Supplement 1

Acoustic telemetry overview

Study lake

The study was carried out in Lake Ledoux (46° 38' N, 73° 15' W), Mastigouche Wildlife Reserve, Québec, Canada. Lake Ledoux is a typical small oligotrophic temperate zone lake with respect to surface area (11.9 ha), mean depth (5.5 m), maximum depth (17.0 m), and general physicochemical characteristics (Magnan 1988). The summer stratification of the lake provides a heterogeneous thermal habitat and thus favourable conditions for fish behavioural thermoregulation. This lake has been the site of many studies on brook charr (*Salvelinus fontinalis*) ecology, including diel activity patterns and habitat use (e.g., Bourke et al., 1996, 1997), resource polymorphism (e.g., Dynes et al. 1999, Pépino et al. 2018, Proulx and Magnan 2004), and behavioural thermoregulation (e.g., Bertolo et al. 2011, Goyer et al. 2014). While previous field studies used radiotelemetry, this is the first to use acoustic telemetry to follow the movements, habitat use, and behavioural thermoregulation of individuals throughout the year. Brook trout are iteroparous, and in our study area they complete their life cycle in the lake (e.g., no migration through tributaries), which offers a good opportunity to study a lacustrine brook charr population moving freely in its natural environment. Reproductive activity occurs from mid-October to mid-November. The spawning ground is a well-defined area easily visible from a boat, with groundwater outflow (personal observations). Brook charr is the only fish species in the lake, and sport fishing is rigorously controlled by the Québec Government (Bourke et al. 1996). The lake was closed to fishing during the study.

VEMCO Positioning System (VPS)

VR2 positioning system

A pilot study was conducted from 5 July 2016 to 22 May 2017 to obtain 3D-localizations and body temperatures of lacustrine brook charr at fine spatio-temporal scales (i.e., every minute; ~1 m accuracy) throughout the year. To this end, we first deployed 10 receivers (VR2Tx-069k-111-BAT; VEMCO Inc., Halifax, NS, Canada) and two references tags (V9TP-2x-069k-1-0034m; VEMCO Inc.) from 5 to 7 July 2016 (Fig. 1). Tag programming was identical for reference and fish tags and was set as follows: the first 90 d with a delay of 30–90 s (setup 1); the following 270 d with a delay of 270–390 s (setup 2). This tag programming was selected to yield fine-scale temporal data during summer (i.e., the first 90 days) and to prolong tag life for the rest of the year. With this programming, tag life was estimated at 720 d. Following the company's recommendation, we moved one receiver to a new location (station 3) and activated all internal transmitters in "Sync Tag" mode with medium power level on 10 August 2016 to ensure better communication among receivers and improve the conversion of tag detection to fish position. Four additional receivers were deployed between 7 and 8 November 2016 to improve coverage of the study lake during winter (Fig. 1). The VR2 positioning system was operational from 7 July 2016 through 22 May 2017, but with different receiver configurations (Table 1, Fig. 1). At the end of this pilot study, we observed that fish data were occasionally missing, especially during periods of inactivity, when individuals are known to agglomerate during the day (e.g., Bourke et al., 1996). We suspected that "collisions" were occurring between tag signals, which occur when individuals are in proximity, creating interference during tag transmission (personal communication with VEMCO staff). We then decided to switch from the VR2 positioning system to HR2 positioning system, a technology specifically developed to circumvent this problem.

HR2 positioning system

From 7 to 8 June 2017, we deployed 23 receivers (HR2-180k-100; VEMCO Inc.; Fig. 1). The receivers were attached to a floating line (3/8" multifilament polypropylene rope) with four to five tie-wrap cables $(14.6'' \times 0.30''$, Type 21S) and anchored to a 50 lb concrete block equipped with a 5/16" galvanized swivel to ensure free rotation of the floating line. The floating line was attached to a buoy (weight 7 lbs, buoyancy 3,200 g; PESCA N-90/8A) with a stainless steel locking safety snap. Two welded stainless-steel rings were used, one to attach the floating line to the buoy and one at the anchor's swivel to allow the rope to slide. The setup allowed us to retrieve the receiver without moving the anchor, which stayed in place throughout the study period. The buoy was installed below the water surface to avoid boat collision and best maintain the receiver in a vertical position (Photo 1). The geographic coordinates of the receivers and reference tags were determined by VEMCO to obtain full coverage of the lake. Receiver water depths were chosen to represent that mainly used by fish. The receivers' built-in transmitters were activated in "Sync Tag" mode with the "very high" power level, following the company's recommendations, to ensure high detection and synchronization among receivers. On 21 June 2017, we installed four reference tags (V9TP-2x-180k-xxxm; VEMCO Inc.) to complete the HR2 positioning system (Fig. 1). Tag programming was identical for reference and fish tags and was set as follows: the first 30 min with a pulse-position modulation (PPM) delay of 10–20 s and a high residency (HR) delay of 5 s (setup 1); the following 740 d had no PPM and an HR delay of 10–14 s (setup 2); the power level was high in both setups. This HR2 positioning system was operational from 21 June 2017 to 6 November 2018 (Table 1). However, the VPS was occasionally not operational during this period due to maintenance work or battery lifetime. The two main maintenance procedures consisted of data offload and battery replacement (Table 2). Because of the shorter battery life of the HR2 receivers (\sim six months) compared to the VR2Tx receivers (\sim 15 months), and because the lake was not accessible during winter, the late winter and early spring periods could not be monitored. No fish data were collected on 11 October 2017 (battery replacement) and from 15 April to 31 May 2018 (battery failure; Fig. 2).

VPS performance

VR2 positioning system

For the first VPS analysis (VPS-Summer), 65% of synctag transmissions were logged on three or more receivers. The average percent of synctag transmissions detected at a single receiver between 0 and 400 m. Synctag detections were \sim 10 times higher after 10 August 2016, the date on which synctag mode was activated with the "high" power level. After this date, VEMCO reported that receiver time synchronization availability was excellent. An average of 23.7% of fish tag transmissions were detected on at least three receivers, resulting in 115,712 fish tag positions calculated by the VPS on 11 different fish. The number of fish tag positions was low until 10 August 2016, after which the number increased linearly until the end of September, then dropped and stabilized in October following the shift in tag programming. The average horizontal position error (HPE) was lower for fish tags than for synctags, and the estimate was below 16.5 for 95% of fish positions (Table 3).

For the second VPS analysis (VPS-Winter), 96% of synctag transmissions were logged on three or more receivers. The average percent of synctag transmissions detected at a single receiver between 0 and 400 m was 66.6%. Synctag detections were stable throughout the study, except for a ~25% decrease in May 2017. VEMCO reported that receiver time synchronization availability was very good throughout this period (7 November 2016 to 22 May 2017). The amount of fish tag transmissions detected on at least three receivers was 71.1% on average, resulting in 239,721 fish tag positions reported by the VPS for nine different fish. The number of fish tag positions dropped by 33% at the beginning of January 2017, and then linearly decreased by 50% from the end of March to the end of May. The average horizontal position error (HPE) was lower for fish tags than for synctags, and the estimate was below 16.1 for 95% of fish positions (Table 3). Detections of reflections (i.e., an object between a transmitter and a receiver that obstructed the straight-line path, or the signal was reflected off an object) were observed in winter, probably due to ice cover. These reflections introduced some poor-quality positions despite low HPE values.

HR2 positioning system

For the third VPS analysis (HR2), synctag detections were stable over time, except for the two last weeks of September, when syntag detection dropped by \sim 20%. A total of 11,951,479 fish tag positions were calculated by the VPS. Globally, we observed a linear increase in fish tag positions over time from June to mid-September (there were five times more fish tag positions in mid-September compared to mid-June). The HPE of fish tag positions showed a right-skewed distribution, with a mode around 0.6, and 96.6% of positions had HPE < 10. HPE was mostly uniform throughout the array (HPE < 2.5), except near the shore (i.e., the first 20 m from the shore). In the narrow area between the eastern and western parts of the lake, HPE values were high because positions in this area lie outside all possible receiver triangles. For transmitters whose positions were known (i.e., synctags and references tags), the measured horizontal error (i.e., the horizontal distance between the calculated position and the known position of the transmitter; HPE_m) could be calculated. The relationship between HPE and HPE_m can be used to predict the actual error of animal tag positions from HPE estimates (Meckley et al. 2014). Overall,

the 95th percentile of HPE_m was < 10 m for HPE < 10. Another measure of position error is the root-mean-squared error (RMSE), which is a time-based measure with units in milliseconds. The RMSE is a measure of detection time error for a given position, where detection time error is primarily caused by signal detections from acoustically reflective surfaces (i.e., multipath). The RMSE distribution for fish tags is also a right-skewed distribution, with a mode near 0, and 99.8% of positions had RMSE < 5. RMSE can be used in combination with HPE to filter data. For instance, by filtering synctag and reference tag positions by RMSE < 2 , HPE_m was lower than 5 m for HPE $<$ 7.5.

For the fourth VPS analysis (HR2), we obtained much the same results, with positioning errors (HPE and RMSE) slightly higher than for the third analysis. A total of 11,915,876 fish tag positions were calculated using the VPS. Globally, we observed a linear decrease of \approx 33% of fish tag positions over time from mid-October to December 2017; positions were then stable until mid-January 2018, when they increased by ~20% for the rest of the study period. The HPE of fish tag positions was a right-skewed distribution, with a mode around 0.8, and 96% of positions had HPE < 10. In general, the 95th percentile of HPE_m was < 15 m for HPE < 10. The RMSE for fish tags showed a right-skewed distribution, with a mode near 0, and 99.7% of positions had RMSE < 5. By filtering synctag and reference tag positions by RMSE < 2, HPE_m was below 10 m for HPE < 7.5.

For the fifth VPS analysis (HR2), VPS performance was highly comparable to the previous year for the same period. A total of 16,864,929 fish tag positions were calculated with the VPS. We observed an overall linear increase of ~25% of fish tag positions over time from June to September 2018 followed by a 50% decrease during the last month of the study (i.e., October 2018). The HPE for fish tag positions showed a right-skewed distribution, with a mode around 0.6, and 96.9% of positions had HPE < 10. Globally, the 95th percentile of HPE_m was < 10 m for HPE < 10. The RMSE of fish tags showed a right-skewed distribution, with a mode near 0, and 99.9% of positions with

RMSE < 5. By filtering synctag and reference tag positions with RMSE < 2, HPE_m decreased to 5 m for HPE $<$ 7.5.

Fish capture and tagging protocol

Fish were caught in both the littoral (< 3.0 m) and pelagic (> 4.5 m) zones of the lake using Alaska traps (opening 1.0 m \times 1.8 m, two 1 m \times 15 m wings, mesh size $\frac{1}{2}$ inch; Fipec Industries, Gaspé, Québec, Canada). The littoral zone traps were always in the epilimnion layer. Fish were collected during three consecutive years (28 June – 12 July 2016; 12–20 June 2017; 5–7 June 2018), mostly during night so that brook charr were captured when they are most active (Bourke et al. 1996). After capture, brook charr were kept in an enclosure (3 m \times 4 m \times 6 m depth) and tagged within the same day.

The tagging procedure was adapted from the methods described by Adams et al. (1998), Bélanger and Rodriguez (2001), Bridger and Booth (2003), Mellas and Haynes (1985), and Thiem et al. (2011). The same procedure (as described below) was also used with success in previous studies (Goyer et al. 2014, Pepino et al. 2015 a, b). In 2016, 12 brook charr (mass: 282–438 g; fork length: 296–342 mm) were equipped with 6.2 g acoustic transmitters (V9TP-2x-069k-1-0034m; VEMCO Inc.). In 2017 (*n* = 30) and 2018 (*n* = 15), 45 brook charr (mass: 226–600 g; fork length: 272–385 mm) were equipped with 4.0 g acoustic transmitters (V9TP-2x-180k-xxxm; VEMCO Inc.). Transmitter mass was always ≤ 2% of the fish body mass, as recommended in previous studies (Bridger and Booth 2003, Mellas and Haynes 1985). Both types of transmitters have two sensors, one for pressure and one for temperature. Fish were anaesthetized with clove oil (50 mg/L) in an 80 L StackNest container containing water from the metalimnion (mean temperature: 13.4°C; range: 10.8–17.5°C) until fish stopped swimming (~ 5 min). Fish were then placed dorsal side down

on a V-shaped surgical board covered with synthetic foam soaked in Aquarium Pharmaceuticals Stress Coat to reduce mucus loss. Continuous water flow was provided to the surgical board to ensure constant irrigation of the gills with a clove oil solution (20 mg/L) to maintain a regular rate of opercular beating (Photo 2). Transmitters were implanted in the peritoneal cavity through a 10 mm incision on the left side of the ventral midline between the pectoral and pelvic fins. To reduce the risk of infection, oxytetracycline (50 μg/g of fish mass; Oxyvet 100LP; CDMV Inc. Saint-Hyacinthe, QC, Canada) was injected into the peritoneal cavity before closing the incision with two to four separate sutures (synthetic absorbable PDS II clear monofilament: Ethicon 4-0 + FS-1; Z441). All surgical equipment, including the transmitter, was sterilized for 10 min with a germicidal detergent (WESCODYNE, West Peneton, Montréal, QC, Canada) before each surgery. The complete surgery (including morphological measurements and photography) lasted on average 6 min in 2016 and less than 5 min in 2017 and 2018. Before surgery, we measured fork and total length $(\pm 1 \text{ mm})$ and mass $(\pm 0.1 \text{ g})$ of brook charr, and sex was determined by visual inspection. After surgery, we photographed fish lying on their left side in a lateral position next to a reference scale for post-processing standardization in future morphological analyses (Photo 3). Brook charr were then placed in an 80 L StackNest container half-filled with metalimnion water with malachite green chloride (0.1 mg/L; 2016) or 1% salt water (2017 and 2018) for 20–30 min to disinfect the surgical area before returning tagged fish to an enclosure in the lake $(3 \text{ m} \times 4 \text{ m} \times 6 \text{ m}$ depth). During this time of recuperation, we measured mouth width $(\pm 0.1 \text{ mm})$ and body width $(\pm 0.1 \text{ mm})$ mm) with an electronic caliper, and we collected 1 cm² of pelvic fin preserved in 95% ethanol for further genetic analyses (Table 4). Fish were released from the enclosure to the lake the day after all fish tagging was completed (2016) or the day following surgery (2017 and 2018). All fish released into the lake were in apparently good condition and behaved normally, except for one fish found dead in the enclosure in 2016.

Fish positions

VR2 positioning system

Start and end times of fish positions were defined in the same way as for the HR2 positioning system (see details in the subsection below). The fate of each fish fell into one of four categories: alive (n = 5), predation (n = 4), movement (n = 2), or dead (n = 1). The first three categories are the same as for the HR2 positioning system. The fourth category is specific to the VR2 VPS because one individual died immediately after tagging, so no position data were collected (Table 5).

The median number of positions per individual was 13,556 (range: 0–44,049). The median number of survey days per individual was 131 days (range: 0–318 days). Of the 12 individuals tagged in 2016, five were still being recorded at the end of their first winter (Table 5), but we were not able to follow these individuals when we switched to the HR2 VPS system in 2017. The median number of daily fish positions was low at the start of the study (< 100 positions/day), then increased starting from the beginning of August to reach \sim 400 positions/day at the end of September (Fig. 3a). The increase at the beginning of August corresponded to "Sync Tag" mode activation on 10 August. The number of fish that were tracked simultaneously ranged from nine individuals/day in July 2016 to five individuals/day at the end of the survey in May 2017 (Fig. 3b). No pattern was apparent in mortality rate.

HR2 positioning system

Fish positions started being recorded as soon as fish were placed in the enclosure, immediately after tagging. To determine the last day of fish positions, we classified the fate of each individual into one of the following categories to ensure that we only used fish data when fish were alive

and still moving (Table 5). (i) Alive: the individual was still moving at the end of the study (i.e., when we removed the receivers); we kept all available data ($n = 8$). (ii) Recapture: the individual was recaptured during the 2018 spawning season using Alaska traps (opening 1.0 $m \times 1.8$ m, two 1 m \times 15 m wings, mesh size $\frac{1}{2}$ inch; Fipec Industries, Gaspé, Québec, Canada) and then euthanized for subsequent laboratory analyses; we kept all available data before recapture (n = 9). (iii) Predation: the transmitter stopped sending a signal before the end of study (i.e., the individual was probably no longer in the lake) or stopped sending a signal for at least 24 h, after which no movement was observed. In both cases, we attributed the fate of the individual to predation (since the lake is considered as a closed system), either by natural predators or illegal fishing that occurred during our survey (personal communication). Natural predators of brook charr in Lake Ledoux include loon during summer and mink during the fall spawning period. We believe that natural predation on adult brook charr is minimal given the size of the fish relative to these natural predators. However, illegal fishing was observed in Lake Ledoux (M. Pépino, personal observation; time-lapse photography) and was probably responsible for the majority of what we classified as "predation." We then considered the end of fish positions as the day before we lost the transmitter signal $(n = 21)$. (iv) Movement: in some cases, we continued to register positions, but with no observed movement for the rest of the study (i.e., the tag was still in the lake but at a fixed position). This could happen after tag expulsion or if the fish died and sank to the lake bottom (n = 7). In this case, we identified the time of shift (i.e., the time when the tag was at a fixed location) based on X and Y coordinates as well as the temperature time series. We used the function 'GetBestBreak' of the R package *bcpa* (Gurarie et al. 2009, Gurarie 2014) and manually adjusted the last day of positions, if necessary, to ensure that the individual was still moving at the time of shift.

For HR2, the median number of positions per individual was 439,459 (range: 4,283–1,881,442). The median number of survey days per individual was 141 days (range: 2–509 days). Among the 30 individuals tagged in 2017, seven individuals were still present during the 2018 spawning season, which means that we tracked two complete summer seasons for these individuals (Table 5). The median number of daily fish positions increased from mid-July (~2,500 positions/day) to mid-September (~5,000 positions/day); it then dropped in October, stabilized until January (~2,000 positions/day), and then rapidly increased to stabilize in February and March (~4,500 positions/day). This pattern was consistent for the two years of survey (Fig. 3a). The number of fish tracked simultaneously ranged from 30 individuals/day in June 2017 to four individuals/day the last day of the survey (Fig. 3b). The mortality rate seemed to increase during the spawning season (mid-October to mid-November), and no mortality was apparent during winter. Recapture contributed to the large increase in mortality rate in the 2018 spawning season (nine recaptures of the 17 fish alive on 15 October 2018). Compared to the VR2 VPS, the number of positions per day was at least one order of magnitude higher for the HR2 VPS (Fig. 3a). The number of fish recorded with the HR2 VPS was 2–3 times higher during summer compared to the VR2 VPS but similar during winter (Fig. 3b).

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Characteristics of the three VPS arrays deployed in Lake Ledoux from 2016 to 2018. Device refers to the reference tag (V9TP) or receivers (VR2Tx and HR2). Device depth is given from the water surface. Maps of these VPS arrays are given in Fig. 1 and field maintenance activities in Table 2. See also the main text for details.

Overview of field maintenance on the three VPS arrays deployed in Lake Ledoux from 2016 to 2018. The VR2 positioning system (VR2-Summer and VR2-Winter VPS) was operational from 7 July 2016 to 22 May 2017. The HR2 positioning system was used from 22 June 2017 to 6 November 2018. No fish data were collected on 11 October 2017 (battery replacement) or from 15 April to 31 May 2018 (battery failure). Five full analyses were completed by the VEMCO company, two from the VR2 VPS and three from the HR2 VPS.

Average horizontal position error (HPE) estimates. The primary purpose of HPE is to provide a relative measure of the error potential of a calculated position. HPE values are relative and unitless.

Brook charr tagging overview. A total of 57 brook charr were tagged from 2016 to 2018. ID: tag identification; Zone: captured in the littoral (L) or pelagic (P) habitat; FL: fork length (mm); Mass: fish mass (g); MW: mouth width (mm); BW: body width (mm); Sex: visual determination of sex (F: female; M: male); Temp: water temperature during surgery (°C); Sutures: number of sutures used to close surgical site; t: duration of surgery (minutes). Only one fish (2857-58) was found dead at the time of release.

Overview of brook charr positions. A total of 57 brook charr were tagged from 2016 to 2018. VPS: VEMCO positioning system; ID: tag identification; Fate: fate of individual (alive, dead, movement, predation¹, or recapture; see text for details); Start date: first day of fish position; End date: last day of fish position; Npos: number of fish positions; Nday: number of consecutive days between first and last positions.

 1 We attributed the fate of the individual to predation, either by natural predators or illegal fishing that occurred during our survey (see "Fish position" section).

Figure 1

Overview of the three VPS arrays deployed in Lake Ledoux. Receivers (red) and reference tags (yellow) are shown. VR2-Summer: ten VR2tx receivers and two reference tags were in operation from 5 July to 8 November 2016. Note that receiver 3 (in grey) was moved to another location (in red) on 10 August 2016 following the company's recommendation to improve performance of the VPS array. VR2-Winter: four additional receivers were added at lake extremities to improve coverage of the lake. A total of 14 VR2tx and two reference tags were in operation from 8 November 2016 to 22 May 2017. HR2: 23 HR2 receivers and four reference tags were in operation from 21 June 2017 to 6 November 2018.

Figure 2

Daily number of positions for the 45 HR transmitters (black lines) from 2017 to 2018. The red line shows the median of daily positions. Note that transmitters could continue to provide position information that no longer reflected fish tracking (i.e., transmitter expulsed from the fish or the fish was dead; see details in Fish positions section).

Figure 3

Daily number of positions of fish equipped with a VR2 or HR2 transmitter (a) and number of fish alive followed simultaneously in lake Ledoux (b). In a, black lines refer to individual fish and the red line to the median of daily positions. Blue dots indicate days when no fish positions were collected.

Photo 1

Overview of the HR2 VPS equipment and installation. Floating line attachment to the buoy (a), HR2 receiver attachment to the rope (b), installation of the HR2 receiver in shallow water (c), V9TP transmitter (d), and 50 lb concrete block equipped with galvanized swivel and welded stainless ring (e). Details of the equipment and installation setup are given in the main text.

Photo 2

Insertion of 4.0 g acoustic transmitter (V9TP-2x-180k-xxxm; VEMCO Inc., Halifax, NS, Canada) in the abdominal cavity of a brook charr (a) and a brook charr after surgery (b). The sutures are located above the orange spot in (b).

Photo 3

Brook charr equipped with an acoustic transmitter that was photographed for future morphological analyses.

