibited less behavioural thermoregulation than

394   salmon in the upstream river sector and salmon tagged after the high temperature events

395   exhibited less behavioural thermoregulation than fish tagged earlier in the summer. A linear

396   relationship with a slope that differed from 1:1 was observed between mean hourly mainstem

397   river temperature and mean hourly internal fish temperature for all individuals (1-tailed Student's

398   t-test, $\alpha = 0.05$, Fig. 3) and no substantial departure from the assumptions of normality and

399   homogeneity were evident in residual plots. In the downstream sector, greater than 70% of the

400   variation in internal fish temperature was explained by the mainstem river temperature

401   (measured at rkm 30). In the upstream sector, however, mainstem river temperature (measured at

402   rkm 45) explained little of the variance in internal fish temperature for individuals that

403   experienced the high temperature events (Fig. 3). The remaining variation in fish temperature (42

404   to 73%) was explained by fish using areas of the river that were cooler or warmer than the

405   ambient mainstem temperature. Ambient mainstem river temperature explained approximately

406   80% of variation in fish temperature for the two individuals that were tagged during late August,

407   after the high temperature events (Fish U-09 and Fish U-10).

408   In the upstream sector, ΔT_{Hmed} was significantly greater than zero when ambient

409   mainstem river temperature was between 14.0 and 16.9°C (bootstrapped 95% CI did not overlap

410   zero, Fig. 4a), indicating that salmon were using warm patches at these cooler temperatures.

411   When ambient mainstem river temperature was between 17 and 18.9°C, median ΔT_{Hmed} was not

412   significantly different than zero (bootstrapped 95% CI contain zero), indicating that salmon did

413   not seek out warm or cool patches. Once ambient mainstem river temperature reached 19°C,

414   however, ΔT_{Hmed} began to skew significantly negative, indicating that fish were actively using

415   cool patches. At water temperatures $\geq 21.0^\circ$C, nearly 100% of detections of tagged salmon were

416   in cool refuges, and ΔT_{Hmed} was approximately -2°C.

417   Fish in the downstream sector showed no evidence of warm patch use, and cool patch use

418   began at lower ambient mainstem river temperatures than in the upstream sector (Fig. 4b). When

419   mainstem river temperature was between 14 and 16.9°C, ΔT_{Hmed} was not significantly different

420   than zero (bootstrapped 95% CI contain zero). Once mainstem river temperature reached 17°C,

421   ΔT_{Hmed} began to skew significantly negative, a threshold that was two degrees cooler than in the

422   upstream habitat. At water temperatures $\geq 19.0^\circ$C, nearly 100% of detections of tagged salmon

423   were in cool refuges and ΔT_{Hmed} was greater than -2°C.

424   There was a clear diurnal pattern in behavioural thermoregulation in the upstream sector

425   and the thermal habitat type occupied (cool patch, ambient mainstem temperature, or warm

426   patch) was not independent of the hour of the day (Fig. 5a; $\chi^2_{(46, N = 4526)}$, P < 0.05). Cool patch

427   use increased throughout the afternoon, when river temperature generally increases due to solar

428   heating, and was greatest at 16:00 before decreasing again. Warm patch use increased during the

429   early morning, and was greatest at 06:00. There was no clear diurnal pattern in behavioural

430   thermoregulation in the downstream sector and no warm patch use was observed (Fig. 5b),

431   however, water type occupied (cool patch or ambient mainstem temperature) was not

432   independent of the hour of the day ($\chi^2_{(23, N = 1765)}$, P < 0.05).

433

434

435   *Within-pool refuge use*

436   We examined within-pool behavioural thermoregulation in the pools where the majority

437   of cool patch use occurred (D3 and U8). During August 2016, three salmon (2 females, 1 male)

438   were detected in D3 on ≥ 18 days. During the same period, seven salmon (3 females, 4 males)

439   were detected in U8 on ≥ 19 days. Measured ambient temperature in pool D3 was always cooler

440   than downstream ambient mainstem temperature (mean = 0.42°C; range = 0.27° to 0.77°C).

441   Measured ambient temperature in pool U8 was 3.4°C cooler to 2.4°C warmer than the upstream

442   ambient mainstem temperature (on average, U8 was 0.12°C cooler than the mainstem). The

443   temperature range recorded at the bottom of U8 was 15.1°C to 21.5°C, whereas the temperature

444   range recorded in D3 was slightly broader (15.7°C to 22.2°C). The locations of thermal refuges

445   within these pools, however, were not known before deployment of temperature loggers.

446   Consequently, temperature loggers were not placed directly in the thermal refuges and recorded

447   pool temperature did not represent the coolest or warmest temperatures available in each pool.

448   The initiation of cool patch residences in pool D3 increased steadily between 16.5°C and

449   20.5°C (Fig. 6a) and no warm patch residences were observed. In pool U8, cool patch residences

450   increased dramatically between 18.9°C and 19.7°C, and reached a plateau at approximately 21°C

451   (Fig. 6b). Initiation of warm patch residences increased steadily between 16°C and 19°C,

452   however, very few warm patch residences began when pool temperature was > 19°C (Fig. 6c).

453   **Discussion**

454   All adult Atlantic salmon that were tagged with temperature-sensing transmitters engaged

455   in behavioural thermoregulation. Although mainstem river temperature in the Nord-Est was

456   relatively cool and did not exceed 23°C, adult salmon used mainstem pools as cool refuges

457   during the warmest period of the summer. Individuals exhibited behavioural thermoregulation at

458 substantially cooler ambient river temperatures (17°C in the downstream sector and 19°C in the

459 upstream sector), than expected from previous studies that described thermal refuge use by

460 juvenile (Gibson 1966; Cunjak et al. 2005; Dugdale et al. 2016) and adult (Shepard 1995)

461 Atlantic salmon. These results support the literature and metabolism-based assessment by Breau

462 (2013) that temperatures above 20°C are stressful to adult Atlantic salmon. Adult salmon

463 predominantly used cool refuges during the afternoon when the warmest ambient mainstem

464 water temperatures were recorded, and during refuge use body temperatures were as much as 5°C

465 cooler than ambient mainstem river temperature.

466   Our combination of TIR imagery, river temperature monitoring, and acoustic telemetry

467 enabled us to obtain a more holistic understanding of how adult Atlantic salmon use thermal

468 habitat than would have been possible with a single-pronged approach. Use of TIR imagery

469 allowed us to map potential thermal refuges at the riverscape scale in a system where large

470 sections of the river are inaccessible. The resulting refuge maps, when applied in concert with

471 optical imagery and first-hand knowledge of the river system, helped target acoustic receiver

472 deployment to potential cool refuges, which we were able to monitor continuously over the

473 course of the summer using acoustic telemetry. Additional deployment of acoustic receivers in

474 deep pools allowed us to identify subsurface cool refuges that were important refuges for adults

475 (e.g. pool D3) but that were missed via evaluation of TIR imagery because the cool water did not

476 extend to the river surface. Because internal salmon body temperature was recorded when

477 salmon were in proximity of a receiver, we obtained near continuous records of salmon body

478 temperature in potential refuge sites. When coupled with long-term measurement of ambient

479 river temperature from temperature loggers, telemetry records allowed us to assess behavioural

480 thermoregulation via thermal refuge use over finer temporal and larger spatial scales than would

481 have been possible using snorkel counts or radio-telemetry, techniques that have been previously

482 applied to study of thermal refuge use by salmonids (e.g. Torgersen et al. 1999; Ebersole et al.

483 2001; Brewitt and Danner 2014).

484 Physiological stress has been observed under laboratory conditions in adult Atlantic

485 salmon at 23°C (Wilkie et al 1997). Ambient mainstem river temperature in the Nord-Est first

486 approached 23°C during the high temperature event that occurred 4-6 August (day 217-219). We

487 observed mass movement of individual Atlantic salmon into pools containing cool refuges

488 during or immediately after this event. The first heat wave likely induced thermal stress, which

489 served as trigger for individuals to search out pools containing cool refuges where they could

490 recover physiologically (Breau 2013). Once fish entered pools containing refuges, they remained

491 in residence for ≥ 2 weeks, similar to the residence time observed for steelhead (*Oncorhynchus*

492 *mykiss*) using cold tributaries as thermal refuges in the Columbia River Basin (Keefer et al.

493 2009).

494 The degree of behavioural thermoregulation observed varied among individuals, and

495 appeared tied to location and timing of release. Individuals in the upstream sector exhibited a

496 greater degree of behavioural thermoregulation than salmon in the downstream sector, which

497 may be explained by the difference in ambient mainstem temperature between the two river

498 sectors. In the upstream sector, measured ambient mainstem temperature exceeded 20°C and

499 approached 23°C during all three high temperature events, whereas in the downstream sector,

500 ambient mainstem river temperature only approached 23°C during the first event. Salmon that

501 were present in the upstream sector before at least one of the three high temperature events that

502 occurred during August 2016 exhibited a greater degree of behavioural thermoregulation than

503 salmon that were tagged near the end of August, when water temperature was cooler (Fig. 3).

504 Use of cool refuges has also been linked to run timing in Columbia River steelhead. Steelhead

505 that migrated during the warmest part of the summer had the greatest rates of refuge use,

506 whereas fish that migrated either early or late in the season experienced lower mainstem

507 temperatures and had lower rates of refuge use (Keefer et al. 2009). Salmonids that migrate

508 during periods when heat stress-inducing warm water events occur seem to have a greater need

509 to use cool water refuges than salmonids migrating later in the summer when water is cooler.

510 　　　In our study, tagged adult Atlantic salmon routinely used cool patches when river

511 temperature in the Nord-Est was less than 19°C, which is substantially cooler than previously

512 reported for Atlantic salmon. Juvenile Atlantic salmon moved into cold water refuges in eastern

513 Canadian rivers when water temperature was 22-24°C (Gibson 1966; Cunjak et al. 2005;

514 Dugdale et al. 2016), and adults stopped migrating and congregated in cold water tributaries in

515 the Penobscot River in Maine at 23°C (Shepard 1995). In the upstream sector, adult salmon used

516 thermal refuges when ambient mainstem river temperature was as cool as 19°C, and nearly 100%

517 of detections were in thermal refuges when ambient mainstem temperature was ≥ 21°C. These

518 results are similar to the patterns of thermal refuge use exhibited by summer steelhead in the

519 Columbia River Basin, where steelhead began using cold water tributaries as thermal refuges

520 when mainstem river temperature reached 19°C, and 70% of tagged fish were detected in refuges

521 when river temperature exceeded 21°C (Keefer et al. 2009). In the downstream sector, however,

522 adults used thermal refuges at ambient mainstem river temperatures as low as 17°C, with 100%

523 of detections occurring in cold refuges when river temperature was ≥ 19°C.

524 　　　Interestingly, in the upstream sector, adult Atlantic salmon used warm water patches

525 when river temperature was < 17°C. Although use of warm water patches by salmonids during

526 winter has been documented (Craig and Poulin 1975; Cunjak and Power 1986; Brown and

527   McKay 1995), use of warm water patches during summer by migrating adult salmon has not

528   been previously reported in the literature. Temperatures are more stable in thermal refuges

529   created by thermal stratification or groundwater inputs than in surrounding river habitat that is

530   more susceptible to diurnal heating and cooling cycles. Warm patch use could result from

531   salmon remaining in refuges during periods when ambient river temperature decreases relative to

532   refuge temperature. Alternatively, adults may actively seek out warm water patches when

533   ambient river temperature is low. Regardless of the mechanism, warm patch use could enable

534   adults to remain within an optimal temperature range, allowing them to balance energy

535   conservation with gamete maturation, which can be slowed if temperature is too low (Berman

536   and Quinn 1991; Newell and Quin 2005; Hasler et al. 2012). Taken together, the temperatures at

537   which cool and warm patch use occurred in the Nord-Est suggest that adults may have a narrow

538   optimal temperature range similar to that previously described to optimize growth in juvenile

539   Atlantic salmon, i.e. approximately 16-20°C (Elliott 1991; Jonsson and Jonsson 2009). Bull trout

540   (*Salvelinus confluentus*) tagged with temperature-sensing V13 acoustic tags were found to

541   occupy a narrow temperature range comparable to laboratory-derived optimal temperatures for

542   growth and metabolism, despite a much broader range of temperatures available to free-

543   swimming individuals in a British Columbia reservoir (Gutowsky et al. 2017). Sockeye salmon

544   have also been observed using a narrow temperature range (9-11°C) while over-summering in a

545   stratified lake, despite the availability of warmer and cooler temperatures (Newell and Quinn

546   2005).

547   We observed a diurnal pattern in refuge use in the upstream sector of the Nord-Est. Cool

548   refuge use was greatest in the afternoon and warm patch use was greatest in the early morning,

549   corresponding to daily maximum and minimum water temperatures. The absence of a clear

550   diurnal pattern in behavioural thermoregulation in the downstream sector may be because the

551   sample size was smaller downstream (5 individuals) than upstream (10 individuals). Peak refuge

552   use by rainbow trout (*O. mykiss*) in the Snake River (Oregon) drainage also occurred in the late

553   afternoon when ambient temperature was greatest (Ebersole et al. 2001). Observations by Breau

554   et al. 2007, however, indicated that aggregation of juvenile Atlantic salmon in cool refuges was

555   influenced more by water temperature than by time of day. Observation of cool refuge use by

556   Breau et al. (2007) was based on snorkel counts in one study reach, whereas our observations

557   were based on near-continuous records of adult body temperature and river temperature, and

558   spanned multiple potential refuges, which could explain the discrepancy between our findings

559   and those of Breau et al. (2007).

560   Although cold water tributaries and confluence plumes served as important thermal

561   refuges for adult salmonids in the much larger Columbia and Penobscot river systems, (Shepard

562   1995; Goinea et al. 2006; Keefer et al. 2009), the two confluence plumes in Nord-Est that were

563   equipped with acoustic receivers did not serve as thermal refuges for adult Atlantic salmon.

564   Mainstem discharge greatly influences confluence plume volume, which subsequently influences

565   refuge carrying capacity (Gendron 2013). The monitored confluence plumes in the Nord-Est

566   were shallow (≤ 1.5 m deep at river discharge of 21 m^3s^{-1} but ≤ 0.75 m deep when river

567   discharge was 9 m^3s^{-1}). Discrete measurements made during August 2016 indicated that the

568   cooling influence of confluence plumes did not extend more than 3 m from the mouth of each

569   tributary, despite temperature differences between tributaries and the mainstem of 10-12°C

570   (Frechette, personal observation). Thus it is unlikely that shallower unmonitored confluence

571   plumes were used as refuges. Our findings are similar to those of Beguin et al. (2000), who

572   found that adult steelhead over-summering in Steamboat Creek (a mid-order stream in Oregon)

573   did not use shallow (< 0.8 m) confluence plumes. Cool water confluence plumes in the Nord-Est,

574   therefore, were likely too shallow to be used by adult Atlantic salmon for long-term behavioural

575   thermoregulation (Torgersen et al. 1999; Beguin et al. 2000; Ebersole et al. 2001).

576   The most important thermal refuges used by over-summering Atlantic salmon adults in

577   the Nord-Est were stratified alluvial pools (D3 and U8). Thermal stratification may form in pools

578   where groundwater seeps or hyporheic flow is present, or in areas where channel morphology

579   causes water velocity to slow sufficiently to prevent mixing throughout the water column

580   (Nielsen et al. 1994). In the absence of mixing, surface water heats up during the day while

581   bottom water maintains a relatively constant temperature. During the night, surface water cools

582   and sinks to the bottom, maintaining a temperature gradient (Nielsen et al. 1994). Although

583   mainstem alluvial pools are rarely stratified (Nielsen et al. 1994; Gendron 2013), pool U8 is

584   unusually large relative to other pools in the Nord-Est. The pool inlet is shallower than the outlet,

585   the maximum depth exceeds 6 m, and a gravel bar island in the centre of the pool may entrain

586   cool bottom water, characteristics that are ideal for formation of thermal stratification via solar

587   heating (Matthews et al. 1994; Nielsen et al. 1994; Gendron 2013).

588   We identified hyporheic flow associated with a small gravel bar near the head of pool U8

589   from the TIR imagery, which may enhance thermal stratification (Gendron 2013). At present, it

590   is not possible to identify solar heating or hyporheic flow as the main mechanism producing

591   thermal stratification in pool U8, however the temperature at the substrate was as much as $1.7°C$

592   cooler than at the surface, which provides evidence of strong thermal stratification (Matthews et

593   al. 1994; Gendron 2013). The source of thermal stratification in pool D3 is still under

594   investigation, but is thought to result from resurgence of cool water originating in a cool tributary

595   located upstream of the pool. Although the confluence plume was clearly evident in TIR images,

596 surface evidence of the plume did not extend into pool D3. Regardless of the source of thermal

597 stratification, pools D3 and U8 were extremely important to over-summering Atlantic salmon, a

598 finding which corresponds with previous studies of thermal refuge use by steelhead and Chinook

599 salmon (*Oncorhynchus tshawytscha*). Thermally stratified pools were the most heavily used pool

600 type by summer run steelhead in the Middle Fork Eel River, a river system in California that is

601 comparable in size to the Nord-Est (Nielsen et al. 1994), and both over-summering spring run

602 Chinook and summer run steelhead disproportionately used pools as cool refuge habitat, relative

603 to their availability in Oregon rivers (Torgersen et al. 1999; Baigun et al. 2000).

604   Deep pools with stable thermal refuges should allow individuals to thermoregulate with

605 relatively little expenditure of energy (Berman and Quinn 1991). Adults holding in large pools

606 may maintain a stable body temperature either by changing position in the pool or by remaining

607 in a stable thermal refuge while ambient water temperature around the refuge changes

608 throughout the day. Such behaviour would buffer adults from large temperature fluctuations

609 associated with daily warming and cooling cycles in the river and allow them to retain valuable

610 energy stores for spawning (Sutton et al. 2007; Hasler et al 2012). It must be noted, however,

611 that temperature is not the only factor driving habitat selection during the in-river pre-spawning

612 period. River discharge, proximity to spawning sites, season, and density of conspecifics may

613 also influence choice of holding habitat. For example, large pools likely serve not only as

614 thermal refuges, but also as hydraulic refuges, allowing fish to hold and maintain optimal

615 temperature while waiting for discharge to increase and permit resumption of migration

616 (Gendron 2013). Such pools are likely to be particularly important in shallow, gravel-bed rivers

617 that are prone to low river discharge during the warmest periods of the summer when adult

618 Atlantic salmon are present, and their abundance and location along a riverscape may influence

619 migratory patterns of adult Atlantic salmon. Although we measured the maximum depths of the

620 pools containing receivers, we did not make detailed measurements of physical habitat. A

621 detailed characterization of available pools, confluence plumes, and intervening habitat would

622 permit the effects of temperature to be disentangled from other factors that influence habitat

623 choice by adults during in-river holding, and is the subject of on-going research in the Nord-Est.

624　　　　Limitations inherent in our study give rise to potential improvements for future research.

625 We based evidence of thermal refuge use on the difference between ambient river temperature

626 and internal fish temperature. We selected river temperature monitoring sites in well-mixed areas

627 of the river that were representative of mainstem temperature, therefore, choice of temperature

628 monitoring sites was unlikely to overly influence our identification of the timing, location, and

629 temperatures at which fish initiated behavioural thermoregulation. It is possible, however, that

630 choice of temperature monitoring sites introduced a minor bias into our results (Torgersen et al.

631 1999). Such a bias could be mitigated by deploying multiple mainstem temperature loggers from

632 which an average reach-specific ambient mainstem temperature could be calculated and used for

633 identifying behavioural thermoregulation. Our study also represents a limited number of

634 individuals tagged during one year in a medium-sized river that is relatively cool compared with

635 more southerly rivers within the range of Atlantic salmon. Since thermal tolerance in salmon is

636 related to the temperature at which they are acclimated (Elliott 1991), salmon populations in

637 rivers that are warmer (or cooler) than the Nord-Est likely have different thresholds for initiation

638 of thermal refuge use. Inter-annual differences in temperature regimes might also produce

639 differences in thermal habitat use within a given river system. Additionally, refuge types that are

640 important in large river systems (e.g. confluence pools) may be less important in smaller

641 systems. Application of our methods in catchments of varying sizes across the range of Atlantic

642   salmon and over multiple years would facilitate identification of refuge types and thresholds for

643   behavioral thermoregulation under different hydrological and temperature conditions. This

644   would further improve our understating of thermal habitat requirements of Atlantic salmon,

645   which could prove invaluable in predicting responses of Atlantic salmon populations to climate

646   change.

647   River temperatures are rising in northern latitude rivers within the range of Atlantic

648   salmon, a trend that is expected to continue under current climate change projections (Ferrari et

649   al. 2007; Webb and Nobilis 2007; van Vliet et al.2013). Although optimal temperature for adults

650   likely differ among river systems due to different thermal regimes and acclimation temperatures,

651   it is clear that adult salmon used a narrow temperature range and required access to cool refuges

652   at much lower temperatures than are regularly measured in salmon rivers (Shepard 1995; Breau

653   et al. 2007; Dugdale et al. 2016). Increased temperatures and reduced river discharge can serve

654   as physical barriers to salmon migration, preventing adults from reaching the thermal refuges

655   that are crucial to survival and energy maintenance when river temperatures become stressful

656   (Torgersen et al. 1999; Hasler et al. 2012). Thus, the ability for adult salmon to access suitable

657   holding pools and cool refuges will become even more important for maintaining populations in

658   a warming climate because of the negative impacts of high temperature on survival and

659   reproductive success. It is therefore imperative to identify, protect, and maintain connectivity

660   among suitable thermal refuges in salmon rivers to ensure population persistence. Such

661   protections may include controlling releases of water from dams on regulated rivers, restricting

662   fishing and other human activities in refuges, and limiting groundwater extraction or other land

663   use practices that reduce riverbed shading or groundwater inputs (Sutton et al. 2007; Breau and

664   Caissie 2013; Kurylyk et al. 2015). In certain cases, enhancing existing thermal refuges or

665 creating new refuges via channel modification or groundwater pumping may be warranted

666 (Kurylyk et al. 2015). Presence of suitable adult thermal habitat is also essential for enhancement

667 programs like the habitat expansion initiative currently in place on the Rivière Sainte-Marguerite

668 Nord-Est, where adult salmon are introduced to previously inaccessible habitat via fish ladders or

669 transport. Inventory of potential thermal refuges should therefore be included in feasibility

670 studies before the implementation of such conservation translocation programs to ensure their

671 success.

672

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690

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865 Table 1. Size and sex of Atlantic salmon implanted with temperature sensing acoustic

866 transmitters (Vemco V13T–H) during summer 2016, by river sector, site of release, and dates

867 individuals were first available for detection in each river sector, and number of times each

868 individual was detected (number of records). Tagging data for fish removed from analyses are

869 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

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871

872   **Figure Captions**

873   Figure 1. Map of study area. Panel A indicates the fish ladder where adult salmon were captured,

874   sites where salmon were released after transport, and placement of acoustic receivers and

875   temperature loggers. Impassable waterfalls bounding the upstream and downstream river sectors

876   are indicated with an X. Receiver positions are indicated with solid grey circles. Receivers in the

877   upstream sector of the river are labelled U1-U9 and receivers in the downstream sector are

878   labelled D1-D4. Temperature loggers are indicated with small dark black circles and were

879   deployed in the river mainstem (at rkm 30 and rkm 45), or in association with acoustic receivers

880   in confluence plumes (U1 and U5) or deep pools (1 logger in D3; 2 loggers in U8 to test for

881   thermal stratification). Panel B depicts the location of all cool patches identified from TIR

882   imagery. Boundaries delineating each study sector are identified with solid black lines. The map

883   was created using ArcGIS software by ESRI.

884

885   Figure 2. Temporal variation in behavioural thermoregulation by individual adult Atlantic

886   salmon tagged with temperature-sensing acoustic tags during summer 2016 from June 29 (day

887   181) to August 31 (day 244). Behavioral thermoregulation is represented as the difference

888   between body temperature and instantaneous mainstem temperature (ΔT_I, where mainstem river

889   temperature was measured at rkm 45 (upstream sector) and rkm 30 (downstream sector). Fish

890   identity corresponds to Table 1 and is presented in the gray box above each individual plot along

891   with sex (M = male, F = female). Each point marks a detection of an individual by a given

892   receiver, with receiver location coded by color. Habitat type is specified for each receiver (DP =

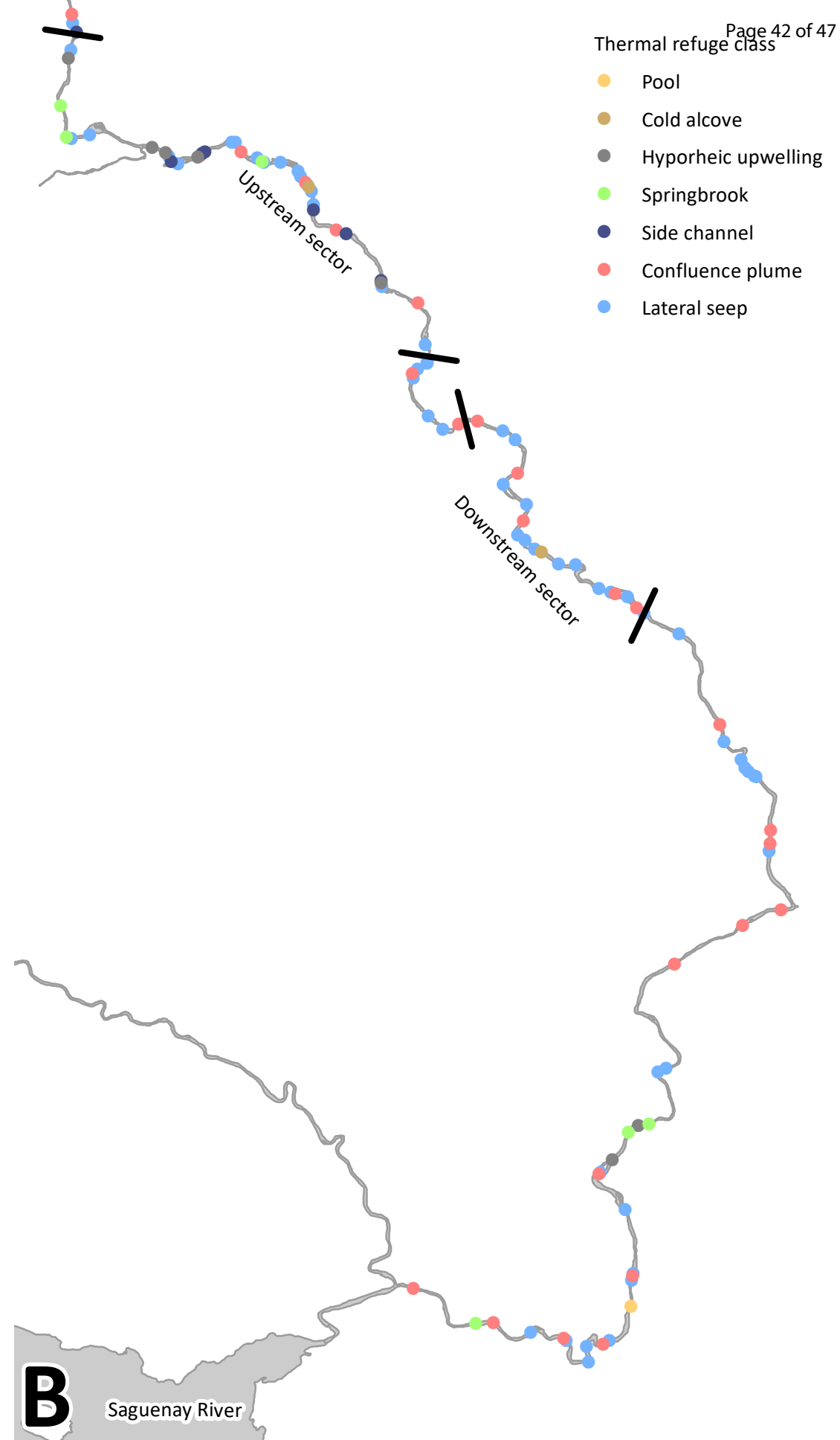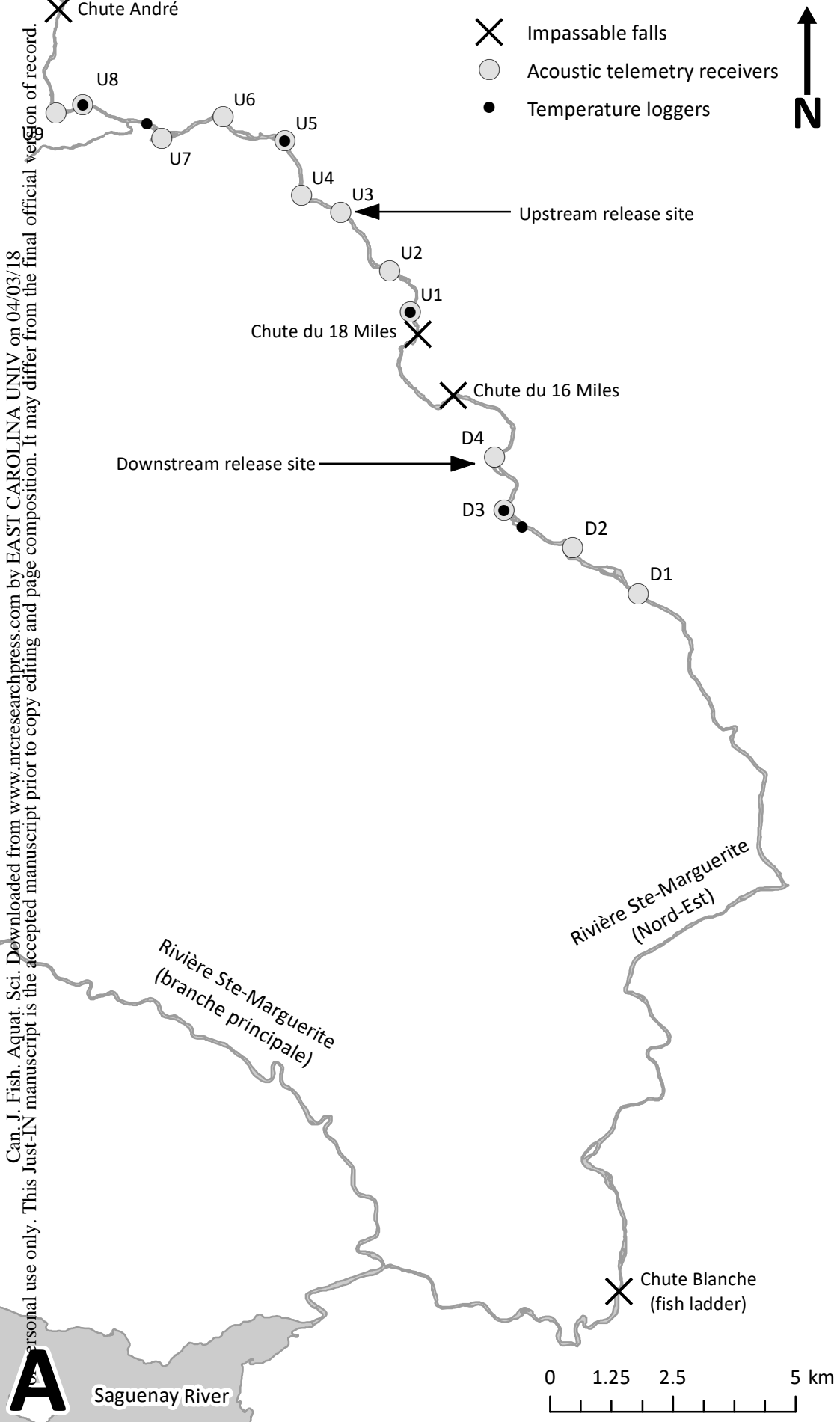893   deep pool; GW = groundwater seep; CP = confluence plume).

894

895   Figure 3. Slope plots depicting the median slope and median intercept obtained via bootstrapping

896   (1000 replicates) for the linear relationship between mean mean hourly internal fish temperature

897   (T_{BH}) and mean hourly ambient mainstem river temperature (T_{RH}) in the upstream and

898   downstream river sectors for all fish included in analyses. The solid black line represents a 1:1

899   relationship between T_{BH} and T_{RH}. Males are represented by dashed lines in shades of blue,

900   females by solid lines in shades of red. Fish identity corresponds to Table 1.

901

902   Figure 4. Frequency histograms of the difference (ΔT_H) between mean hourly internal fish

903   temperature and mean hourly mainstem river temperature for the A) upstream sector (n = 10

904   salmon) and B) downstream sector (n = 6 salmon), binned by mainstem river temperature

905   (measured at rkm 45 for the upstream sector and at rkm 30 for the downstream sector), for

906   August 2016. Solid red vertical lines denote the median (ΔT_{Hmed}) and dashed black vertical lines

907   denote the 95% CI, generated using 5,000 Bca bootstrap replicates. Note different y-axes

908   between panel A and panel B, and in the 14-15.9°C bin in Panel B.

909

910   Figure 5. Percent of total hours that salmon used cool patches ($\Delta T_H \leq$ -1°C; black bars) mainstem

911   river temperature (-1°C > ΔT_H > 1°C; light gray bars), and warm patches as ($\Delta T_H \geq$ 1°C; dark

912   gray bars) in the upstream (a) and downstream (b) sectors.

913

914   Figure 6. Empirical cumulative density curves for river temperature (measured at the bottom of

915   pool U8 in the upstream sector and at the bottom of pool D3 in the downstream sector) at the

916   start of each patch residence (use of cool or warm patches $\geq$ 15 min).
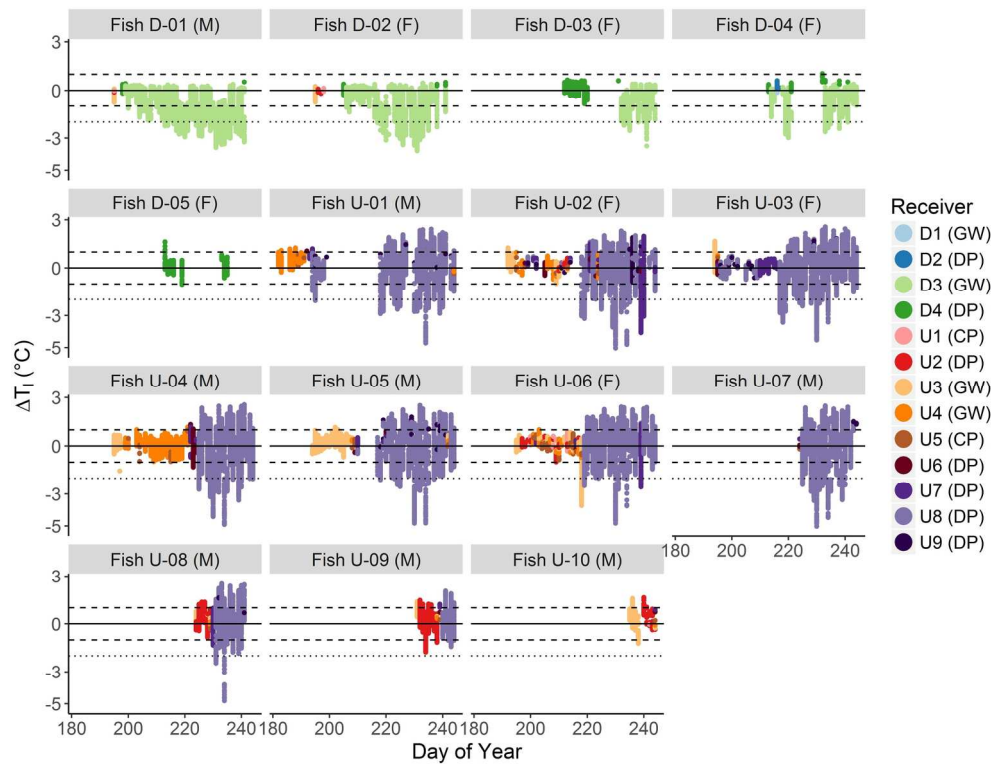
A

- ✕ Impassable falls
- ⬤ Acoustic telemetry receivers
- • Temperature loggers

N

Chute André

U8
U9
U6
U7
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)

0   1.25   2.5   5 km

Saguenay River

B

Thermal refuge class
- ⬤ Pool
- ⬤ Cold alcove
- ⬤ Hyporheic upwelling
- ⬤ Springbrook
- ⬤ Side channel
- ⬤ Confluence plume
- ⬤ Lateral seep

Upstream sector

Downstream sector

Saguenay River
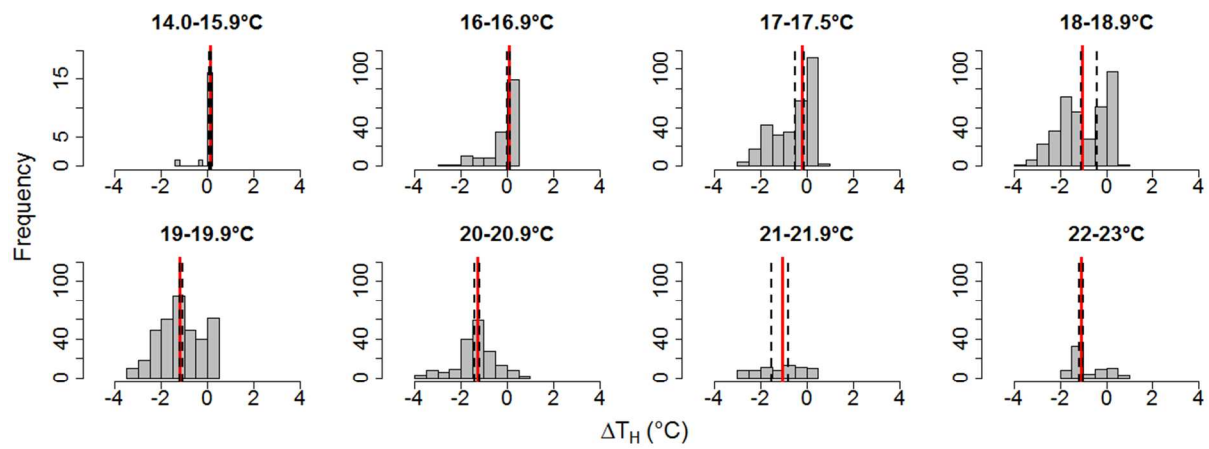
Figure 2

181x139mm (300 x 300 DPI)
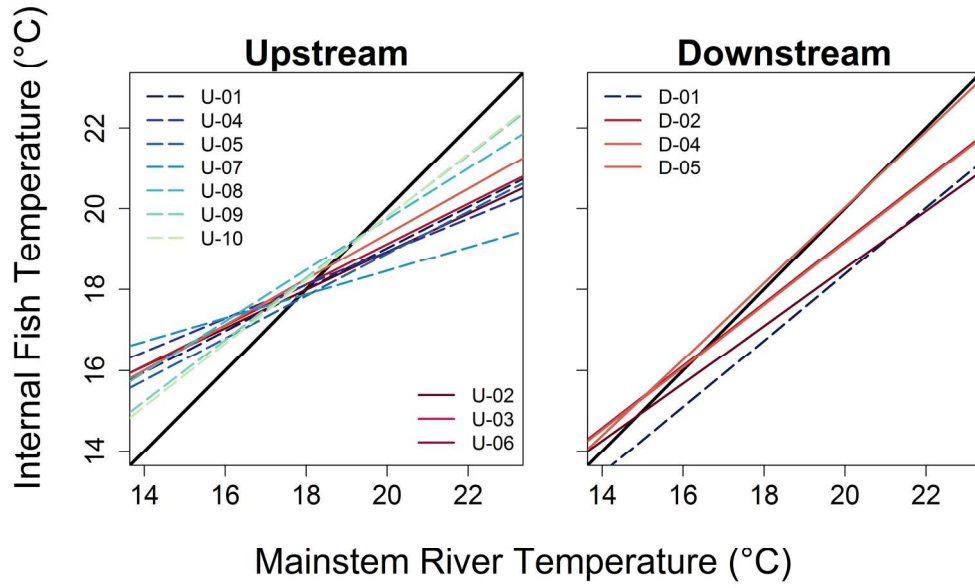
Figure 3

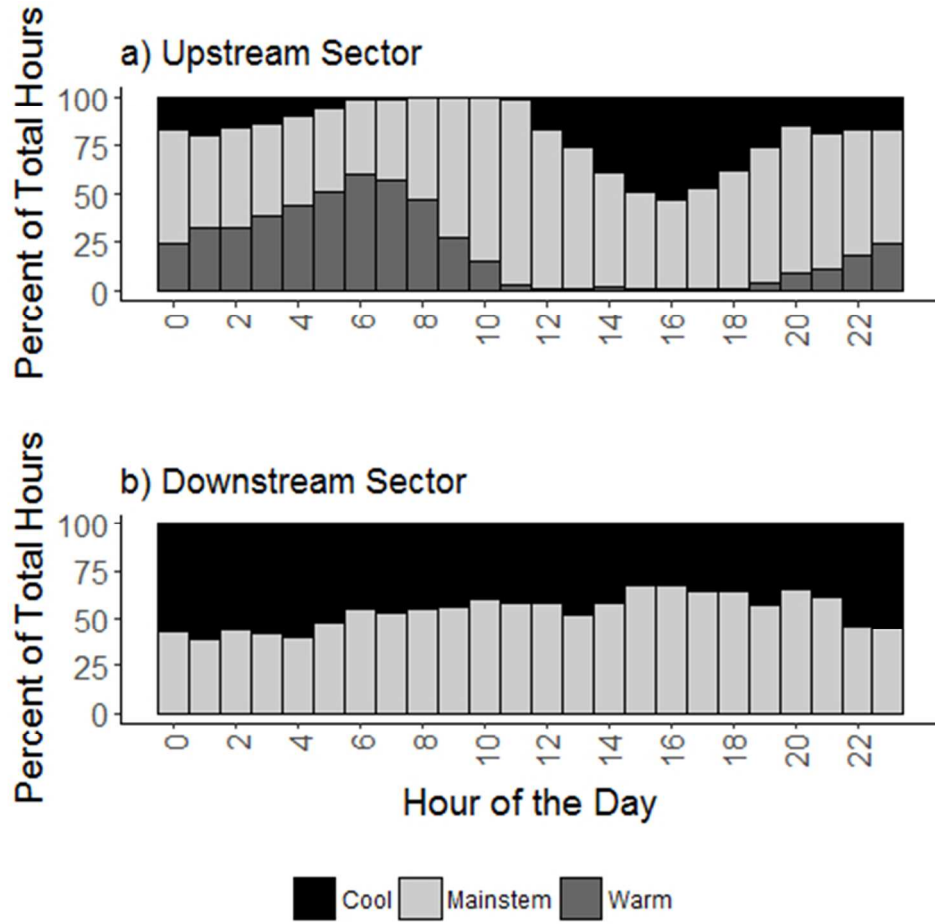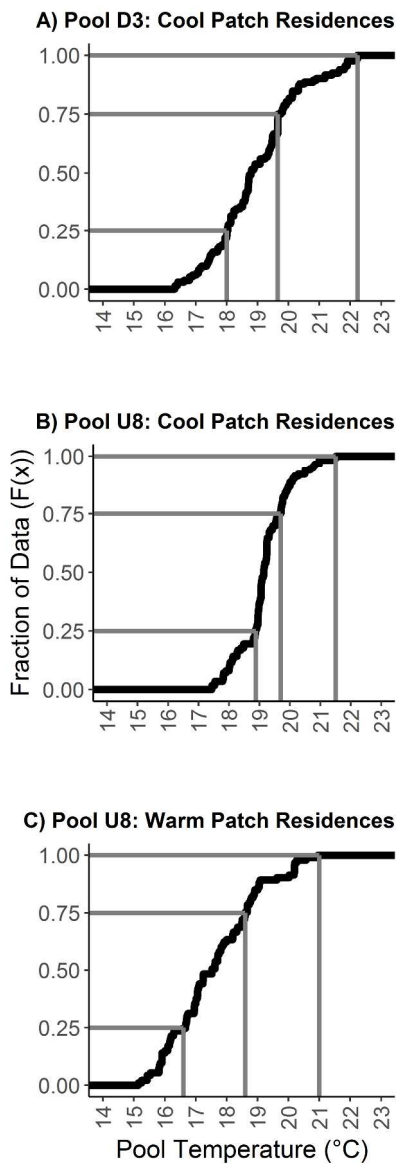181x139mm (300 x 300 DPI)

A) Upstream Sector
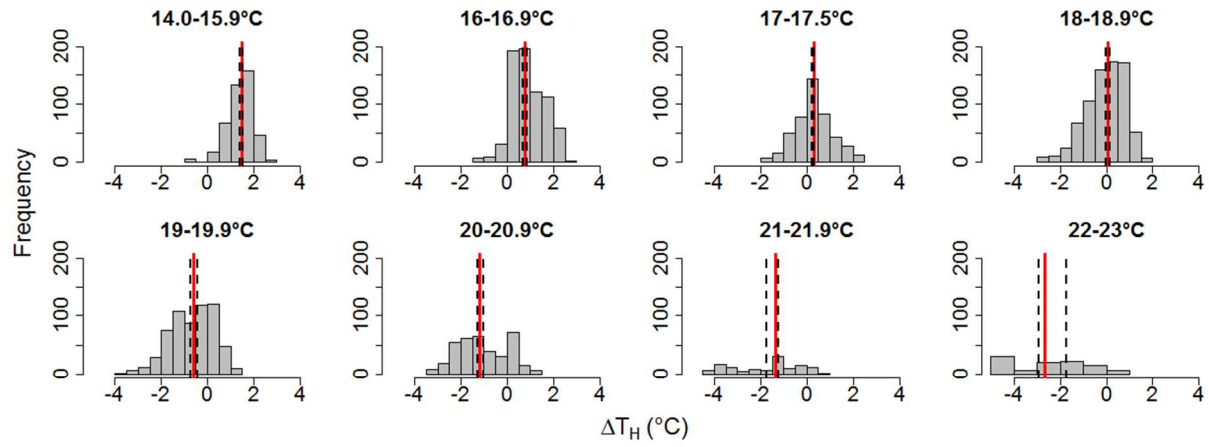
B) Downstream Sector

Figure 5

136x128mm (96 x 96 DPI)

Figure 6

236x653mm (300 x 300 DPI)