

HABITAT SELECTION AND MOVEMENT BEHAVIOUR OF YELLOW
LAMPMUSSEL (*LAMPSILIS CARIOSIA*) IN THE WOLASTOQ RIVER
CATCHMENT, NEW BRUNSWICK

by

Darren Greeley

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Supervisor: Michelle Gray, PhD, Faculty of Forestry and
Environmental Management

Examining Board: Wendy Monk, PhD, Environment and Climate Change
Canada, Canadian Rivers Institute

Mike Duffy, PhD, Department of Biology

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ABSTRACT

The influence of environmental stimuli on the ecology of freshwater mussels is still poorly understood. For Yellow Lampmussel (*Lampsilis cariosa*, YLM), I predicted that there are certain thresholds of environmental stimuli (e.g., temperatures and water levels) that may drive refuge seeking behaviour via vertical or horizontal migration. My thesis objectives were to 1) determine the thermal habitat use and depth of sediment burrowing behaviour in YLM and 2) compare YLM movement behaviour between sites to explore the influence of temperature and water level on movement. YLM (n=40) were tagged with thermal sensors at two sites within the Wolastoq River (W|SJR) catchment. YLM maintained a shallow (<10 cm) burrowed position and their horizontal movement behaviour was substantially different between sites. While both temperature and water level statistically influenced horizontal migration, it is likely that numerous environmental stimuli interact in complex ways to drive movement behaviour in YLM.

DEDICATION

To my wife who believed in me more than I ever did.

To my father who never got to see me become the man I was always supposed to be.

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Chapter 1 : GENERAL INTRODUCTION

Freshwater mussels (Bivalvia: Margaritiferidae, Unionidae) are rapidly declining across North America and are considered one of its most imperiled faunal groups (Hastie et al., 2003). Most of the freshwater mussel species found in North America are either extinct or vulnerable to extinction (Bogan 1993; Williams et al., 1993). Declines in unionid mussel species are attributed largely to anthropogenic causes such as alteration of flow regimes (e.g., dams, diversions, channels), habitat destruction/alteration, pollution, and more recently, climate change (Bolotov et al., 2018; Galbraith et al., 2010; Hastie et al., 2003; Strayer et al., 2004).

Climate warming may lead to altered thermal regimes in aquatic ecosystems, especially when coupled with cumulative human impacts (e.g., thermal effluent, flow alteration; Hester & Doyle, 2011) and resulting changes to natural processes (e.g., reduced ice cover, increased salinization; Jeppesen et al., 2020; Rahel & Olden, 2008). Thermal regime alterations may have adverse impacts on many aquatic organisms from fish (e.g., elevated temperatures causing increased energy requirements in young-of-year trout; McDonald et al., 1996) to amphipods (e.g., higher temperatures leading to a more rapid completion of life cycle; Wilhelm & Schindler, 2000).

Importance of temperature to freshwater mussels

Unionids, as ectothermic organisms, also are influenced by temperature in terms of metabolic processes (Ganser et al., 2015; Haney et al., 2020), reproduction (Schneider et al., 2018; Watters & O'Dee, 2000), survival (Galbraith, 2012; Pandolfo et al., 2010), and migration (Allen & Vaughn, 2009; Amyot & Downing, 1997; Block et al., 2013).

Elevated temperatures may increase filtration and excretion rates which in turn increases energy and oxygen demands (Ganser et al., 2015; Haney et al., 2020). The increase in energetic demand may lead to changes in nutrient cycling at an ecosystem level (e.g., remove nutrient limitation, change algal communities, impact water quality; Atkinson et al., 2013; Atkinson & Vaughn, 2015), since mussels are sometimes so abundant that they can comprise 90% of the biomass in a given aquatic system (Negus, 1966; Strayer, 2015; Vaughn, 2018).

As obligatory parasites, unionids are dependent on interacting with their respective fish hosts. Changes to the thermal regime may affect this crucial part of their lifecycle, since glochidia release depends on temperature (Vaughn & Taylor, 2000). Thus, depending on host species, a change in the timing of the release could cause asynchrony between parasite and host, resulting in reduced reproductive capacity (Vaughn & Taylor, 2000).

Freshwater mussel survival depends on thermal regime since temperatures must be suitable for their host fish (species-dependent) and all lifecycle stages (larval, juvenile, adult) of the mussel (Pandolfo et al., 2010, 2019). Although the average median lethal temperature (LT_{50}) of most life stages appears to begin at ~ 30 °C, there is variation depending on life stage and species, (Archambault et al., 2014; Mitchell et al., 2018; Pandolfo et al., 2010, 2019). Recent studies have shown that these organisms may be more stenothermic (capable of surviving over only a narrow range of temperatures) than previously considered, where the average temperature difference between LT_5 (median lethal temperature for 5% of the study group) and LT_{50} was ~ 5 °C (Pandolfo et al., 2010, 2019). Extreme temperature events are tied to mass mussel mortality occurrences and are

predicted to increase with climate change (McDowell & Sousa, 2019; Trenberth, 2011). These types of events (e.g., extreme summer high temperatures, droughts) are more likely to be ecologically detrimental than gradual warming (Hastie et al., 2003; Mouchon & Daufresne, 2006).

Thermal stimuli (i.e., extremes, chronic stress, and rapid changes) are crucial to freshwater mussels because temperature is one of the most important factors affecting the ecology of ectothermic organisms (Angilletta et al., 2002; Bennett, 1990). Therefore, when it comes to the conservation and management of threatened mussel species, the thermal ecology must be considered when planning/managing for its longevity, especially in a changing climate (Ganser et al., 2015; Hastie et al., 2003; Pandolfo et al., 2010).

Movement behaviour in freshwater mussels

Although relatively sessile, unionids may undergo either vertical (burrowing) or horizontal migration to avoid or mitigate stressful habitat conditions (Schwalb & Pusch, 2007; Watters et al., 2001). Migrations are most often noted during drought or dewatering conditions where mussels become emersed due to reductions in water level (e.g., Golladay et al., 2004; Gough et al., 2012; Mitchell et al., 2018; Newton et al., 2014). However, the type and order of movement (e.g., horizontal only, horizontal then vertical, or vertical only) depends on the species (Galbraith et al., 2015; Gough et al., 2012). Emersion represents a mortality threat through desiccation and/or suffocation, although some species can maintain gas exchange (e.g., up ~1.8 days via gaping behaviour; Byrne & McMahon, 1994) even when emersed (Byrne & McMahon, 1994). Near-emersion, (i.e., where water levels are very low, but the mussel remains submerged) can result in

several sub-lethal stressors such as elevated temperature, increased energetic demand, and lowered dissolved oxygen (Galbraith et al., 2010; Gough et al., 2012). These sub-lethal stressors induced by low water events (e.g., drought, dewatering, diversions of regulated rivers) can exceed the physiological tolerance of a given mussel and thus elicit movement behaviour (Galbraith et al., 2010).

Freshwater mussels are reported to undergo vertical migration seasonally (e.g., endobenthic (subsurface) during winter, epibenthic (surface) during warmer months; Amyot & Downing, 1997). Regular vertical migration has also been reported during the warmer months, but is species-dependent (Eads & Levine, 2019; Schwalb & Pusch, 2007). The direct cause of non-seasonal vertical migration is still debated, but it is suggested that the use of thermal niches (e.g., vertical migration into the sediment seeking cooler temperatures) could be important to mussel abundance and population success (Spooner & Vaughn, 2009). The utilization of thermal refugia in sediment by unionids as a driver for vertical migration is relatively unstudied, however, thermal refugia has been shown to be available for use in mussel beds (Briggs et al., 2013; Newton et al., 2013).

Several studies have reported that temperature affects mussel movements (e.g., Amyot & Downing, 1997; Block et al., 2013; Schwalb & Pusch, 2007; Waller et al., 1999), however, they also suggest other factors such as discharge (e.g., Schwalb & Pusch, 2007), photoperiod (e.g., Amyot & Downing, 1997; Schwalb & Pusch, 2007), substrate particle size (e.g., Eissenhauer et al., 2023) and reproduction (e.g., Amyot & Downing, 1997) also play a role.

Objectives and Overview

Fisheries and Oceans Canada requires any proponent that may alter an aquatic environment (e.g., bridge construction or culvert installation) to make special consideration to mitigate impacts to aquatic species-at-risk (SAR; Government of Canada Fisheries Act, 1985). Additional work that is required to mitigate effects to SAR (e.g., mussel relocation) can cause proponents to experience significant delays to project initiation, since the permit approval is dependent on the mitigation of impacts on SAR.

To assist in the identification of sites that would require SAR consideration, and the expedition of the permitting process, the New Brunswick Department of Transportation and Infrastructure (NB DTI) has partnered with researchers from the Canadian Rivers Institute (CRI). They are working to develop a science-based, weight-of-evidence (WOE) risk assessment framework to establish the presence/absence of SAR aquatic species. The WOE framework will be developed using SAR habitat, behaviour, and distribution research done at three spatial scales (i.e., micro, meso, and macro). This thesis relates to the micro-scale aspect of the WOE framework, where the primary focus is to increase the understanding of the fundamental ecology of a New Brunswick aquatic species-at-risk: the Yellow Lampmussel (*Lampsilis cariosa*; YLM). Centered on the lower portion of the Wolastoq | Saint John River (i.e., from the Mactaquac generating station near Fredericton to the mouth of Washademoak Lake), this research looks to investigate the following objectives:

- 1) Determine the thermal habitat uses and depth of sediment burrowing behaviour in YLM.

- 2) Compare YLM horizontal movement behaviour between sites/ivers and explore the influence of temperature and water level on movement.

Chapter 2 focuses on observations made at Canaan River, where tagged YLM (n=20) establish a baseline of movement behaviour and thermal habitat selection. The impact of tagging on YLM movement behaviour is investigated, examining the frequency and duration of movement between tagged and untagged mussels. Finally, the effect of water temperature and water level on YLM movement behaviour is examined using binary logistic regression.

Chapter 3 compares the findings of Chapter 2 to a new site at Salmon River. Thermal habitat selection and movement behaviour are compared against the environmental regimes (i.e., surface water temperature, water level) of each site. The logistic regression modelling efforts from Chapter 2 are also improved by incorporating additional data and including site as a categorical variable.

Chapter 4 is a synthesis of research findings and how they relate to the objectives outlined in this Chapter 1. This final chapter focuses on my important findings regarding YLM thermal habitat selection and movement behaviour, tied into a common theme of stability seeking behaviour and preference for stable environments.

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**Chapter 2 : THERMAL HABITAT SELECTION AND MOVEMENT
BEHAVIOUR OF A FRESHWATER MUSSEL SPECIES-AT-RISK:
THE YELLOW LAMPMUSSEL (*LAMPSILIS CARIOSA*)**

ABSTRACT

The Yellow Lampmussel (*Lampsilis cariosa*; YLM) is a freshwater mussel species-at-risk in New Brunswick. Knowledge of their behavioural response to environmental stimuli (e.g., high temperatures and low water levels) will be crucial to informing conservation and management decisions, especially in a warming climate. YLM (n=20) were tagged with thermal sensors and passive integrated transponder (PIT) tags at a high-density site within the lower Wolastoq | Saint John catchment, New Brunswick. Temperature data revealed that 1) YLM remain burrowed a few (<10) centimeters into the riverbed sediment for most of the sampling period and 2) the mussels received a measure of thermal insulation (up to 1.75 °C) from surface water by maintaining that position. From where they were released, tagged YLM moved an average of 7.5 m (\pm 3.78 SD) up to a maximum of 15.39 m. The net distance the YLM moved was not influenced by sex or shell length. Frequency and duration of horizontal movement did not differ significantly between tagged and untagged YLM. The majority (52%, n=61) of the horizontal movement occurred during a 3-day stretch of high temperature and low water in (6-9) August. A logistic regression confirmed that both temperature and water level were significantly associated with the likelihood of a movement response, however the model was not a strong fit for the data (McFadden's pseudo $R^2 = 0.13$). Future work should include studying additional sites and rivers within the catchment, as well as including additional parameters (e.g., discharge, dissolved oxygen) within the model.

INTRODUCTION

Freshwater mussels (Bivalvia: Margaritiferidae, Unionidae) are declining across North America and the Yellow Lampmussel (*Lampsilis cariosa*; YLM) is no different (Hastie et al., 2003; Nedeau, 2000). YLM are found along the Northeast Atlantic slope of North America, ranging from Georgia (United States) north to Nova Scotia (Nedeau, 2000). In Canada, YLM are present in only one river basin in Nova Scotia and one river basin in New Brunswick.

The Yellow Lampmussel population in New Brunswick (NB) generally inhabits the lower portion Wolastoq | Saint John River (W|SJR) catchment. Its historical range within the W|SJR spans from the Mactaquac Generating Station near Fredericton, down to near the mouth of Washademoak Lake (including major tributaries; Sabine, 2004). In the lower W|SJR, this mussel is believed to be threatened by changes to habitat and water quality (e.g., sedimentation, pollution), invasive species (e.g., *Dreissena* spp.), in concert with low water levels (e.g., risk of emersion and thermal stress; Fisheries and Oceans Canada, 2010). Its narrow geographical range, along with the largely anthropogenic threats facing this species, have led to its designation as a species of ‘*special concern*’ by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC; COSEWIC 2004).

This research aims to examine the role of temperature and water level on the fundamental ecology of YLM, within the lower W|SJR. These mussels are known to favor sandy environments, where they are frequently found to be burrowed up to their apertures in the sediment (COSEWIC, 2004; Sabine et al., 2004). This work attempts to understand not only how deep they are burrowing, but also whether their use of the

sediment habitat is static or dynamic over time. In addition to vertical migration (i.e., burrowing), another goal of this research is to determine the role of water temperature and water level on the horizontal movement behaviour of YLM. It is likely that there are certain tolerance thresholds of environmental stimuli (e.g., high water temperatures or low water levels) that when exceeded, will elicit movement responses in YLM. Therefore, the highest number of YLM horizontal movement responses should occur during periods of lowest water levels and/or highest temperatures. Similarly, burrowing behaviour may also be influenced by the same stimuli and there may be a measure of behavioural thermoregulation associated with YLM burrowing. If this is the case, sediment habitat use would be dynamic, meaning that the mussels would oscillate between sediment and surface as site conditions changed.

Other work with freshwater mussels has shown that water temperature influences many aspects of their biology and ecology, but responses are generally species-dependent (Ganser et al., 2015; Haney et al., 2020; Pandolfo et al., 2010). These studies tend to be done in a laboratory setting and generally fail to account for thermal fluxes and/or gradients (i.e., aquaria are usually uniform in temperature). The impact of environmental stressors on movement behaviour of YLM in the W|SJR have not been studied. Addressing fundamental knowledge gaps in YLM ecology will help improve their long-term management and conservation, for this freshwater mussel species-at-risk.

METHODS

Site selection

The W|SJR is eastern Canada's longest river (~673 km) with a basin area of over 55,000 km². The river flows from its headwaters in northern Maine and southeastern Quebec, where it then flows through NB and into the Bay of Fundy (located south of NB). The lower W|SJR basin (i.e., downstream of the Mactaquac Dam near Fredericton to near the mouth of Washademoak Lake) is where the river broadens and is joined by two major tributaries: Salmon River via Grand Lake and Canaan River via Washademoak Lake. This section of the river is home to many alluvial islands that can provide excellent sandy habitat for a variety of freshwater mussel species.

Intensive directed snorkeling surveys were completed during the summer of 2021 to locate areas of ideal habitat (measured by relative abundance of YLM), using the work of Sabine et al. (2004) as a guide. Snorkeling locations (shallower sandy areas) were then selected remotely using Google Earth 2019 satellite imagery (Figure 2-1).

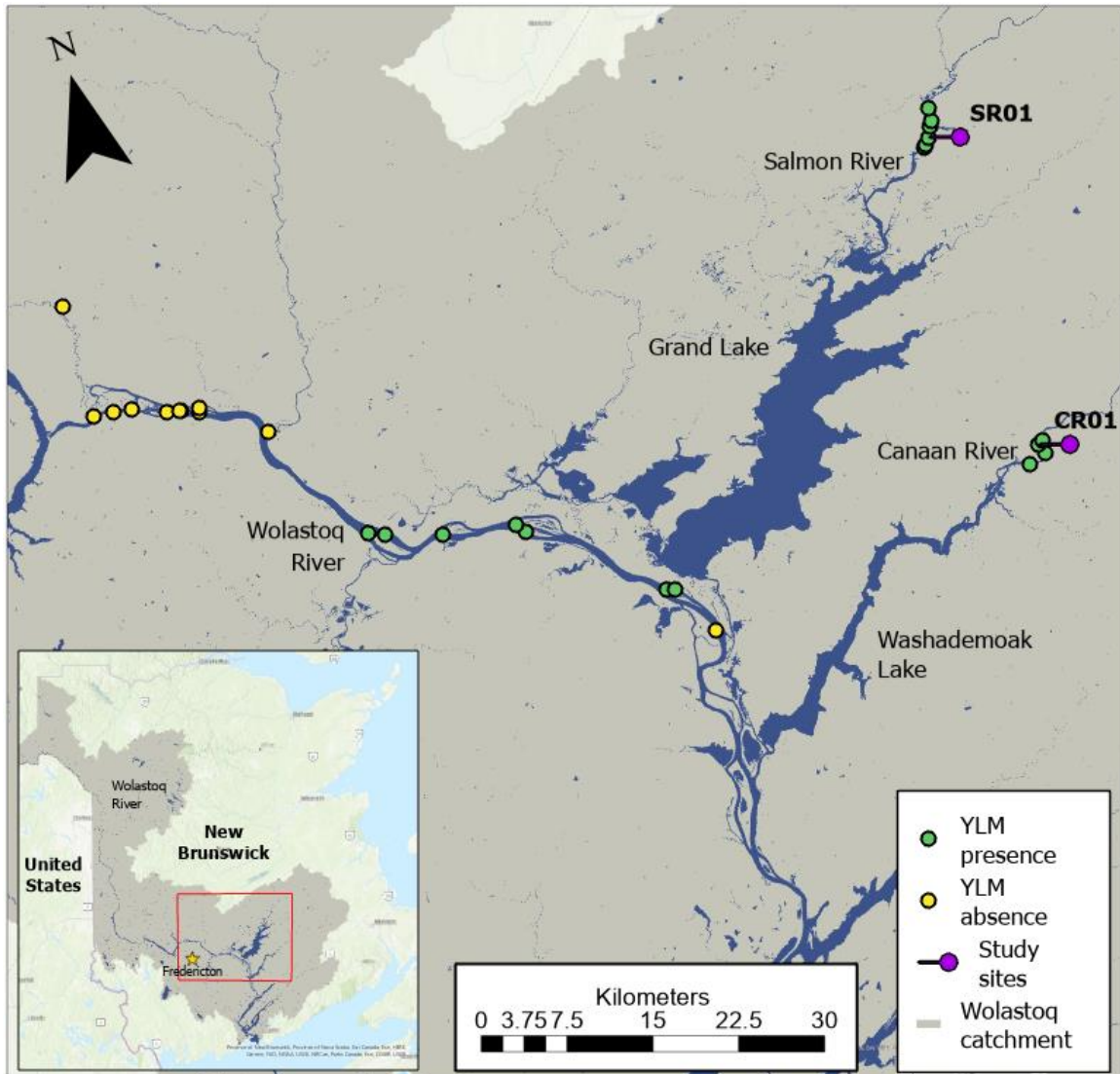


Figure 2-1: Yellow Lampmussel presence (n=17; green dots), absence (n=12; yellow dots), and high-density study site (n=2) locations from snorkeling surveys conducted during 2021. Map source: GeoNB.

The Canaan River site (CR01) was selected for further study due to the abundance and ease of locating YLM specimens. At least 20 YLM were needed for tagging purposes and this site easily provided those specimens. This site's boat access was restricted due to the lack of public boat launches in the area, making it an ideal place to leave expensive monitoring equipment.

Study Area

The Canaan River site (CR01) was located approximately 5.2 km upstream of the Route 10 bridge in Coles Island, NB. It is situated in the Canaan River, a tributary river that drains into Washademoak Lake and then into the main stem of the W|SJR. Canaan River is a highly tannic medium-sized river (5th order stream) that was ~116 m wide at the site location. The habitat at this site is shallow and sandy with many patches of dense algae, particularly near the shore (Figure 2-2). Due to its proximity to the Bay of Fundy (the bay the W|SJR drains into; ~87 km), the water level at Canaan River is influenced by the tide, resulting in fluctuations of ~30-40 cm twice daily.

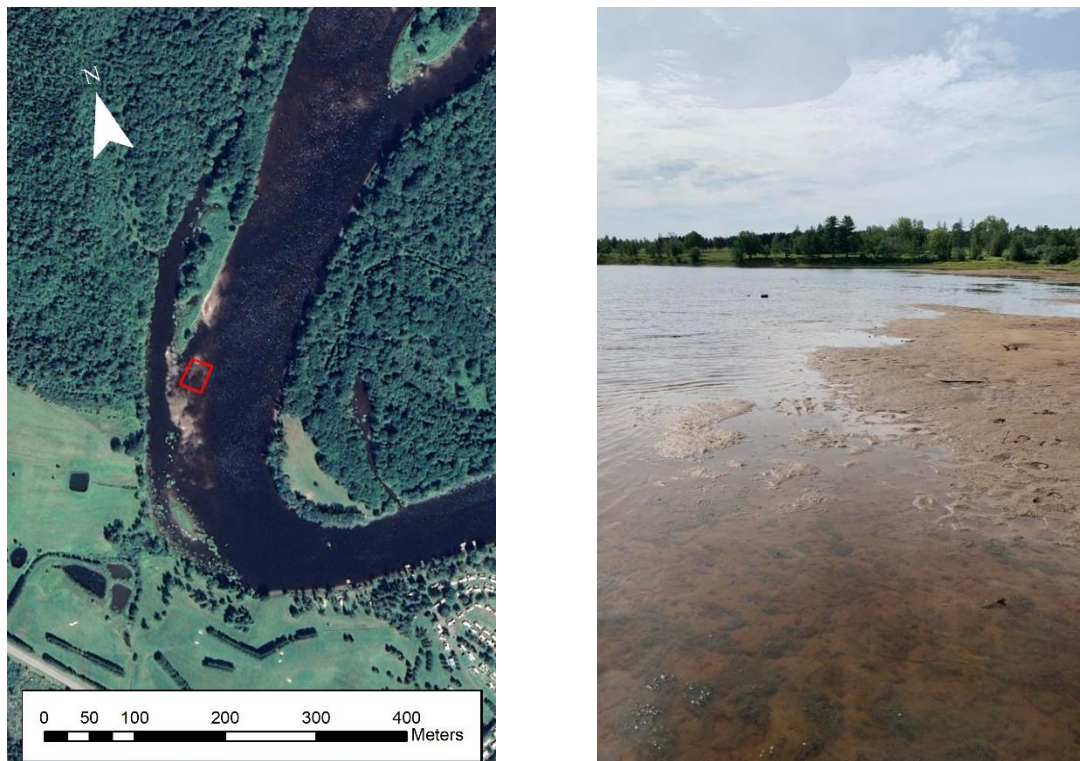


Figure 2-2: Canaan River study site (CR01) aerial imagery with the study area bordered in red (left) and on-site downstream view (right). Map source: Google Earth.

Hyporheic grid setup and site monitoring

Hyporheic monitoring took place from 12 July – 2 September, 2022. A grid of 20 hyporheic wells were installed to characterize the thermal regime within the riverbed sediment (Figure 2-3). With a well installed every 10 m, the grid ran 40 m (parallel to flow) x 30 m (perpendicular to flow) for a total area of 1200 m². Each well consisted of a capped uPVC plastic tube with a string of Onset HOBO pendant temperature loggers hanging (one at each of 10 cm, 20 cm, and 40 cm) from the cap (Figure 2-4). These depths were chosen because mussels have been found to burrow up to a depth of 20 cm, but are usually within the first 10 cm of the riverbed sediment (Balfour & Smock, 1995; Schwalb & Pusch, 2007). All wells also had a series of flow-through holes drilled into the tube and silt screen wrapping to prevent clogging. A passive integrated transponder (PIT) tag was attached to each cap so that the wells could be found in the event of shifting substrate. Each well was installed vertically into the sediment up to the cap.

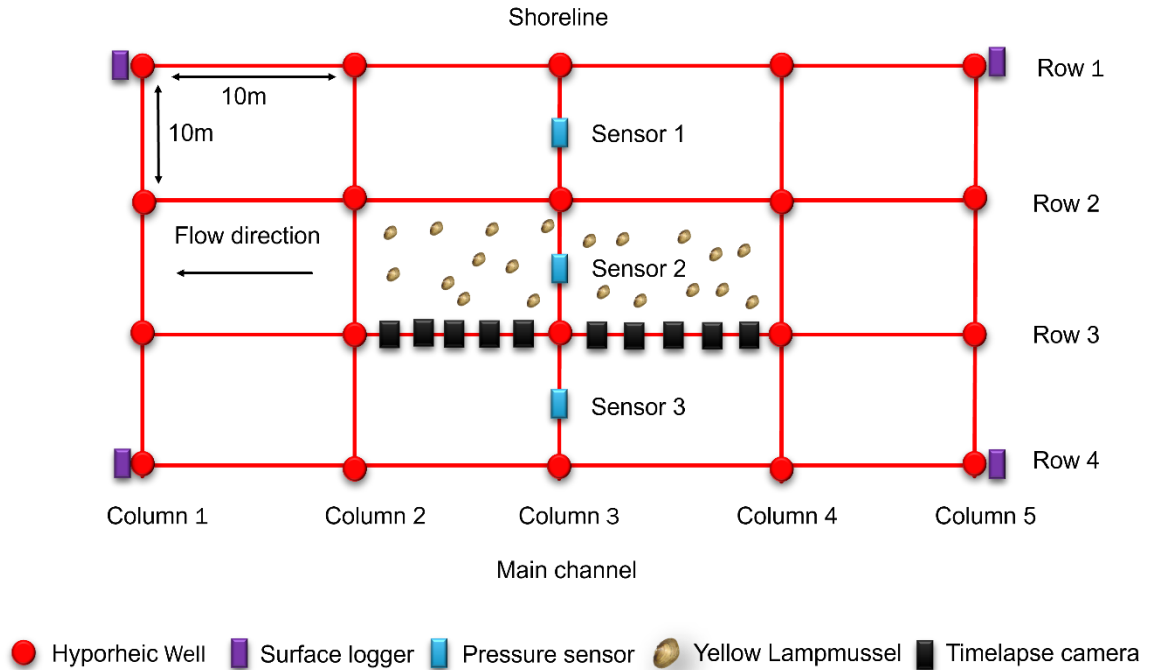


Figure 2-3: Schematic showing the placement of temperature loggers, pressure sensors, and camera placement for the hyporheic well grid installed in the Canaan River, New Brunswick

River water temperatures were monitored with additional pendant loggers (n=4) attached to bricks, one at each corner of the grid (Figure 2-3). Pressure sensors (Onset Hobo U20 level loggers) were placed through the center of the grid to record water level data as well as river water temperature. A fourth pressure sensor was installed in a nearby tree measuring air pressure that was then used to calibrate pressure sensors placed on the grid. All surface water loggers and pressure sensors were installed within a section of white uPVC tube to act as a solar shield (i.e., measuring ambient temperatures). All Hobo pendant loggers and pressure sensors were set to record temperature and/or pressure readings at 30-minute intervals. Data from all sensors was downloaded and then trimmed (12 July, 13:00 – 2 September, 9:00) to ensure that all observations were recorded while

the sensors were deployed. Data of similar types (e.g., well temperatures, surface water temperatures, pressure readings) were compared in a time series so that any erratic or erroneous data could be excluded from analysis. Sensor 2 data (both pressure and temperature readings) were determined to be after becoming emersed repeatedly over the duration of the field season. Emersion was observed at the site visually and empirically through pressure readings that distinctly matched air pressure. Placement of the hyporheic grid was chosen to center on the area and depth that YLM were known to occupy based upon the previous year's snorkeling efforts.



Figure 2-4: Example of PVC tubing used as hyporheic wells with temperature loggers suspended at 10 cm, 20 cm, and 40 cm from the cap.

Thermal sensor tagging and mussel placement

Twenty mussels were collected via snorkeling at Canaan River. Each mussel was outfitted with an Alpha Mach Biskit temperature logger (25 mm diameter x 8.2 mm depth, 7.2 g) as well as a small (11 mm) passive integrated transponder (PIT) tag. The

Biskit temperature loggers were set to record at 30-minute intervals. Each Biskit was affixed to the anterior portion of the right valve and the PIT tags were attached to the posterior portion of the right valve (Figure 2-5).

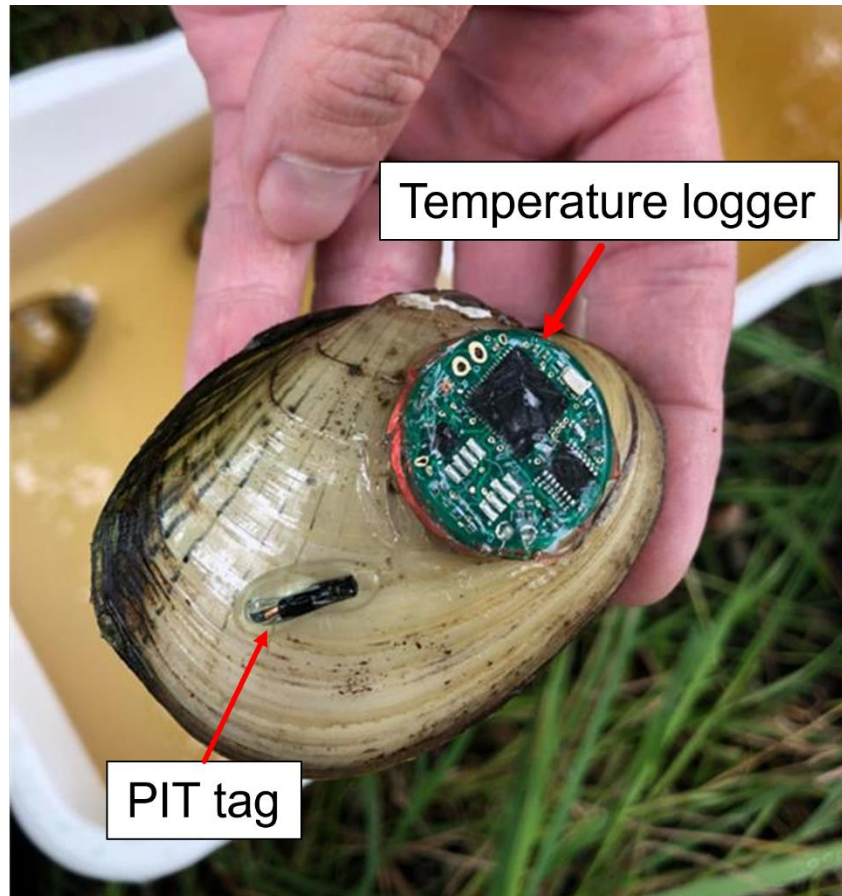


Figure 2-5: Images of passive integrated transponder (PIT) tag (11 mm x 2.1 mm) and Alpha Mach Biskit thermal sensor (25 mm x 8 mm) attachment to an adult Yellow Lampmussel.

Both temperature loggers and PIT tags were attached using a thin layer of Gorilla Super Glue Gel. This product contains ethyl-cyanoacrylate as its main bonding agent,

which is considered aquarium safe and commonly used in such applications. PIT tag orientation on the shell was chosen such that when the mussel was burrowed, the tag would be in a vertical orientation, providing a larger detection field than a horizontal orientation (Linnansaari et al., 2007)

After application of the glue, the mussels were immersed in a separate holding container, where the glue cured for a minimum of five minutes. The maximum time spent in the holding container was the duration of time it took to tag five mussels, plus five minutes curing after the last mussel was placed in the holding container (~12 minutes). Once a group of five mussels had been tagged and the glue cured, they were put out into flow-through laundry baskets and placed back into the Canaan River. This process was repeated until all 20 mussels were in four laundry baskets weighed down by rocks and zip-tied together. The mussels were left overnight to fully cure the glue, inspected for tag retention in the morning, then released onto the hyporheic grid. Mussels were placed back into the area and depth from which they were predominately found, to ensure as little artificial movement was introduced as possible. This depth and area were located between rows 2 and 3, and between column 2 and 4 of the hyporheic grid, bordered by deeper water outside those columns (Figure 2-6).

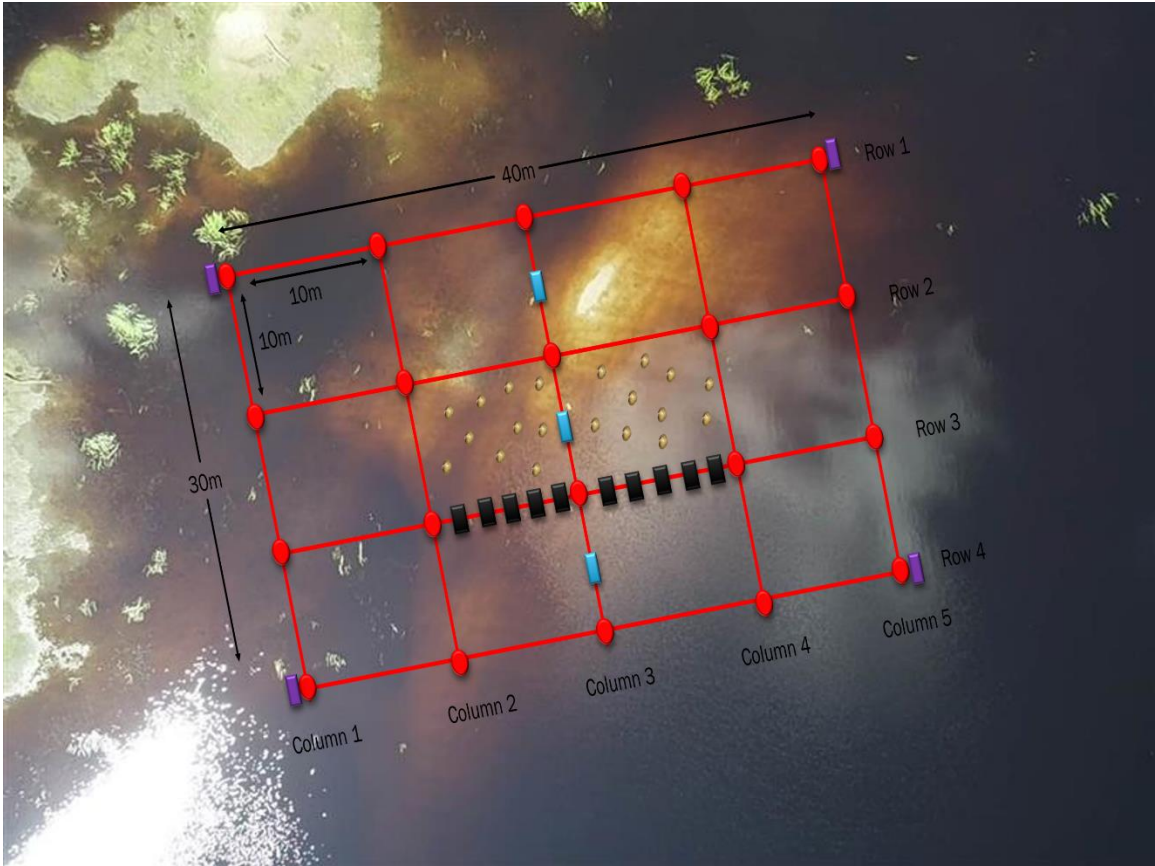


Figure 2-6: Hyporheic grid overlaid on drone imagery taken at Canaan River, New Brunswick. Yellow Lampmussel were released after tagging between rows 2 and 3 and between columns 2 and 4.

A mobile radio frequency identification (RFID) PIT tag reader was used to detect tagged mussels for data recovery at the end of the field season (2 September, 2022). After detection, the mussels were excavated by hand and their Biskit loggers were scanned with an Alpha Mach PITStop RFID antenna. This antenna is designed to specifically interact and program the Biskit loggers. Mussel position was recorded upon release (12 July, 2022) and again when they were recovered for data downloading (2 September, 2022). YLM position was determined using a Bad Elf Pro + (BE-2300-GPS \pm 2.5 m) handheld GPS unit. YLM horizontal distance was calculated by measuring the distance (m)

between release and recapture points for each individual mussel in ArcGIS Pro (v 3.03). Of the 20 YLM tagged at Canaan River, only 16 mussels were recovered and had their data downloaded. The 4 YLM that were not recovered were presumed to have moved to deeper water that the backpack RFID unit could not reach.

Mussel movement observations

Mussel movement was tracked using ten Brinno TLC-200 time-lapse cameras (Figure 2-7). Cameras were set up to record an image at 5-minute intervals, which the camera would then string together into a video. Each camera was set to record from 5:00 am until 10:00 pm. To deploy the cameras underwater, each camera was outfitted with a custom waterproof housing made from uPVC piping. The housing and cameras were then strapped to standard 6" cinderblocks and placed on the bottom of the river. Cameras were placed along the third row of the grid, outside of where the mussels were released (Figure 2-3). All cameras underwent weekly maintenance, where the lenses were cleared of any algae/detritus and the data could be downloaded. The camera position was marked with two pieces of rebar (one for each of the front and the rear) prior to the camera being taken out of the water, so that they could be placed back in their original position.

Camera observations were reviewed frame by frame, where the type (i.e., vertical burrowing, horizontal, and vertical emergence), duration (min), date, and time of the movements was recorded. YLM movement initiation was considered as the first frame in which a movement behaviour was observed, while the end of the movement was the final frame where the movement was observed. YLM movement was observed for the entirety of the time the hyporheic grid was deployed.

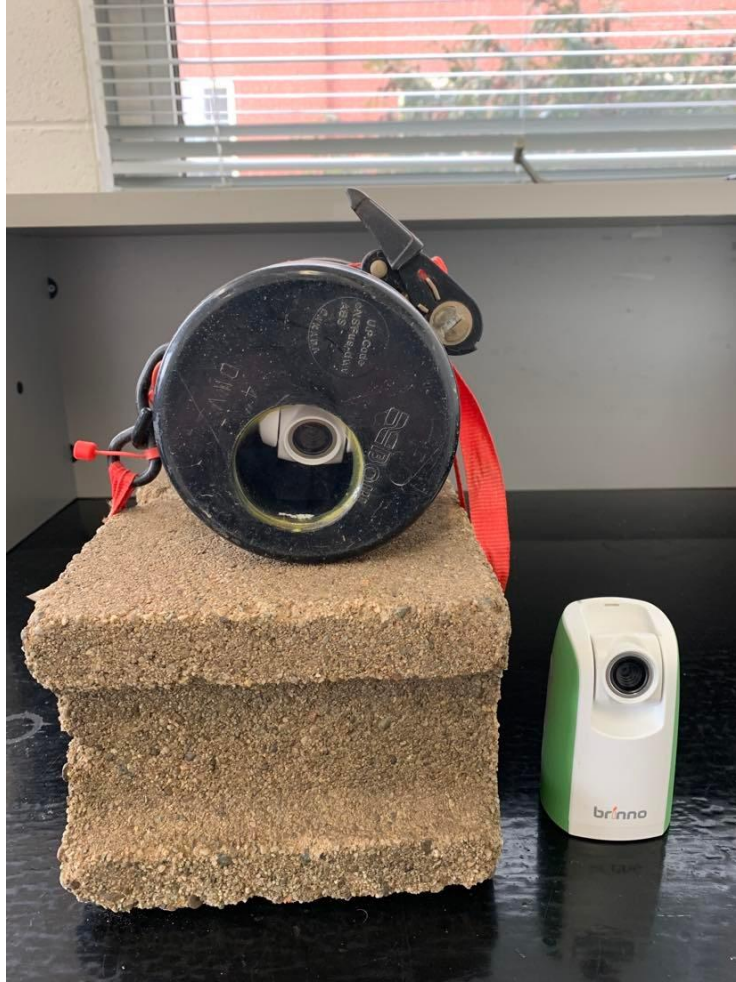


Figure 2-7: Example of Brinno TLC-200 time-lapse cameras and custom waterproof housing used to record mussel movement observations.

Data Analysis

To ensure that the observed YLM horizontal movement was not due to physical differences (i.e., mussel length or sex), an analysis of co-variance (ANCOVA) was used to test the effect of mussel length and its categorical covariate, mussel sex, on distance travelled. The distance data used in the ANCOVA was measured using GIS (ArcGIS Pro v 3.03) using release and relocation GPS points. As such, the net distance metric only

refers to the difference between where they were released versus where they were relocated. It is probable that the mussels moved additional times during the field season and this metric likely underestimates total movement for the season. It is more representative of net movement/directionality over the sampling period. Similarly, a t-test was conducted between tagged and untagged mussels to understand the influence of the tags on YLM horizontal movement.

To analyze the influence of temperature and water level on the likelihood of a YLM horizontal movement response, a multiple binary logistic regression was conducted. Binary logistic regression was chosen due to the binary nature of the movement observations (i.e., movement or no movement) for both temperature and water level data. Movement observations were determined as the first camera frame where the YLM entered the screen. Due to the difference in units between temperature and water level, both variables were scaled in R (using the `scale` function) so that the magnitude of change within variables would be comparable. The logistic regression also had a statistically significant result when compared to a null model, establishing that it fit the data better than the null model.

All data was analyzed using R programming software (version 4.2.2; R Core Team, 2020) in the RStudio (version 2022.12.0; RStudio Team, 2022) environment using *pscl* (v1.5.5; Simon Jackman, 2020), *corrplot* (v0.84; Wei & Simko, 2017), *rstatix* (v0.7.2; Alboukadel Kassambara, 2023), *multcomp* (v 1.4-23; Torsten Hothorn, 2023), *xts* (v0.13.0; Ryan, Ulrich, Bennet, Joy, 2023) and *MuMIn* (v1.47.5; Kamil Bartoń, 2023) packages.

RESULTS

Hyporheic grid monitoring

Due to the tidal nature of Canaan River, the site experienced significant (~30-40 cm) daily fluctuations in water level (Figure 2-8A). The site experienced a cold snap during early August, substantially dropping the water temperature (~9 °C) during that time (Figure 2-8B). Before this event water temperatures averaged 24.8 ± 1.5 °C, while post event they averaged 22.1 ± 1.1 °C, a difference of almost 3 °C.

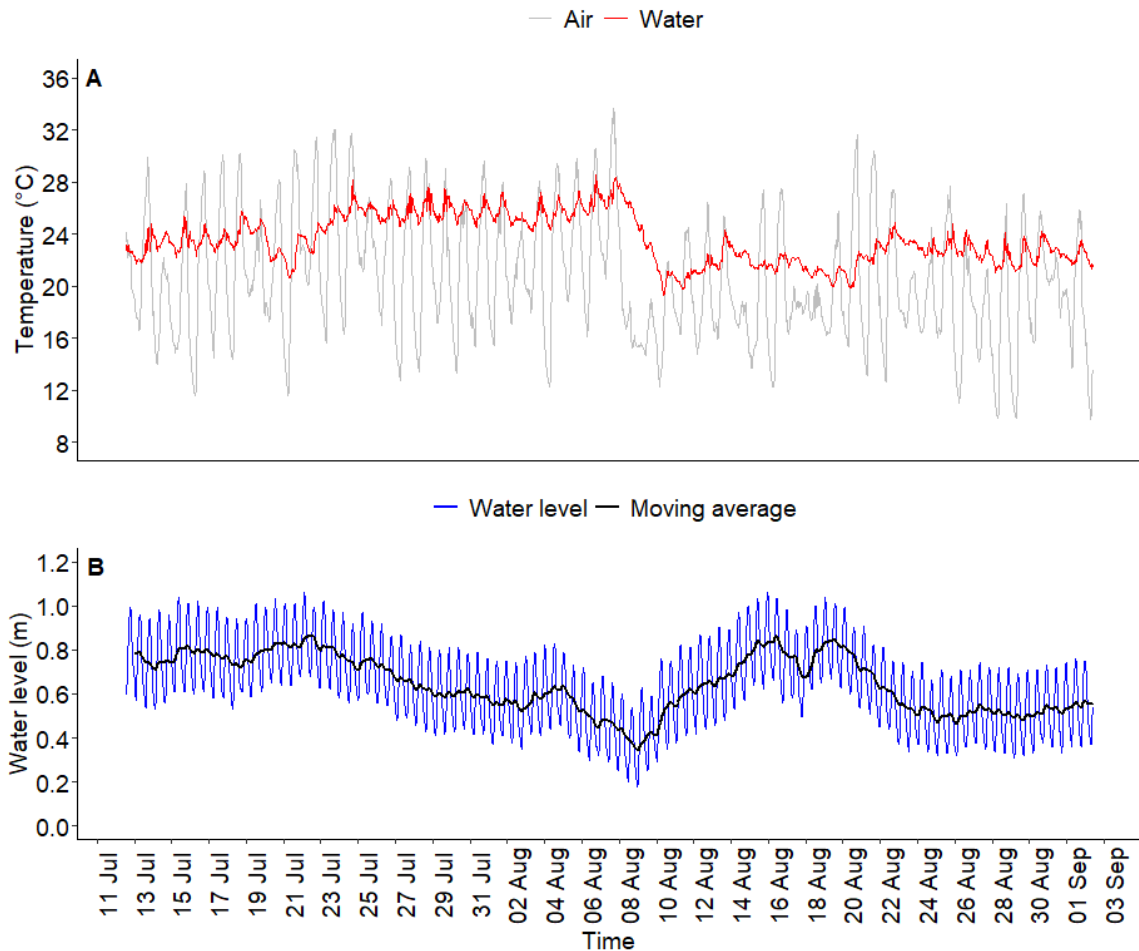


Figure 2-8: Temperature (°C; A) and water level (m; B) measured at CR01 for the duration of the field season. Sensor 3 was used for both temperature and water level data.

Within the grid, most metrics reported almost identically (for temperature and water level respectively) outside of the sensor 2 readings (Table 2-1). Sensor 2 reported the greatest extremes in min/max temperature, temperature variation, minimum water level and water level variation.

Table 2-1: Air and surface water temperature summaries for the Canaan River grid. Sensors 1-3 (recording water pressure and surface water temperature) were deployed through the center of the grid between noted rows. Surface water loggers (recording only surface water temperature) were deployed at each corner of the grid at noted locations. Min = minimum temperature, Max = maximum temperature, SD = standard deviation, CV = coefficient of variation.

Logger Type	Min (°C)	Max (°C)	Range (°C)	Mean (°C)	SD (°C)	CV (%)
Air sensor	9.77	33.64	23.87	20.52	4.66	22.71
Sensor 1 (Row 1-2)	19.38	28.26	8.88	23.26	1.82	7.82
Sensor 2 (Row 2-3)	16.81	29.65	12.84	23.53	1.99	8.45
Sensor 3 (Row 3-4)	19.28	28.56	9.27	23.55	1.88	7.99
Surface 1 (Row 1, Col 1)	19.77	27.86	8.09	23.27	1.76	7.54
Surface 2 (Row 1, Col 5)	19.22	28.04	8.83	23.27	1.83	7.87
Surface 3 (Row 4, Col 5)	19.24	28.2	8.97	23.49	1.87	7.94
Surface 4 (Row 4, Col 1)	19.29	28.26	8.97	23.38	1.85	7.93
Logger type	Min (m)	Max (m)	Range (m)	Mean (m)	SD (m)	CV (%)
Sensor 1 (Water level)	0.22	1.08	0.86	0.67	0.18	26.35
Sensor 2 (Water level)	0.06	0.77	0.70	0.39	0.16	42.54
Sensor 3 (Water level)	0.18	1.06	0.88	0.64	0.18	28.32

This type of consistency (i.e., within grid variation generally differing by < 1 %, min and max temperatures differing by < 1 ° C) was also noted within the well temperatures for each respective depth (i.e., 10 cm, 20 cm, 40 cm; Table A-1, A-2, A-3). Overall, the data shows that the site is dynamic due to its tidal nature, but conditions within the grid are equally dynamic, outside of major topographic differences (e.g., the prominent sandbar near sensor 2).

Thermal habitat selection

The results of hyporheic monitoring showed that there is a measure of thermal decay within the riverbed sediment, resulting in cooler temperatures as sediment depth increases (Figure 2-9). Mussel temperature loggers (n=16) reveal that their position throughout the season is generally cooler than surface water, but warmer than temperatures 10 cm in the sediment (Figure 2-9). This suggests that this species spends most of its time burrowed within the first few centimeters (i.e., < 10 cm).

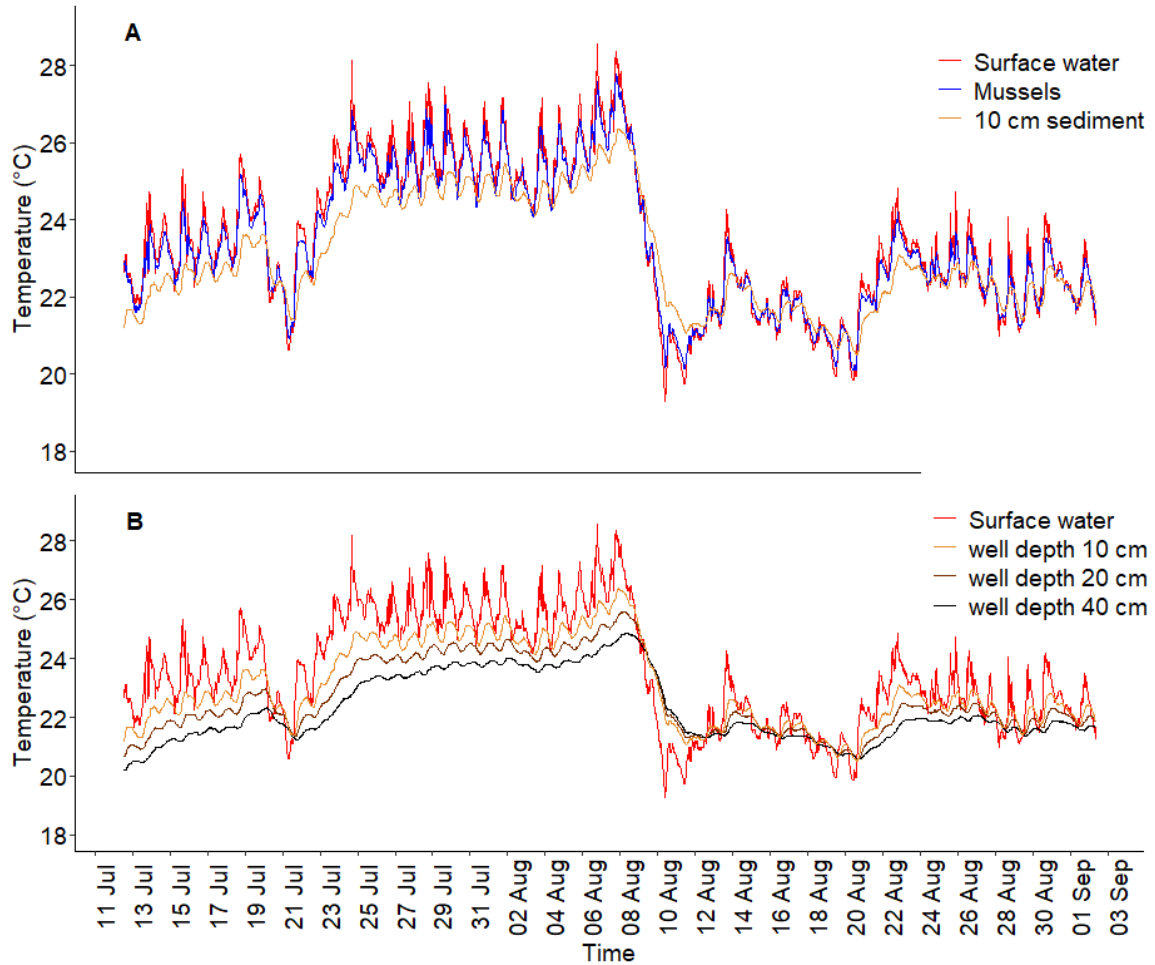


Figure 2-9: Temperature time series of (A) mean mussel temperatures (n=16) and (B) hyporheic temperature profile. Wells used for hyporheic temperatures were averaged from row 3 (i.e., mean temperature reading from all five wells), closest to where the mussels were released. Sensor 3 surface water, also the closest to where mussels were released, was used for comparison.

In general, the tagged YLM recorded similar temperatures across all 16 specimens.

The mussels were able to receive a cooling effect of $-0.5\text{ }^{\circ}\text{C}$ to $-1.75\text{ }^{\circ}\text{C}$ (relative to surface water at sensor 3) repeatedly throughout the season (Figure 2-10).

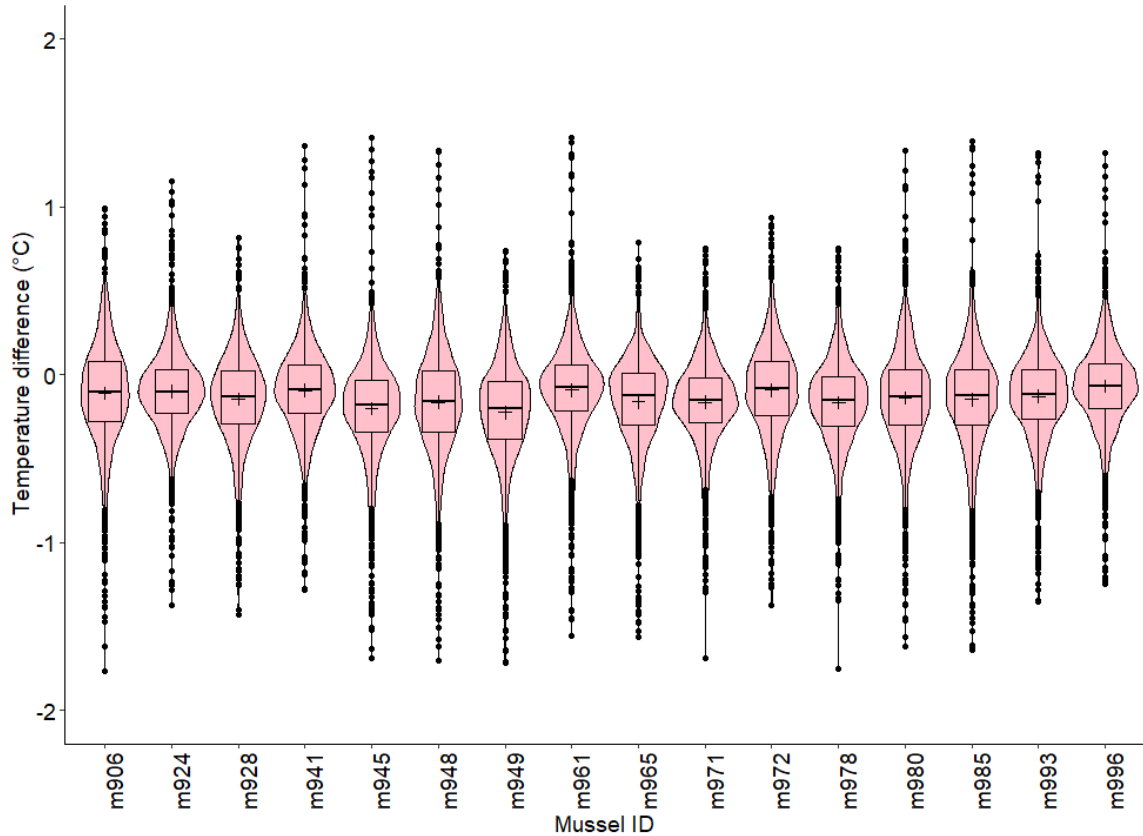


Figure 2-10: Violin wrapped boxplots showing temperature difference of mussel temperatures (n=16) subtracted from surface water temperatures (sensor 3). Positive numbers indicate mussels were warmer than surface water temperatures, while negative numbers indicate mussels were cooler than surface water temperatures. Crosses represent mean values.

Movement behaviour

Over the course of the field season (12 July 12 – 2 September), every mussel that was recovered (n=16) moved from its original release point (Figure 2-11). Movement was mostly uniform in direction towards deeper, cooler water between the 3rd and 4th rows of the hyporheic grid. The YLM also exhibited some clustering or aggregative behaviour at their recapture points.

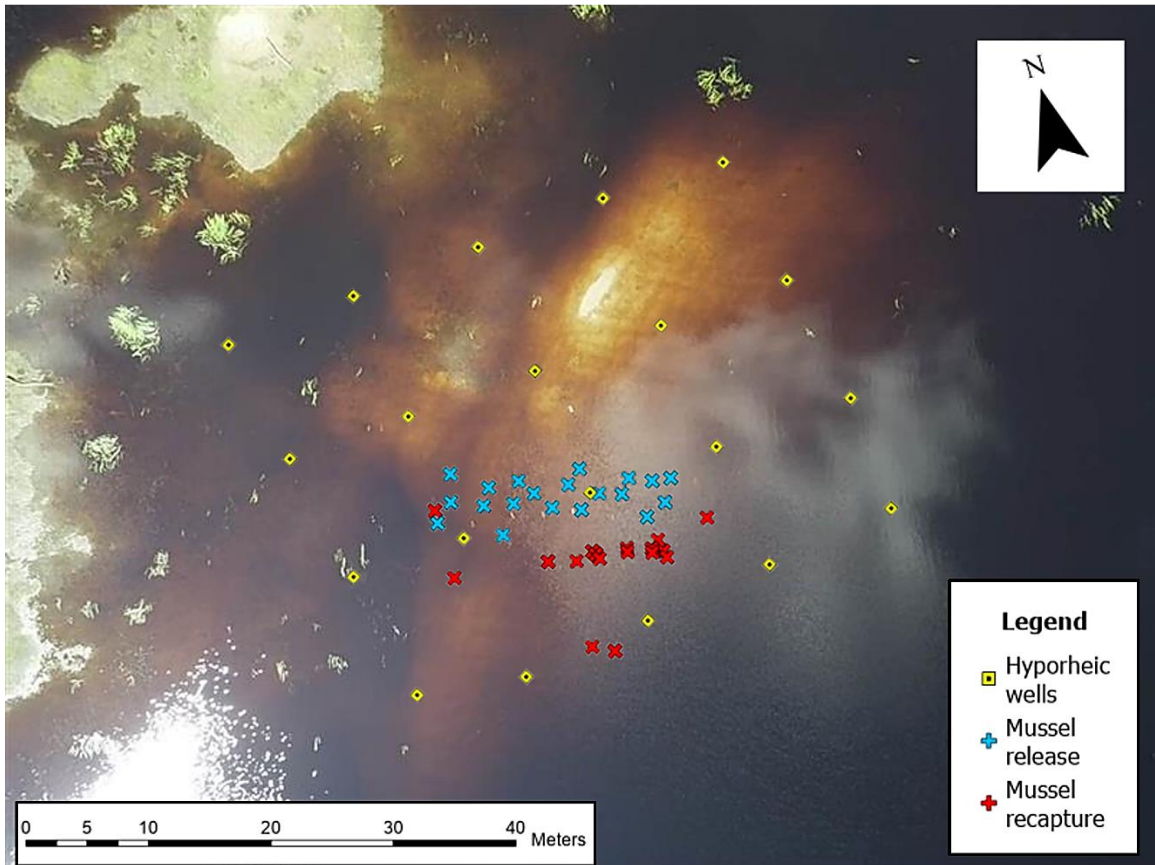


Figure 2-11: Map of mussel release (n=20) and recapture (n=17) points on the hyporheic grid at Canaan River.

Recapture efforts resulted in 16 of 20 mussels being recovered at Canaan River. Average movement was $7.5 (\pm 3.78 \text{ SD})$ m with four mussels moving more than 10 m over the course of the season (up to a maximum of 15.39 m; Table 2-2). An ANCOVA was conducted to test the effect of mussel length and its categorical covariate, mussel sex, on distance travelled. The interaction term was not statistically significant ($F_{1,11} = 1.25, p = 0.29$), indicating that there is no difference in slope (response) for net distance for either male or female mussels. The reduced model (i.e., without interaction term) compared intercepts and found that there was no difference ($F_{1,12} = 1.51, p = 0.12$) in

intercept (mean) for distance, while controlling for sex. Finally, there was no significant effect of length on distance ($F_{1,13} = 0.004, p = 0.95$).

Table 2-2: Summary of net distance moved by each mussel tagged (n=20) at CR01. Net distance was calculated by measuring where they were recovered versus where they were released using ArcGIS Pro version 3.03. Mussels that were not recovered had no distance calculated.

Mussel ID	Sex (M/F)	Length (mm)	Net Distance (m)
m949	M	81.87	15.39
m971	M	90.80	12.85
m961	M	85.97	11.85
m965	F	78.31	11.24
m906	M	89.09	9.13
m972	M	94.22	9.08
m978	M	93.16	7.88
m948	M	89.06	7.81
m924	F	79.00	7.10
m941	F	90.20	6.92
m994	M	86.42	5.99
m928	M	98.49	5.37
m977	M	87.44	5.05
m993	F	78.33	4.66
m985	M	88.54	4.01
m945	M	90.15	2.72
m980	F	79.05	0.93
m932	M	85.23	n/a
m996	F	84.28	n/a
m979	F	77.86	n/a

The underwater cameras were able capture all three types of movement freshwater mussels typically engage in (i.e., horizontal, vertical emergence, vertical burrowing) for both tagged and untagged individuals. Horizontal movement was the most common

behaviour, making up 57% of all movement observations (n=107) and accounting for 72% of the total time YLM were captured moving on camera.

Movement frequency was similar between tagged and untagged mussels (Figure 2-12). A two-tailed t-test determined that there was no significant difference in mean duration of movement ($t_{85} = 1.38, p = 0.17$) for all behaviours collectively, or horizontal movement alone ($t_{47} = 1.003, p = 0.32$). This data suggests that YLM movement behaviour was unaffected by the addition of the Biskit tags for the duration of the field season.

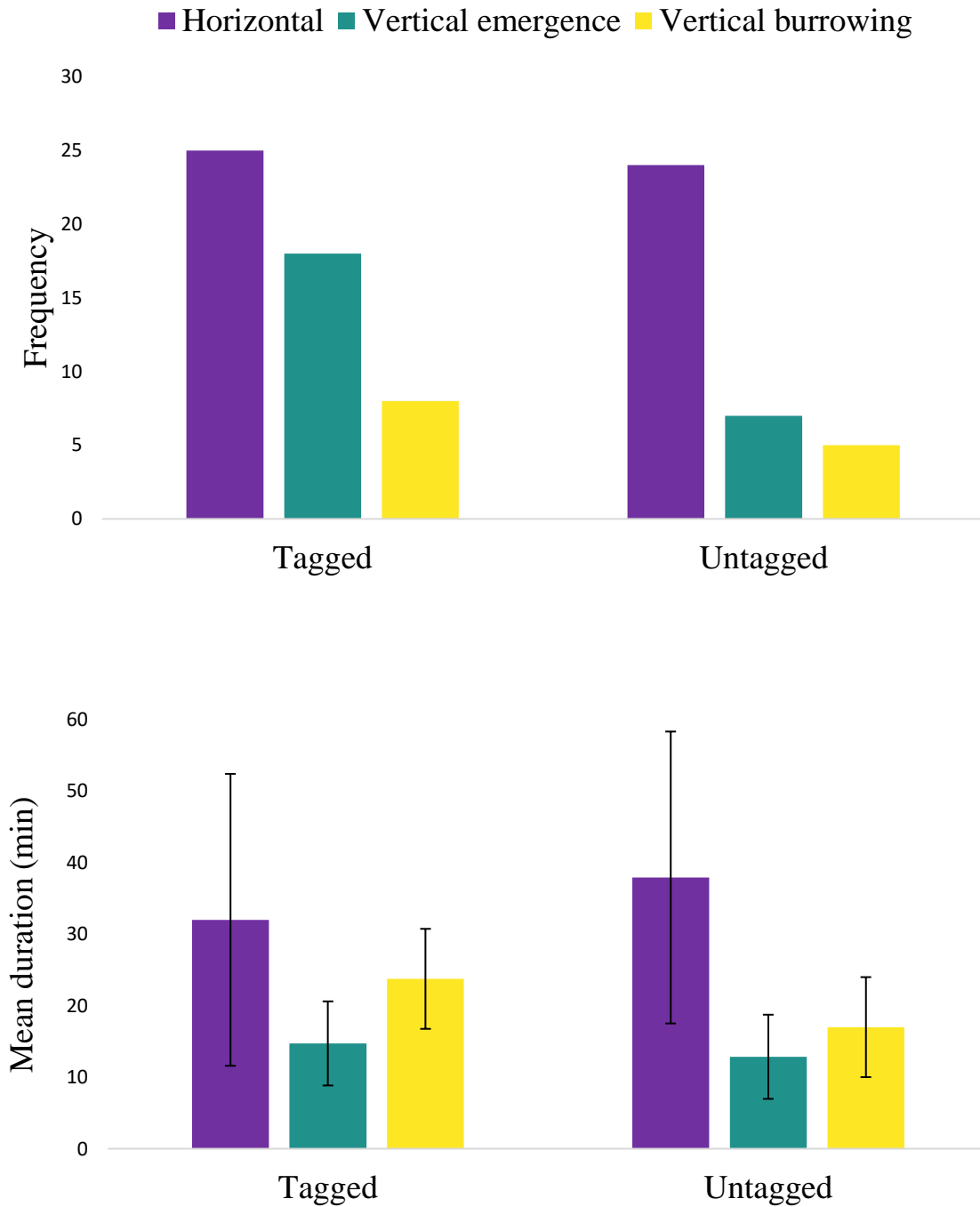


Figure 2-12: Frequency (top) and mean duration (bottom) of mussel movement behaviours observed by underwater cameras. Some (n=12) mussels did not have their right valve visible to determine if they were tagged or not and were excluded from this figure. Error bars represent one standard deviation.

Onset temperatures (i.e., the surface water temperature recorded at the time of a movement observation) for horizontal movement showed a bimodal distribution, however 52% of these movements happened when surface water temperatures were at their warmest (i.e., > 25 °C, Figure 2-13). The bimodal distribution is due to the movement observations occurring predominately before and after the cold snap during August, where mean temperatures were separated by almost 3 °C (Figure 2-8).

Most horizontal movements (52%) occurred when water levels were at their lowest (i.e., < 40 cm), however, they were still moving during times where emersion is not a significant threat (i.e., water level >50-60 cm, Figure 2-14). Recorded vertical movements (i.e., emergence and burrowing) always occurred within 5-10 minutes of the cessation or initiation of horizontal movements and as such, did not record different onset temperatures.

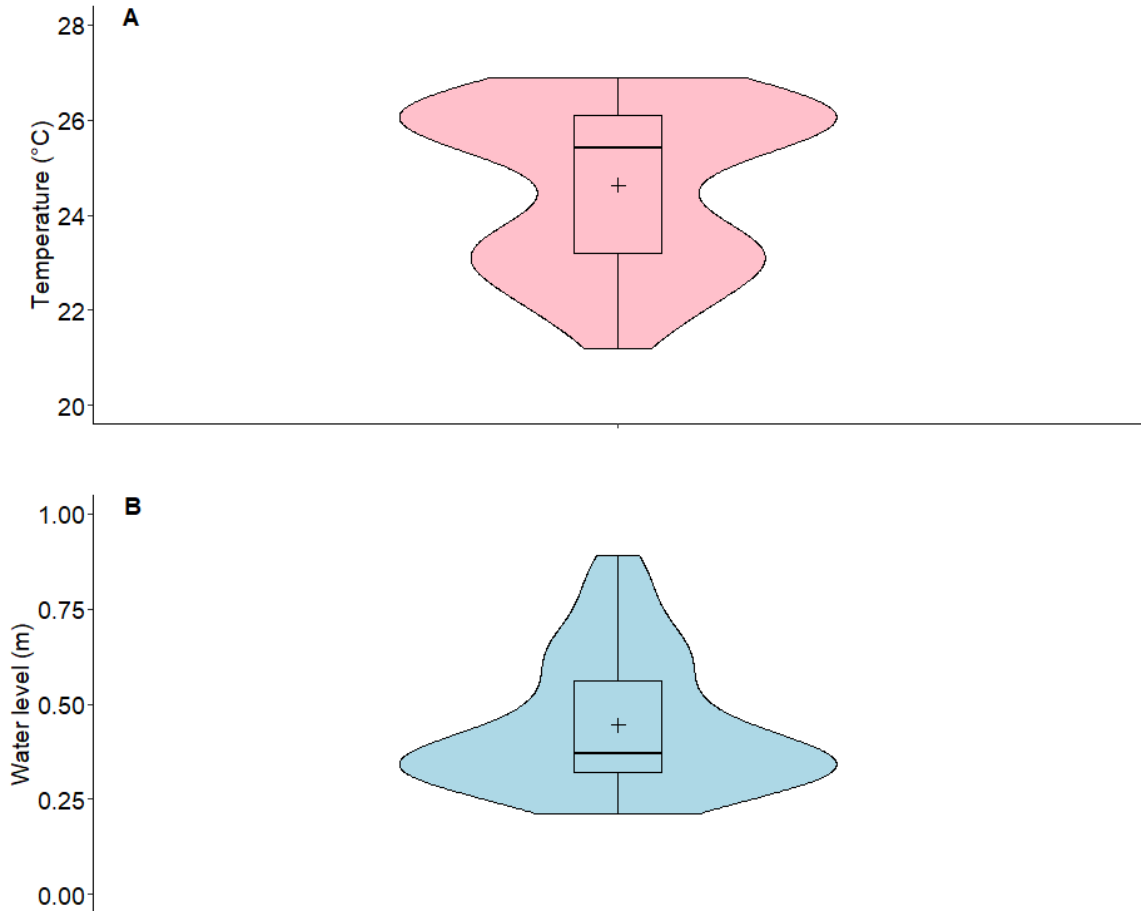


Figure 2-13: Violin wrapped box plots showing onset surface water temperature (A, n=61, sensor 3) and water level (B, n=61, sensor 3) of horizontal movement observations at Canaan River. Crosses represent mean values.

Drivers of movement

Water level appears to be an acute stressor for YLM where 52% of all horizontal movements occurred between 6-9 August, when water level had reached its lowest point of the season (Figure 2-14). Several underwater cameras (n=5) in the center of the camera line became exposed during this period, generally for a period of 3-4 hours. Cameras on either end of the camera line were in deeper water and did not become

exposed at any point during the season. The tagged mussels traversed the camera line to deeper water, close to sensor 3 (Figure 2-11). The uniformity of direction and measure of clustering of the relocation points suggests that there was a strong stimulus to move to their final location (Figure 2-14). The very low water levels during that period of 6-9 August would represent such a stimulus since the low water levels would pose a significant emersion threat.

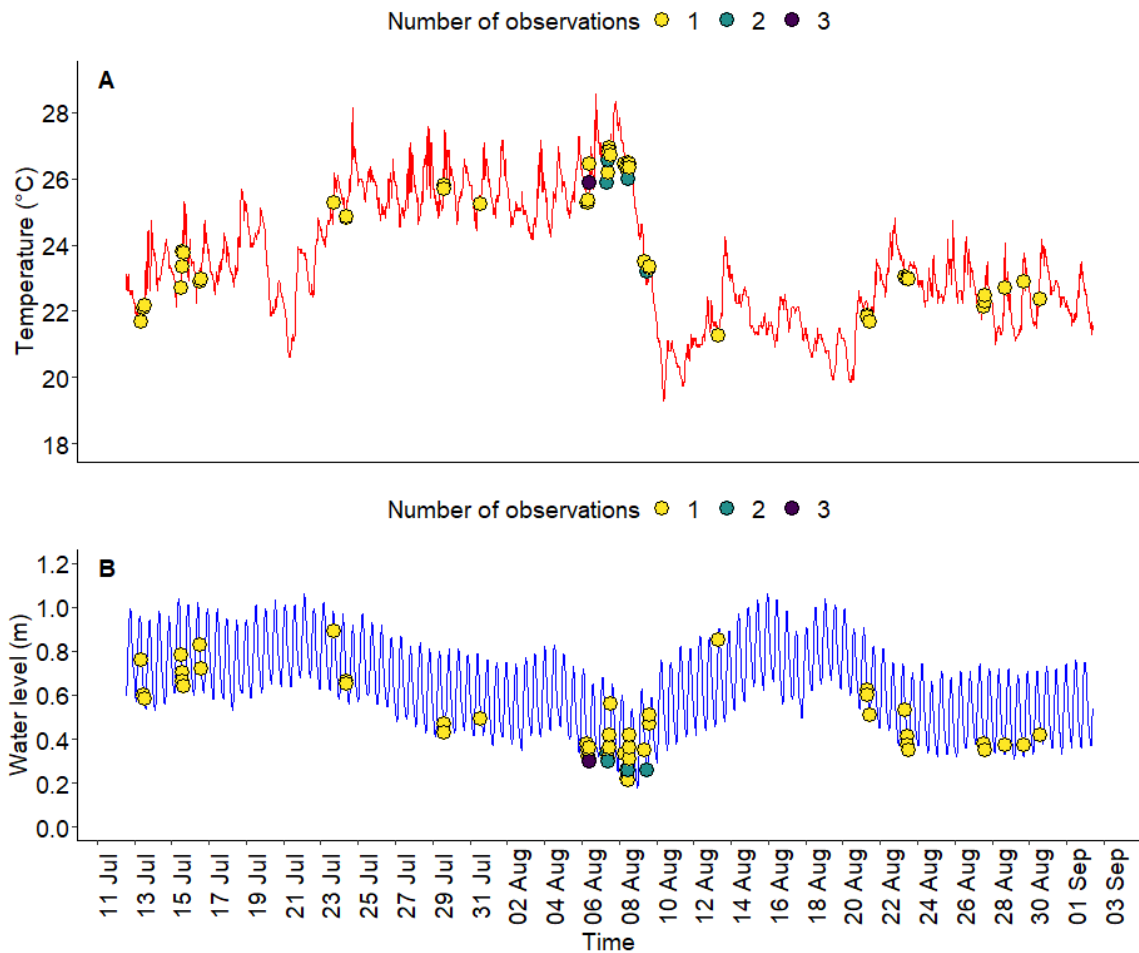


Figure 2-14: Horizontal mussel movement observations plotted onto temperature (A) and water level (B) at time of observation (n=61) at Canaan River. Temperature and water level values taken from sensor 3, nearest to the mussels.

The multiple logistic regression results for the model: movement ~ temperature + water level showed that both explanatory variables were significantly associated with a horizontal mussel movement response (Table 2-3). Temperature range had a significantly positive association with a movement response, while water level had a significantly negative association with a movement response.

The logistic regression had a statistically non-significant ($p \geq 0.05$) result from a Hosmer-Lemeshow test, indicating the model's estimate fit the data at an acceptable level. A Stepwise Akaike information criterion (AIC) comparison showed that a reduced model (i.e., movement ~ temperature or movement ~ water level) would not be a better fit for the data. As such, the full model was used in the calculation of beta values, intercept, and confidence intervals (Table 2-3). The model intercept shows that the log odds of a response when all variables are set equal to zero are significantly different than zero. McFadden's pseudo R^2 (based on the log-likelihood kernels for the intercept-only model and the full estimated model) was calculated to be 0.13, meaning that the model is not a strong fit for the data. McFadden's pseudo R^2 values are generally lower than traditional R^2 values where values of 0.2-0.4 indicate a good-excellent fit (Louviere et al., 2010; McFadden, 1974).

Overall, results of logistic regression show a weak (based on the model fit), but significant, relationship between temperature, water level, and the likelihood of a horizontal movement response. Each unit of increase in temperature was associated with an increase in the chance of a movement response of 5-79%, with 95% confidence. Each unit increase in water level was associated with an effectively zero percent chance of a

movement response. However, with each unit decrease in water level would be an associated 56-87% chance of a mussel movement response, with 95% confidence.

Table 2-3: Results of multiple logistic regression for the model of horizontal mussel movement (1/0) = temperature + water level. A bolded *p* value indicates a statistically significant result.

Explanatory variable	Estimate	Std error	z-value	<i>p</i> value
Intercept	-4.999	0.224	-20.100	<0.001
Temperature	0.315	0.135	2.322	0.0202
Water level	-1.165	0.178	-6.515	<0.001

Explanatory variable	β-value	Chance of response (%)	Lower CI (95%)	Upper CI (95%)
Intercept	0.011	1.10	0.69	1.64
Temperature	1.370	36.99	5.20	79.27
Water level	0.312	-68.81	-87.31	-56.21

DISCUSSION

In the attempt to identify drivers of YLM movement behaviour, both water level and temperature were found to have a statistically significant influence on the likelihood of a YLM horizontal movement. YLM did not appear to be substantially affected by the addition of tags and their distance travelled did not appear to be from physical differences (e.g., length or sex). The mussels exhibited direction selection towards deeper water, selecting habitat closer to the center of the main channel. The sediment habitat use by YLM was observed to be almost completely static rather than dynamic as predicted. The mussels appeared to spend long periods of time burrowed punctuated by infrequent movements, which the data suggests was due to temperature and/or water level.

Drivers of movement behaviour

The effects of low water levels on mussel movements have been studied (e.g., Newton et al., 2015; Schwalb & Pusch, 2007; Sullivan & Woolnough 2021), where freshwater mussels exhibit two general behavioural responses. The first response being horizontal migration seeking deeper water, and the second being vertical migration into the sediment where they were able to withstand periods of emersion (Byrne et al., 1994; Gough et al., 2012). The prominent threat presented by low water levels is mortality via emersion, but other sub-lethal stressors such as increased temperature and hypoxia can also occur (Byrne et al., 1994; Haney et al., 2020; Ganser et al., 2015).

The influence of temperature on horizontal movement in freshwater mussels has been studied but generally only seasonal/monthly trends are described (e.g., Amyot & Downing, 1997; Schwalb & Pusch, 2007). Schwalb & Pusch (2007) reported that horizontal distance (cm/wk) was strongly correlated to mean monthly temperature. In these studies was correlated with increasing mean monthly water temperature. Block (2013) observed that mussels were more likely to burrow, and movement (i.e., righting and burrowing) intensity increased with temperature. Similarly, Amyot & Downing (1997) reported that proportion of the population found at the sediment surface was closely related to monthly water temperature. These studies are finding that temperature can influence movement behaviour in mussels, however, they also suggest other factors such as discharge (e.g., Schwalb & Pusch, 2007), photoperiod (e.g., Amyot & Downing, 1997; Schwalb & Pusch, 2007), substrate particle size (e.g., Eissenhauer et al., 2023) and reproduction (e.g., Amyot & Downing, 1997) also play a role. While the direct influence of temperature on horizontal migration of mussels remains poorly understood, there is

evidence that physiological responses to temperature appear to be species-specific (Ganser et. al, 2015).

Most horizontal movement observations were likely in response to a significant emersion threat since 52% of those observations occurred during a 3-day (6-9 August) span of the season's lowest water levels. This data, along with the model significance and negative slope, suggests water level is an important driver of movement for YLM, when it is low. At higher (i.e., >50-60 cm) water levels, there is no longer a threat of emersion, and there were still numerous observations of YLM movement. Those higher water observations are likely where temperature (or another unmeasured/correlated variable) is providing the main stimulus for movement.

The effects of varying rates of temperature change, like those observed at Canaan River, can significantly affect thermal tolerance in other ectothermic organisms (Mora & Maya, 2006; Terblanche et al., 2007). The magnitude of the effect of temperature change is dependent on organism type and species, but the observations and model significance of temperature suggest that mussels are similarly affected. Binary logistic regression showed that increases in temperature were significantly and positively associated with an increase in the likelihood of a mussel movement response.

Overall, the data suggests that water level and water temperature (and likely combinations thereof) may be driving the movement behaviours for YLM, in this system. It is also possible that the mussels are moving due to non-stressful stimuli such as to facilitate fertilization/reproduction and/or other stressors/variables that were not measured at Canaan River (e.g., discharge or dissolved oxygen). Mussels have been reported to move more during periods when the discharge variability is lower (e.g.,

Sullivan & Woolnough, 2021). It is possible that discharge was similarly influential at CR01, given that there would be correlation between discharge and water level. Water levels, outside of lows threatening emersion, could certainly be working as a proxy for factors such as discharge or more complex hydrologic variables (e.g., Froude and Reynolds numbers, relative sheer stress). Increases in water temperature, along with periods of low water levels, can lead to hypoxic conditions and thus, stressful conditions for mussels. However, the tidal nature of Canaan River and the mixing of water that happens during a given tidal cycle (i.e., water level will rise and lower ~30 to 40 cm twice within 24 hours) suggests that periods of hypoxia were unlikely.

Accounting for additional variables in the model may have improved the fit and resulting McFadden's pseudo R^2 , but the lack of fit is more likely a result of the short sampling duration (~51 days) and lack of additional sites. Since there were no extended periods of time of low movement during the study, the distribution of binary observations almost overlapped. Mussel movement in general has been found to increase with mean monthly temperature (e.g., Amyot & Downing, 1997; Schwalb & Pusch, 2007), so presumably there would have been less movement outside of the summer months, that would have provided a greater separation between movement and no movement data points. Additional sites would allow for a better fit and greater model defensibility, since it would allow for inference as to whether the observations at Canaan River were typical of YLM or a site-specific adaptation.

Movement behaviour

Overall, the data suggests that the observed YLM horizontal movement is not being driven by the addition of the Biskit thermal sensors. The results of Levine et al. (2014) suggest that mussel burrowing behaviours are not influenced by the addition of artificial shell structure, using a weight/length ratio of 1g: ~5 mm. Since their artificial structure length/weight ratio was more than double the ratio used in this study (i.e., 7.2 g Biskit tag: mean length 86.4 ± 6.1 mm), it is not surprising that the YLM at CR01 were not significantly affected by their tags.

The mean net horizontal distance (7.5 ± 3.78 m over ~52 days) travelled by the tagged YLM aligns with other movement studies (e.g., 120 m annually from Peck et al., 2014; up to 32 cm/day from Schwalb & Pusch, 2007), although, horizontal movement rates have been shown to vary across mussel species (Daniel & Brown, 2014; Gough et al., 2012; Mitchell et al., 2018). Horizontal movements of freshwater mussels are generally attributed/correlated to low water levels or dewatering (e.g., Galbraith et al., 2015; Gough et al., 2012; Mitchell et al., 2018; Newton et al., 2014; Schwalb and Pusch, 2007; Sullivan & Woolnough, 2021), as a mortality threat via emersion. However, other factors such as discharge variability and mussel size class have also been reported to influence horizontal movement (Sullivan & Woolnough, 2021). Directionality of horizontal movements are generally reported as erratic or random (e.g., Balfour & Smock, 1995; Schwalb & Pusch, 2007), this may depend on species or system, since there have also been reports of clear directionality in long-term (i.e., multi-annual) horizontal movement studies (Peck et al., 2014).

Generally, the tagged YLM moved away from the shoreline towards the center of the river channel. They selected for deeper water, almost uniformly, between the third and fourth row of the hyporheic grid. By moving to deeper water, the tagged YLM would be less prone to being threatened by emersion. It is unlikely that the mussels received much thermal insulation from this movement since there were very minor surface water temperature differences throughout the grid. Selecting this habitat would reduce the effect of a stressor identified as a potential driver of horizontal movement and thus, is a plausible explanation for the apparent uniformity of selection. Interestingly, the movement by the tagged mussels did not appear to be random towards deeper water, since it was available both upstream and downstream of the grid, in addition to nearer to the center of the channel. This may be an artifact of the sampling timing, as the movement direction between capture and relocation may have been random, but the net direction of movement was clearly away from shore. Similar directional selection was reported by Newton et al. (2014), where mussels moved perpendicular to shore and Peck et al. (2014), who reported a net downstream directionality.

Thermal habitat selection

The temperature data from the tagged YLM allows for two major inferences: 1) the mussels spend most of their time burrowed within the first few (i.e., < 10) centimeters of sediment and 2) that YLM are insulated from extremes in temperature by maintaining that position in the sediment. The mussels consistently demonstrated temperatures slightly cooler than the surface water while simultaneously being warmer than the 10 cm sediment temperatures during warmer periods, and the inverse during cooler periods.

This data illustrates that for YLM, at this site, being burrowed up to their apertures was the default position. If the mussel temperatures had consistently matched the surface water, it would be interpreted as being above the surface. Similarly, if the mussel temperatures had matched or been cooler than the 10 cm sediment (or some oscillating pattern between surface and 10 cm), it could be inferred that they were burrowing deeper and/or repeatedly burrowing and surfacing. Underwater camera observations confirm their consistent positioning with some YLM remaining stationary for weeks at a time. It was also noted that upon every cessation of horizontal movement observed, burrowing occurred. There was no apparent evidence of alternative scenarios (e.g., YLM mainly being above surface, burrowing deeper than a few centimeters, or burrowing to variable depths and returning to the surface), hence the conclusion that being burrowed up to their apertures is the default position.

The consistent positioning within the sediment was shown to buffer these mussels from extremes in temperature, it provides a measure of thermal insulation. During the highest temperatures, the mussels never reached the same highs as the surface water and the inverse was also true during the coolest periods. The sediment position of YLM exposes them to a narrower range in temperatures than surface water, offering a measure of thermal stability. Throughout the study period YLM received a cooling effect of up to 1-1.75 °C numerous times. While this benefit does not seem significant, it is likely important to these mussels biologically. Ganser et al. (2015) found that some species of freshwater mussels start to show signs of thermal distress at ~25 °C, and other studies have reported reporting oxidative stress as well as reduction of the synthesis of adenosine triphosphate (ATP; Said & Nassar, 2022). Considering the Canaan River reached a

maximum of ~27-28 °C, receiving an almost 2 °C buffer could prevent YLM from hitting more stressful temperatures and allow for a more stable thermal profile. Pandolfo et al. (2010) found that the difference between 5% and 50% mortality of juveniles within a given species was only 5.3 °C, demonstrating that increases in temperature, especially near critical thermal maximums, can be the difference between thermal stress and mortality, albeit in juvenile mussels. Even without experiencing mortality, several sub-lethal thermal stressors such as respiratory pattern alteration, lowered hypoxia tolerance, could be lessened with some insulation versus the surface water temperatures (Ganser et al., 2015; Haney et al., 2020).

YLM positioning within the sediment would also provide a reduction of energetic demand (relative to being on the surface of the sediment) due to the increased metabolic activity of higher temperatures on ectothermic organisms (Haney et al., 2020). Increased energetic demand from higher temperatures, particularly if there are not sufficient nutrients to compensate, has been shown to be a stressor, but its effects are species dependent (Haney et al., 2020).

Between the temperature data and the underwater camera observations, it is apparent that YLM spend most of their time burrowed into the sediment. However, these observations only apply to this site and the conditions (i.e., temperature and depth regimes) present during the monitoring period. These mussels may spend more time at the surface during cooler periods (e.g., Fall or Spring) or at greater depths than were observed. While YLM were buffered from extremes in temperature, there are other reasons that they could be burrowing such as predator avoidance, photosensitivity, or reduced shear stress.

Future work

This research suggests that both water temperature and water level have an influence on the horizontal movement of YLM. The sediment habitat use depth was generally shallow (<10 cm), static, and consistent across all tagged mussels. YLM appeared to spend long periods of time burrowed punctuated by infrequent movements, which the data suggests was due to changes in temperature and/or water level.

Future work that would build upon this research would include adding sites within the same river and adding sites within other rivers. Increasing the number of sites within a river will allow for the examination of whether there are site-specific differences in YLM movement behaviour, possibly driven by the presence/absence of ground water inputs that could affect site-specific thermal regimes. Studying sites in additional rivers could demonstrate if there is river-specific plasticity in YLM movement behaviour. Additional rivers could also provide a gradient of variation in temperature/water level regimes. It would also be insightful to look at a comparison of temperature and water level regimes at sites that have YLM and those that do not. Studying other species would provide insight into whether the observed effects of temperature and water level on the movement of YLM are similar across other native W|SJR species. Different species may also be stressed by different environment variables, so monitoring variables such as discharge and dissolved oxygen would be very informative. Increasing the number of monitored variables could also improve further predictive modelling efforts. Ultimately, any work that improves the understanding of YLM ecology will be beneficial to its long-term conservation and management within the lower W|SJR catchment.

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**Chapter 3 : COMPARISON OF HABITAT SELECTION AND
MOVEMENT BEHAVIOUR OF THE YELLOW LAMPMUSSEL
(*LAMPSILIS CARIOSA*) AT TWO-HIGH DENSITY SITES IN THE
WOLASTOQ RIVER CATCHMENT, NEW BRUNSWICK**

ABSTRACT

Understanding how freshwater mussels respond to environmental stimuli, such as high temperatures and low water levels, is growing increasingly important in the changing climate. This work investigates the behavioural responses of Yellow Lampmussel (*Lampsilis cariosa*, YLM), a species-at-risk mussel in New Brunswick, to temperature and water level changes. YLM were tagged (n=20 at each site) with thermal sensors and passive integrated transponder (PIT) tags at two sites within different rivers: Canaan River (CR01) and Salmon River (SR01). Thermal sensor data revealed that 1) YLM maintained a burrowed position throughout the sampling period (July- September) and 2) being burrowed provided the mussels with an insulating effect (up to 3 °C) from surface water. The thermal insulation at SR01 was occasionally almost twice that of CR01. YLM moved an average of 2.8x further and 5.5x more often at CR01 than SR01, suggesting that movement behaviour can be site/river-specific. The majority (52%, n=61, at CR01 and 82%, n=11, at SR01) of movement occurred during a 3-day stretch during (6-9 August), where water temperatures were high and water levels were low. A logistic regression confirmed that temperature, water level, and site/river significantly influenced the likelihood of a YLM movement response, however the model was not a strong fit for the data (McFadden's pseudo $R^2 = 0.17$). Future work should include studying additional rivers, including other ecologically significant variables in the model, and studying the movement of other mussel species. Increasing the knowledge of the fundamental ecology of YLM will be important to inform future management and conservation decisions.

INTRODUCTION

Freshwater mussels (Bivalvia: *Margaritiferidae*, *Unionidae*) are declining across North America, which makes understanding their ecology fundamental to their long-term management and conservation (Hastie et al., 2003; Nedeau, 2000). Yellow Lampmussel (YLM; *Lampsilis cariosa*) are found along the Northeast Atlantic slope of North America, ranging from Georgia (United States) north to Nova Scotia (Nedeau, 2000).

The YLM population in New Brunswick (NB), Canada, generally inhabits the lower portion of the Wolastoq | Saint John River (W|SJR) catchment. Its historical range within the lower W|SJR spans from the Mactaquac Generating Station near Fredericton, down to near the mouth of Washademoak Lake (including major tributaries; Sabine et al., 2004). In the W|SJR, this mussel is believed to be threatened by changes to habitat and water quality (e.g., sedimentation, pollution), invasive species (e.g., *Dreissena* spp.), and low water levels (e.g., risk of emersion and thermal stress; Fisheries and Oceans Canada, 2010). Its narrow geographical range, along with the largely anthropogenic threats facing this species, have led to its designation as a species of ‘*Special concern*’ by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC; COSEWIC 2004).

Migration, be it burrowing into the sediment (vertical) or moving across riverbed sediments (horizontal), are YLM’s primary means of escaping stressful conditions (Schwalb & Pusch, 2007; Watters et al., 2001). Movements are most often noted during drought or dewatering conditions where mussels may become emersed (Golladay et al., 2004; Gough et al., 2012; Mitchell et al., 2018; Newton et al., 2014), but the type and

order of movement (e.g., horizontal only, horizontal then vertical, vertical only) depends on species (Galbraith et al., 2015; Gough et al., 2012).

Water temperature has been reported to influence many aspects of freshwater mussel biology and ecology, but responses are generally species dependent (Ganser et al., 2015; Haney et al., 2020; Pandolfo et al., 2010). Several studies have found that temperature affects mussel movements (e.g., Amyot & Downing, 1997; Block et al., 2013; Schwalb & Pusch, 2007; Waller et al., 1999) and it has been suggested that the use of thermal niches (e.g., vertical migration into the sediment seeking cooler temperatures) could be important to mussel abundance and population success (Newton et al., 2014; Spooner & Vaughn, 2009).

This research looks to explore and compare the impact of temperature and water level on the movement behaviour (i.e., vertical habitat selection and horizontal migration) of YLM between two high-density sites. YLM likely have certain thresholds of temperature and water level (e.g., high temperatures and low water levels) that when exceeded, will elicit a movement response. Since these sites likely have different temperature and water level regimes, it is likely that YLM horizontal movement will differ according to site-specific environmental regimes. Differences in environmental stimuli between sites would also likely result in YLM using the sediment habitat differently (i.e., burrowing to different depths, more frequent burrowing/emerging).

The effects of environmental stressors on movement behaviour of YLM in the W|SJR have not previously been studied. Addressing these fundamental knowledge gaps in YLM ecology will be crucial to its long-term management and conservation.

METHODS

Site selection

The W|SJR is eastern Canada's longest river (~673 km) with a basin area of over 55,000 km². The river flows from its headwaters in northern Maine and southeastern Quebec, where it then flows through NB and into the Bay of Fundy (located south of NB). The lower W|SJR basin (i.e., downstream of the Mactaquac Dam near Fredericton to near the mouth of Washademoak Lake) is where the river broadens and is joined by two major tributaries: Salmon River via Grand Lake and Canaan River via Washademoak Lake. This section of the river is home to many alluvial islands that can provide excellent sandy habitat for a variety of freshwater mussel species.

Intensive directed snorkeling surveys were completed during the summer of 2021 to locate areas of ideal habitat (measured by relative abundance of YLM), using the work of Sabine et al. (2004) as a guide. Snorkeling locations (shallower sandy areas) were then selected remotely using Google Earth 2019 satellite imagery (Figure 3-1).

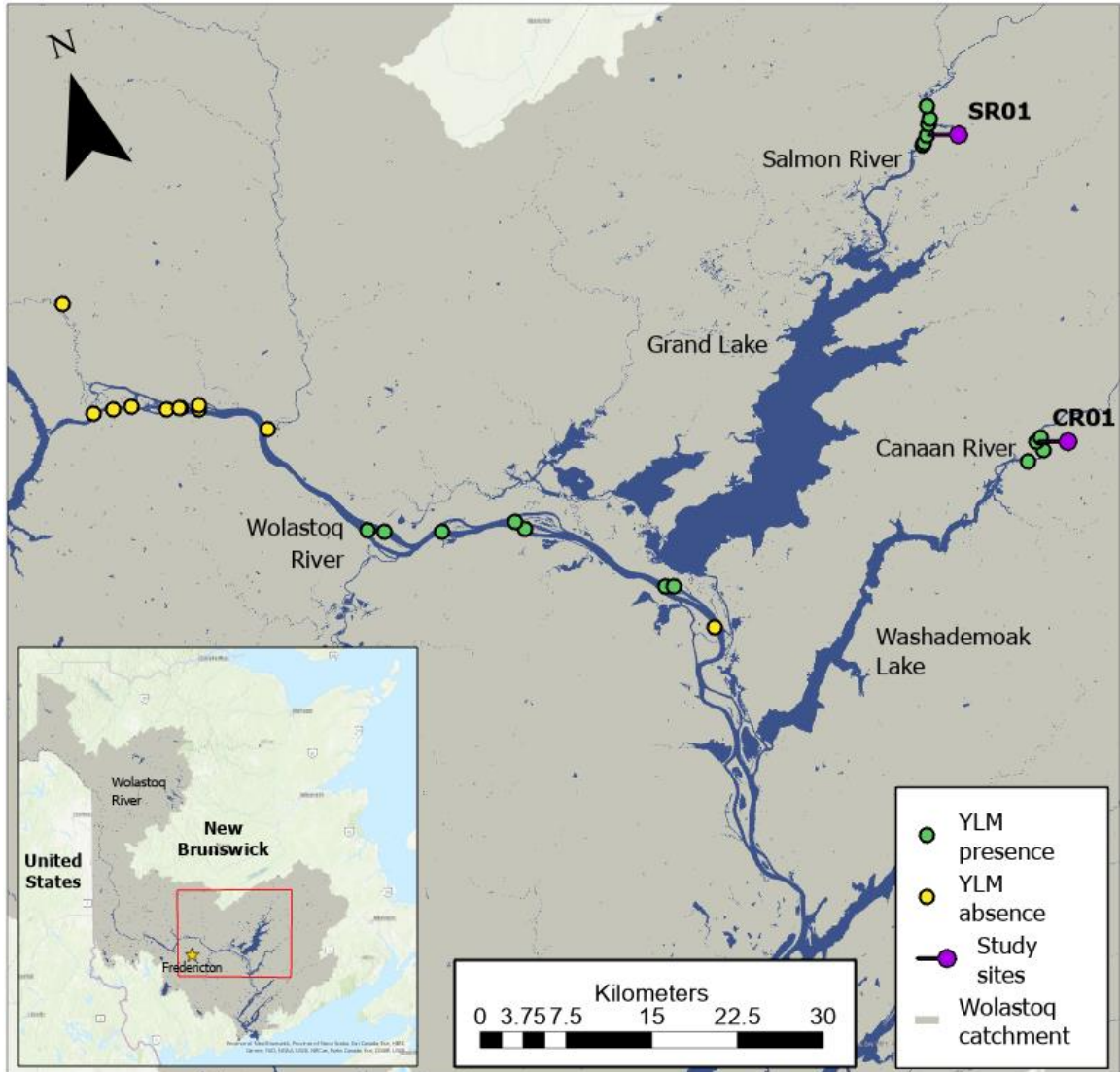


Figure 3-1: Yellow Lampmussel presence (n=17; green dots), absence (n=12; yellow dots), and high-density study site (n=2) locations from snorkeling surveys conducted during 2021. Map source: GeoNB.

No high abundance sites were located on the main stem of the W|SJR, but two high abundance sites were located: one in the Salmon River (SR01) near Chipman, NB and one in the Canaan River (CR01) near Coles Island, NB. These tributaries accounted for 89% (54% at Canaan and 35% at Salmon) of the total specimens located.

Study Area

SR01 is located approximately 1.12 km northeast of the Route 10 bridge in Chipman, NB. It resides within the Salmon River, a tributary river that drains into Grand Lake and then into the main stem of the W|SJR. This is a highly tannic, medium-sized river (6th order stream) that was ~100 m wide at the site location. The habitat at SR01 is best described as a shallow sandy area that extends upstream into a series of sandbars (Figure 3-2). There is sparse emergent vegetation present at this site.

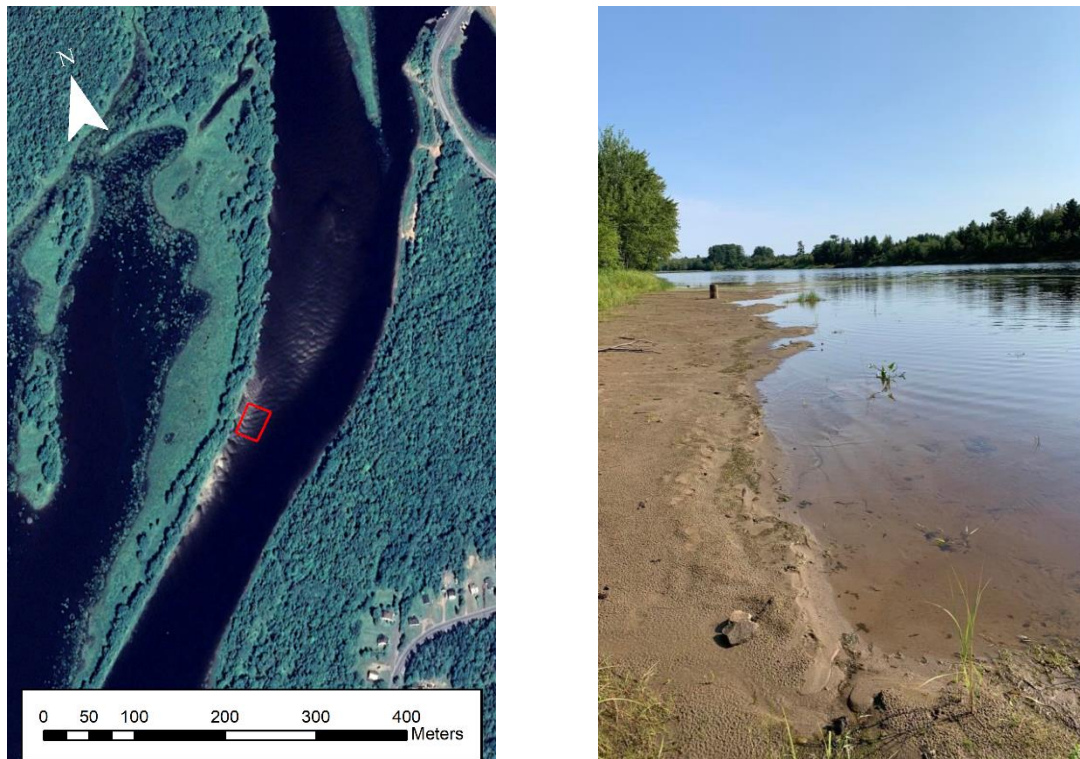


Figure 3-2: SR01 aerial imagery with the study area bordered in red (left) and on-site upstream view (right). Map source: Google Earth.

CR01 is located approximately 3.51 km northeast of the Route 10 bridge in Coles Island, NB. It is situated in the Canaan River, a tributary river that drains into

Washademoak Lake and then into the main stem of the W|SJR. Like the Salmon River, this is also a highly tannic medium-sized river (5th order stream) that was ~116 m wide at the site location. The habitat at this site is shallow and sandy with many pockets of dense algae, particularly near the shore (Figure 3-3).

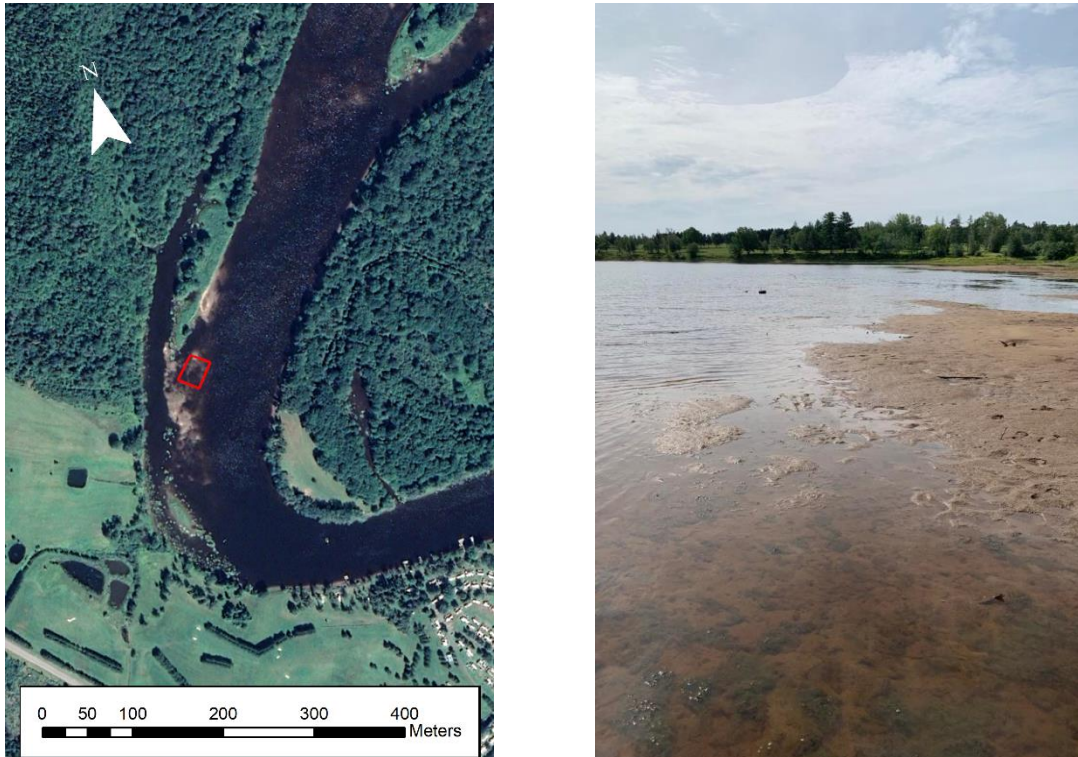


Figure 3-3: CR01 aerial imagery with the study area bordered in red (left) and on-site downstream view (right). Map source: Google Earth.

Hyporheic grid setup and site monitoring

Hyporheic monitoring took place from 20 July – 2 September 2022. A grid of 20 hyporheic wells was installed at both sites to characterize the thermal regime within the riverbed sediment (Figure 2-3). With a well installed every 10 m, the grid ran 40 m

(parallel to flow) x 30 m (perpendicular to flow) for a total area of 1200 m². Each well consisted of a capped PVC plastic tube with a string of Onset HOBO pendant temperature loggers hanging (one at each of 10 cm, 20 cm, and 40 cm) from the cap (Figure 2-4). These depths were chosen because mussels have been found to burrow up to a depth of 20 cm, but are usually within the first 10 cm of the riverbed sediment (Balfour & Smock, 1995; Schwalb & Pusch, 2007). All wells also had a series of flow-through holes drilled into the tube and silt screen wrapping to prevent clogging. A passive integrated transponder (PIT) tag was attached to each cap so that the wells could be found in the event of shifting substrate. Each well was installed vertically into the sediment up to the cap.

River water temperatures were monitored with additional pendant loggers (n=4) attached to bricks, one at each corner of the grid. Pressure sensors (Onset Hobo U20 level loggers) were placed through the center of the grid to record water level data as well as river water temperature. A fourth pressure sensor was installed in a nearby tree measuring air pressure that was then used to calibrate pressure sensors placed on the grid. All river water loggers and pressure sensors were installed within a white uPVC tube to act as a solar shield (i.e., measuring ambient temperatures). Every Hobo pendant logger and pressure sensor was set to record temperature and/or pressure readings at 30-minute intervals. Data from all sensors was downloaded and then trimmed (20 July, 17:00 – 1 September, 9:00) to ensure that all observations were recorded while the sensors were deployed. Data of similar types (e.g., well temperatures, surface water temperatures, pressure readings) were compared in a time series so that any erratic or erroneous data could be excluded from analysis. Data from sensor 2 was determined to be unreliable after

becoming emersed repeatedly over the duration of the field season. Emersion was observed both visually and empirically through pressure readings that distinctly matched air pressure. Placement of the hyporheic grid was chosen to center on the area and depth that YLM were known to occupy based upon the previous year's snorkeling efforts.

Thermal sensor tagging and mussel placement

Twenty mussels were collected via snorkeling at each site. Each mussel was outfitted with an Alpha Mach Biskit temperature logger (25 mm diameter x 8.2 mm depth, 7.2 g) as well as a small (11 mm) passive integrated transponder (PIT) tag (Figure 2-5). The Biskit loggers were affixed to the anterior portion of the right valve and the PIT tags were attached to the posterior portion of the right valve. Both temperature loggers and PIT tags were attached using a thin layer of Gorilla Super Glue Gel. This product contains ethylcyanoacrylate as its main bonding agent, which is considered aquarium safe and commonly used in such applications. PIT tag orientation on the shell was chosen such that when the mussel was burrowed, the tag would be in a vertical orientation, providing a larger detection field than a horizontal orientation (Linnansaari et al., 2007)

After application of the glue, the mussels were immersed in a separate holding container, where the glue cured for a minimum of five minutes. The maximum time spent in the holding container was the duration of time it took to tag five mussels, plus five minutes curing after the last mussel was placed in the holding container (~12 minutes). Once a group of five mussels had been tagged and the glue cured, they were put out into flow-through laundry baskets and placed back into the river. This process was repeated until all 20 mussels were in four laundry baskets weighed down by rocks and zip-tied

together. The mussels were left overnight to fully cure the glue, inspected for tag retention in the morning, then released onto the hyporheic grid. Mussels were placed back into the area and depth from which they were predominately found, to ensure as little artificial movement was introduced as possible.

A backpack radio frequency identification (RFID) PIT tag reader was used to detect tagged YLM for data recovery at the end of the field season. After detection, the mussels were excavated by hand and their Biskit loggers were scanned with an Alpha Mach PITStop RFID antenna. This antenna is designed to specifically interact and program the Biskit loggers. The mussel position was recorded upon release (12 July, for CR01 and 20 July for SR01) and again when they were recovered for data downloading (2 September, for CR01 and 1 September for SR01). YLM position was determined using a Bad Elf Pro + (BE-2300-GPS \pm 2.5 m) handheld GPS unit.

Movement observations

YLM movement was tracked using ten Brinno TLC-200 time-lapse cameras. Cameras were set up to record an image at 5-minute intervals, which the camera would then string together into a video. Each camera was set to record from 5:00 am until 10:00 pm. To deploy the cameras underwater, each camera was outfitted with a custom waterproof housing made from PVC piping. The housing and cameras were then strapped to standard 6" cinderblocks and placed on the bottom of the river. Cameras were placed along the third row of the grid, outside of where the mussels were released (Figure 2-3).

Camera observations were reviewed frame by frame, where the type (i.e., vertical burrowing, horizontal, and vertical emergence), duration, date, and time of the movement was recorded. Mussel movement was observed for the entirety of the time the hyporheic grid was deployed.

Sediment sampling

Sediment was collected from a random location within each row (n=4) in the hyporheic grid, for both sites. These sediments samples were collected for particle size analysis to characterize the fine (< 2mm) fraction of the soil (Groenendyk et al., 2015). Samples were collected by filling a Ziplock bag ~ $\frac{3}{4}$ full using a sediment scoop. In the laboratory, the samples were dried at 43° C initially for storage and then cooked at 288° C overnight, before mechanical sieving. A sub sample of 500g of the dried sample was then passed through a series of sieves (4.75mm - 0.075mm) and then the remaining particles (< 0.075 mm) were classified as “fines” (i.e., silt and clay). Mechanical sieving of the sediment samples was done using a sediment shaker and followed the American Society for Testing and Materials (ASTM) Standard (ASTM D422, 2006; Table 3-1).

Table 3-1: Standard sieve sizes used by ASTM International for sediment grain size analysis and particle distribution. Sieves #4 - #200 were used for sediment samples.

Sieve Designation in Accordance with ASTM D422			
Alternative	Standard	Alternative	Standard
Lid		No. 10	2.00 mm
3 in.	75 mm	No. 20	850 μ m
2 in.	50 mm	No. 40	425 μ m
1- ¹ / ₂ in.	37.5 mm	No. 60	250 μ m
1 in.	25.0 mm	No. 100	150 μ m
³ / ₄ in.	19.0 mm	No. 140	106 μ m
³ / ₈ in.	9.5 mm	No. 200	75 μ m
No. 4	4.75 mm	pan	

Classification of the sediment grain size was based on the United States Geological Survey (USGS) classification definitions (Table 3-2). The accuracy of this classification only applies to particle sizes below the #4 sieve since any particle size greater than 4.75 mm could be trapped by that sieve.

Table 3-2: United States Geological Survey (USGS) classification of naturally occurring sediments. Taken from USGS Scientific Investigations Report 2019–5073, page 5.

Classification of Naturally Occurring Sediments—Sediment Classes 5

Sediment grade				Sediment aggregates and abbreviations	Composite gravel grades	Composite sediment grades of this study and abbreviations	
Grain-diameter range, in mm	Scale, phi	Citation of grade name	Grade name			Based on grain-size analysis of sediment samples	Based on visual analysis of seabed imagery
2,048 to <4,096	-11	Blair and McPherson (1999)	Very coarse boulder gravel	Gravel <i>G</i>	Boulder gravel	Gravel ₂ <i>G₂</i>	Boulder gravel bG
1,024 to <2,048	-10		Coarse boulder gravel				
512 to <1,024	-9		Medium boulder gravel		Cobble gravel		Cobble gravel cG
256 to <512	-8		Fine boulder gravel				
128 to <256	-7		Coarse cobble gravel		Pebble gravel		Pebble gravel pG
64 to <128	-6		Fine cobble gravel				
32 to <64	-5		Very coarse pebble gravel				
16 to <32	-4		Coarse pebble gravel				
8 to <16	-3		Medium pebble gravel		Gravel ₁ <i>G₁</i>		
4 to <8	-2		Fine pebble gravel				
2 to <4	-1	Wentworth (1922)	Granule gravel				
1 to <2	0		Very coarse sand				
0.5 to <1	1	Udden (1914)	Coarse sand	Sand <i>S</i>	Coarse-grained sand cgS	Mud <i>M</i>	
0.25 to <0.5	2		Medium sand				
0.125 to <0.25	3		Fine sand		Fine-grained sand fgS		
0.062 to <0.125	4		Very fine sand				
0.031 to <0.062	5		Coarse silt	Mud <i>M</i>	Silt		
0.015 to <0.031	6		Medium silt				
0.008 to <0.015	7		Fine silt	Mud <i>M</i>	Clay		
0.004 to <0.008	8		Very fine silt				
0.002 to <0.004	9		Coarse clay				
0.001 to <0.002	10		Medium clay				
0.0005 to <0.001	11		Fine clay				

Data Analysis

A truncated dataset was used for all CR01 time series, since the SR01 grid was deployed a week after (i.e., 20 July for SR01 versus 12 July for CR01), for clearer comparison and visualization. Observations from the full period of deployment of CR01 were used for the onset temperatures and the binary logistic regression.

The distance data was measured using GIS (ArcGIS Pro version 3.03) using release and relocation GPS points. As such, the metric distance travelled only refers to the absolute difference between where the YLM were released versus where they were

relocated. It is probable that the mussels moved additional times during the field season and this metric likely underestimates total movement for the season. It is more representative of net movement/directionality over the sampling period.

To analyze the influence of temperature and water level on the likelihood of a YLM horizontal movement response, a multiple binary logistic regression was conducted. Binary logistic regression was chosen to predict the likelihood of a mussel movement response due to the binary nature of the movement observations (i.e., movement or no movement) for both temperature and water level data. Movement observations were determined as the first camera frame where the YLM entered the screen. The logistic regression had a non-significant ($p \geq 0.05$) result from a Hosmer-Lemeshow test, indicating the model's estimate fit the data at an acceptable level. Due to the difference in units between temperature and water level, both variables were scaled in R (using the scale function) so that the magnitude of change within variables would be comparable. The logistic regression also had a significant result when compared to a null model, establishing that it fit the data better than said null model.

High temperatures alone may not stimulate YLM horizontal movement behaviour. Accumulated thermal stress (e.g., growing degree days at or above a temperature stressful to freshwater mussels) may be more influential and could explain movement differences between sites. Calculation of growing degree days (GDD) was completed using the *pollen* package. Type "B" was selected where heat units are calculated based on the difference between the mean daily temperature and the threshold (t_{base} ; 25 °C was chosen as a temperature threshold for thermal stress in mussels; Ganser et al., 2015). In the case

when the value of t_{\min} (minimum daily temperature) is lower than t_{base} , then it is replaced by t_{base} .

All data was analyzed using R programming software (version 4.2.2; R Core Team, 2020) in the RStudio (version 2022.12.0; RStudio Team, 2022) environment using *pscl* (v1.5.5; Simon Jackman, 2020), *corrplot* (v0.84; Wei & Simko, 2017), *rstatix* (v0.7.2; Alboukadel Kassambara, 2023), *multcomp* (v 1.4-23; Torsten Hothorn, 2023), *xts* (v0.13.0; Ryan, Ulrich, Bennet, Joy, 2023), *pollen* (v0.82.0; Jakub Nowosad), and *MuMIn* (v1.47.5; Kamil Bartoń, 2023) packages.

RESULTS

Environmental comparison

CR01 and SR01 are both highly tannic, relatively slow flowing, medium-sized rivers, however, there are significant differences in their water level regimes. CR01 occupies a lower position in the W|SJR catchment and is more affected by tidal influences from the Bay of Fundy. As a result, the water level generally fluctuates ~30-40 cm, twice daily (Figure 3-4). Temperature differences were not as dramatic as water level, with SR01 generally being cooler by ~1-2 °C, up to a maximum of ~4 °C, for the sampling duration.

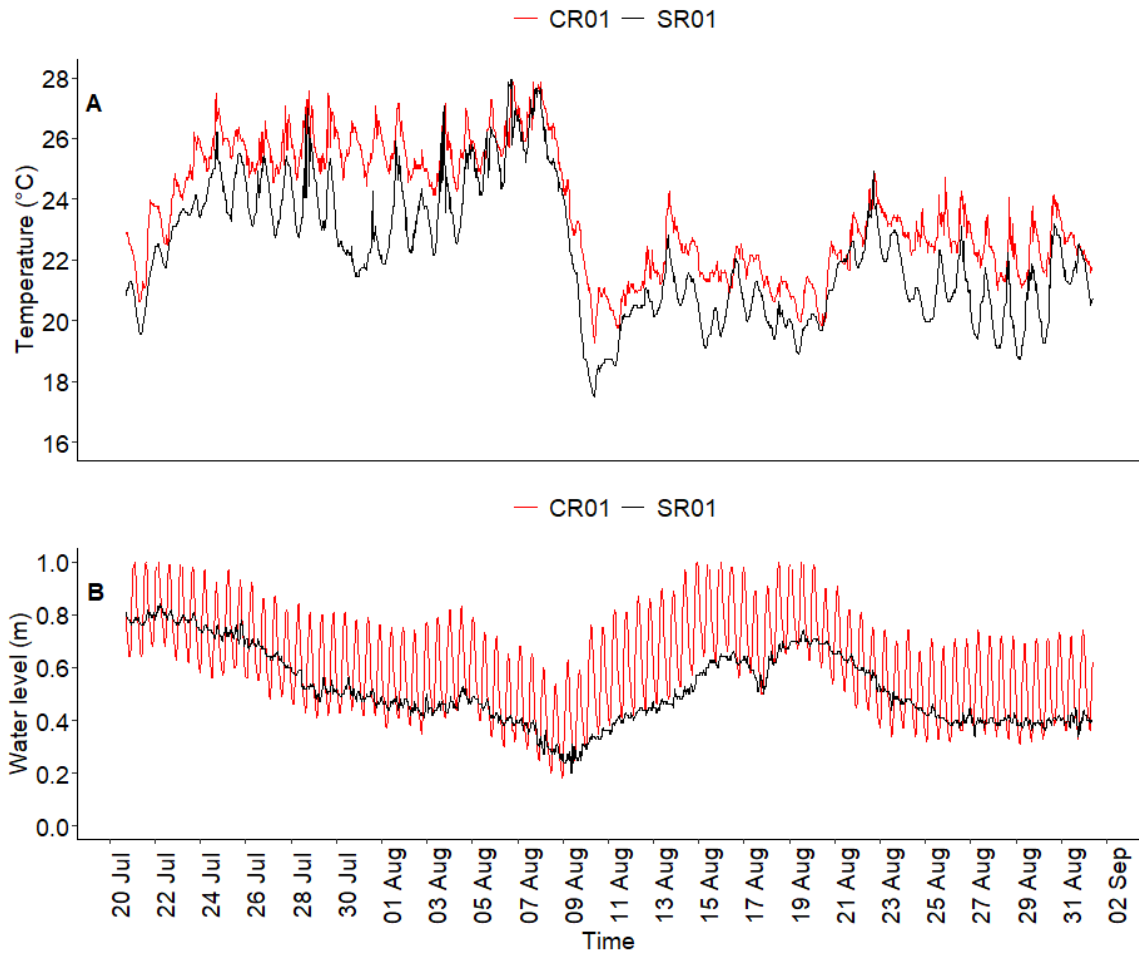


Figure 3-4: Temperature and water level time series for both CR01 and SR01, 20 July – 1 September.

Variability in both environment variables were compared as a unitless measure: coefficient of variation (Figure 3-5). Water level showed the greatest amount of variation, with CR01 regularly varying 2-3 times more than SR01. Variation in surface water temperature was substantially closer between sites. Hyporheic monitoring revealed that sediment temperatures within and between both grids were similar (Table A-1, A-2, A-3).

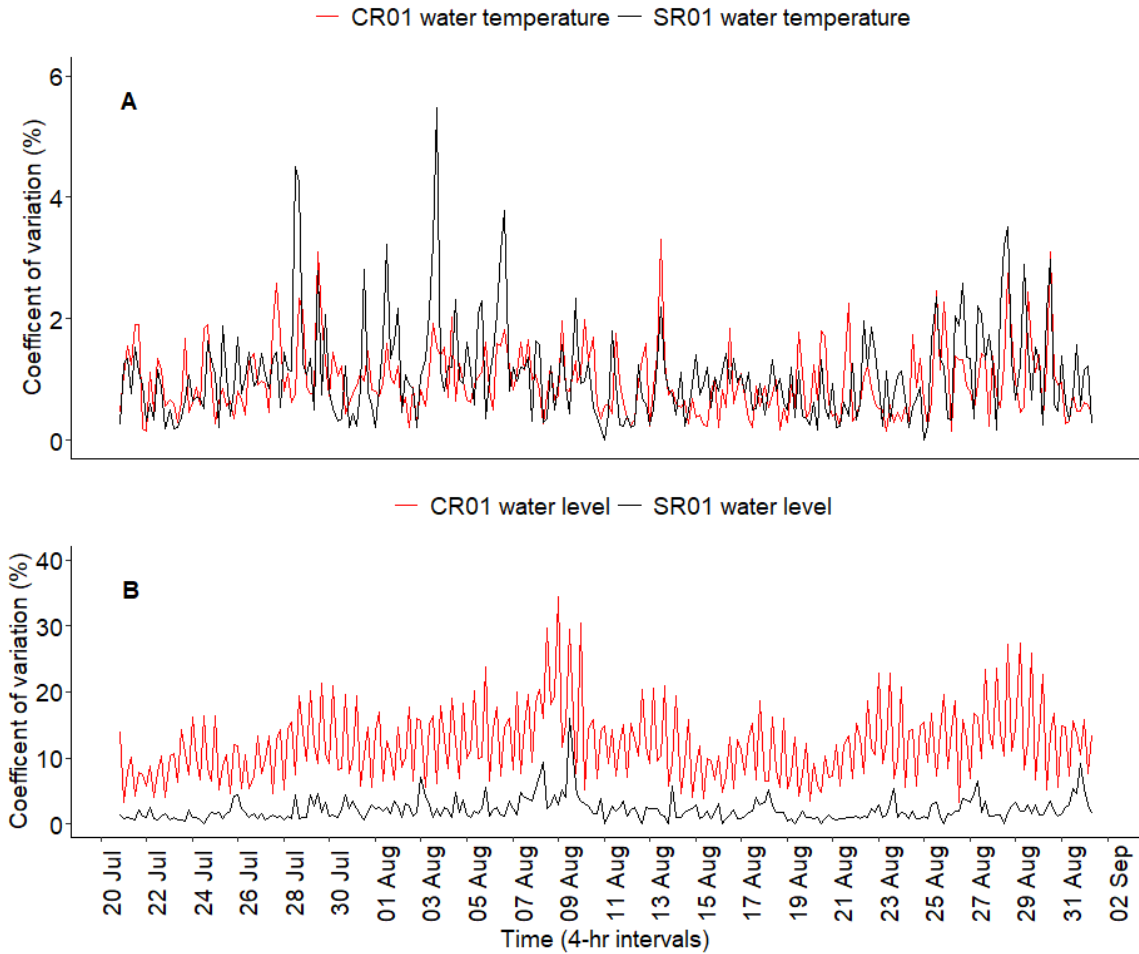


Figure 3-5: Coefficient of variation as a percent for surface water temperature (A) and water level (B).

While surface water temperature variability was similar between sites, accumulated thermal stress did differ (Figure 3-6). Using a base temperature of 25 °C, which is approximately the threshold where freshwater mussels experience thermal stress, CR01 accumulated ~10 additional growing degree days than SR01.

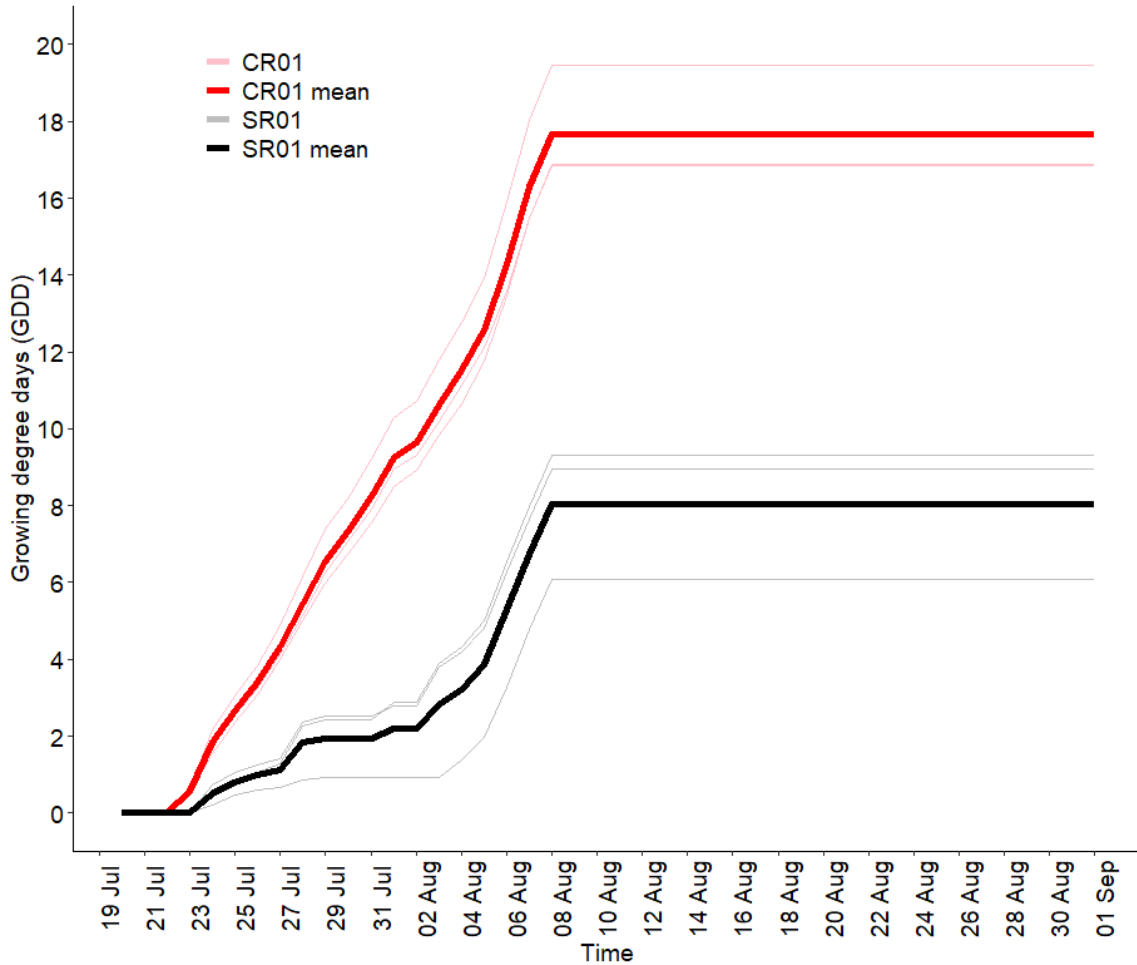


Figure 3-6: Growing degree days using a base temperature of 25 °C, for both SR01 and CR01. Sensor 3 and the deepest two surface water loggers were located closest to the tagged mussels and were used to calculate growing degree days (GDD).

The mean sediment particle size was similar at both sites (Figure 3-7). The dominant sediment type for both sites was medium sand (0.25-0.5 mm), contributing 77.5% and 83.6% of the mean particle size for CR01 and SR01 respectively.

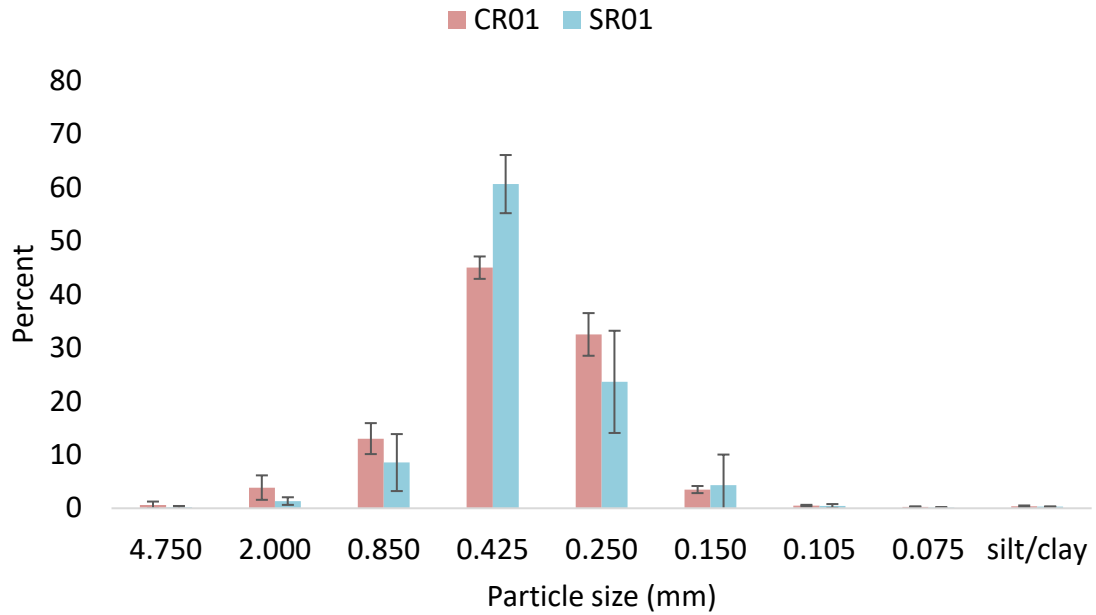


Figure 3-7: Mean particle size distribution (n=4 for each site) of sediment samples taken from the hyporheic grid installed at both CR01 and SR01.

Thermal habitat selection

YLM were observed maintaining a burrowed position, with only their apertures or a small part of the posterior shell visible, for most of the study period. The burrowed position was only abandoned in favour of moving horizontally off camera. No mussels were observed to emerge and stay on the riverbed surface. Certain individuals that burrowed on camera were also observed to remain burrowed in the same location for up to ~14-18 days.

The tagged mussels consistently reported temperatures that were slightly cooler than surface water, while also being warmer than the 10 cm sediment temperatures (Figure 3-8). The ordering of temperatures is consistent not only during peaks in temperature, but

during periods where the temperature drops as well. This is reflective of the positioning of YLM, being burrowed a few centimeters into the sediment. This positioning exposes YLM to a narrower range of temperatures relative to the surface water. The data suggests these mussels spend most of their time burrowed and shows no evidence of deeper burrowing, for the duration of the study.

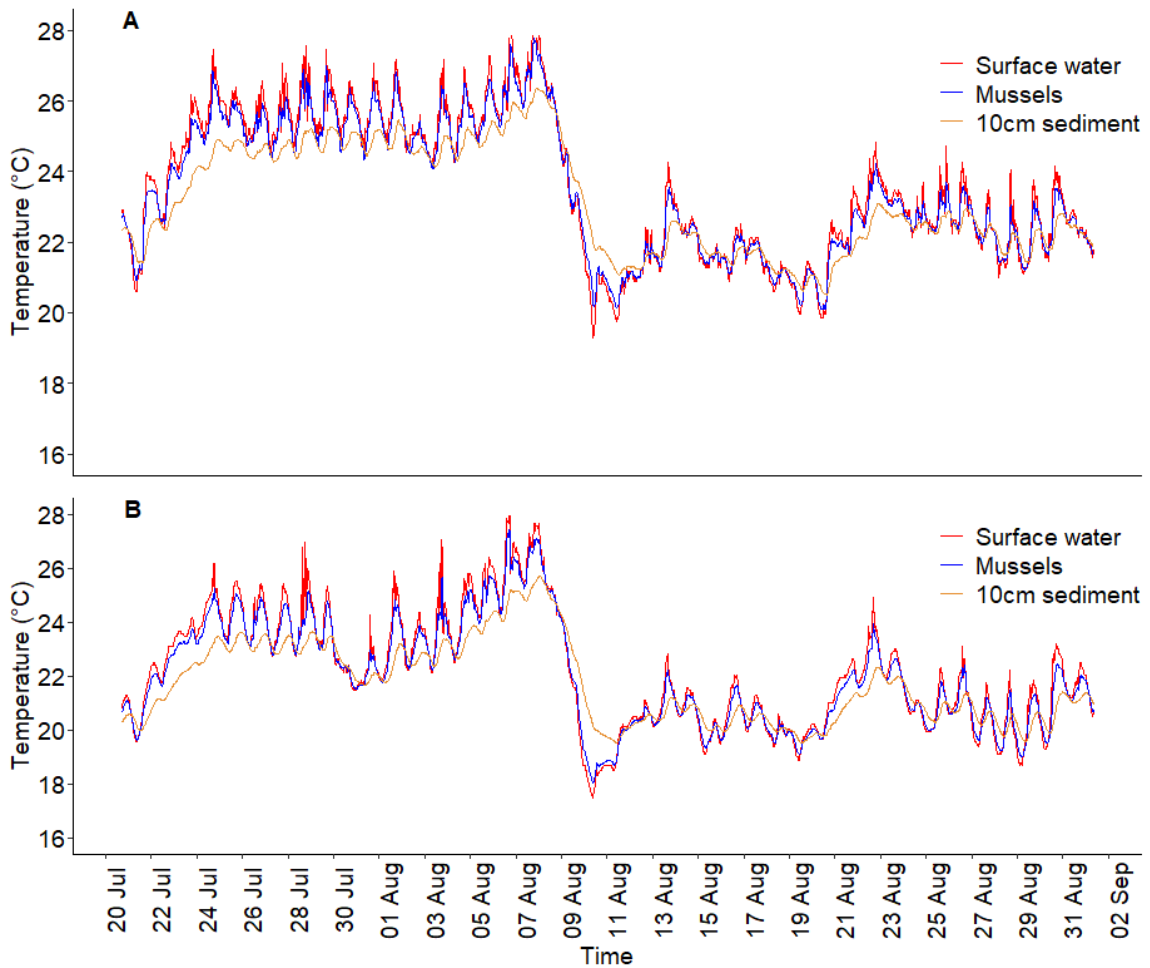


Figure 3-8: Temperature time series of mean mussel temperatures at CR01 (n=16, A) and SR01 (n=20, B). Wells used for 10 cm sediment temperatures were averaged from row 3 (i.e., mean temperature reading from all five wells), closest to where the mussels were released. Sensor 3 surface water, also the closest to where mussels were released, was used for comparison.

The sediment positioning of YLM provided an insulating effect against changes in surface water temperature (i.e., they do not experience the same extremes as surface). However, the magnitude of insulation was different between sites with the effect being almost double at SR01 (1-3 °C) than CR01 (1-1.75 °C), for portions of the sampling period (Figure 3-9).

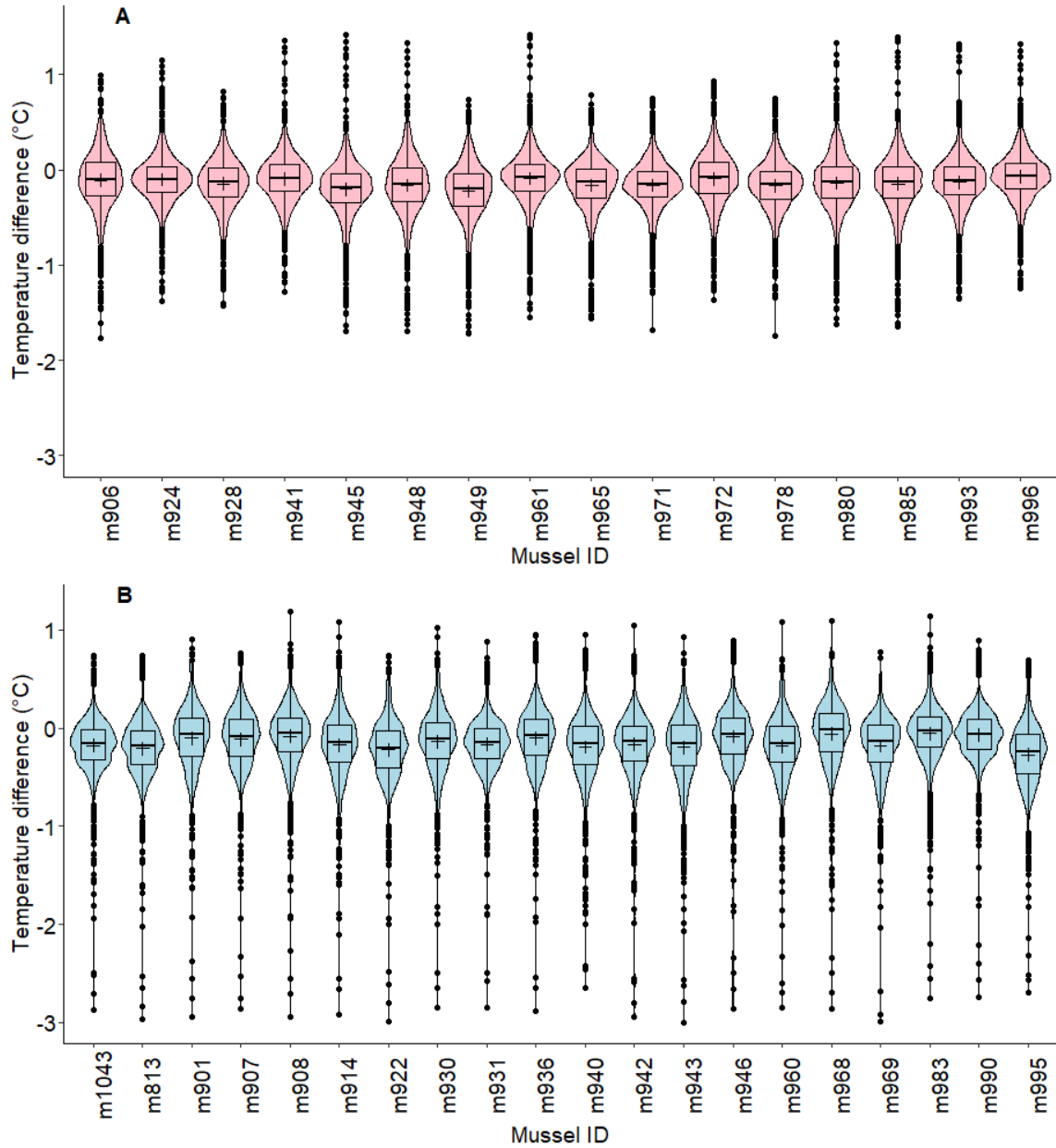


Figure 3-9: Violin-wrapped box plots of the mussel temperatures subtracted from surface water temperatures, for both CR01 (n=16, top) and SR01 (n=20, bottom). Sensor 3 water temperature data was used for both sites. Negative numbers represent cooler than surface water temperatures and positive numbers represent warmer than surface water.

Movement behaviour

Of the 40 tagged YLM, 90% were successfully recovered via the backpack RFID unit. The mussels that were not relocated (n=4) were released at CR01 and presumed to have moved to deeper water (e.g., beyond row 4) that could not be searched with the backpack RFID unit. The YLM only exhibited clear directional selection at CR01, where the release and relocation points are distinctly separate (Figure 3-10). These mussels moved almost uniformly towards deeper water in the direction of the center of the channel. SR01 YLM showed no discernible separation between release and relocation points.

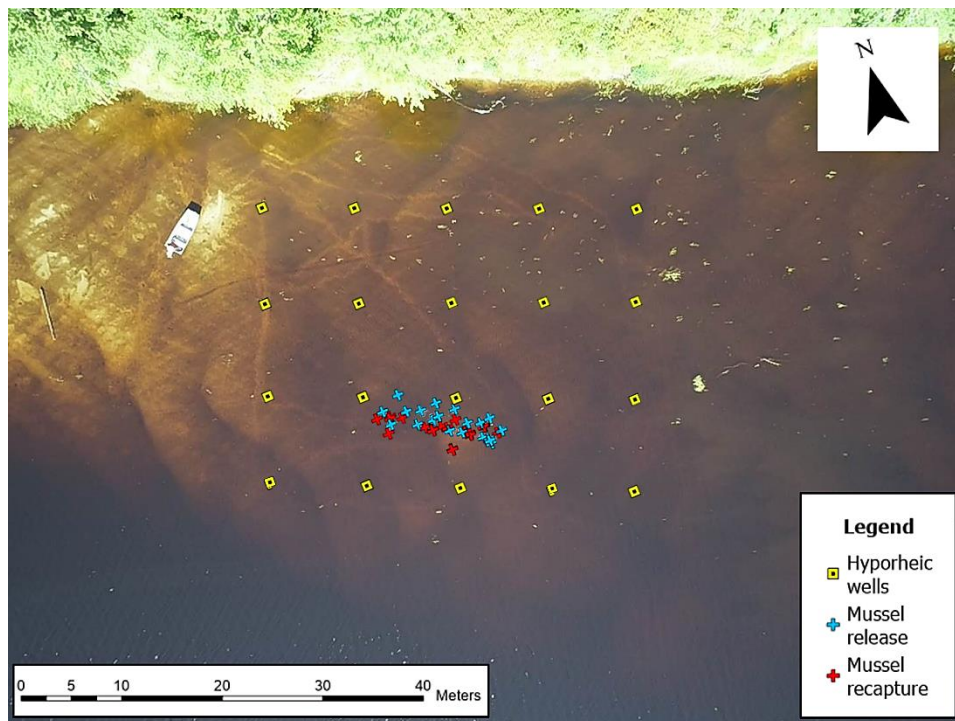
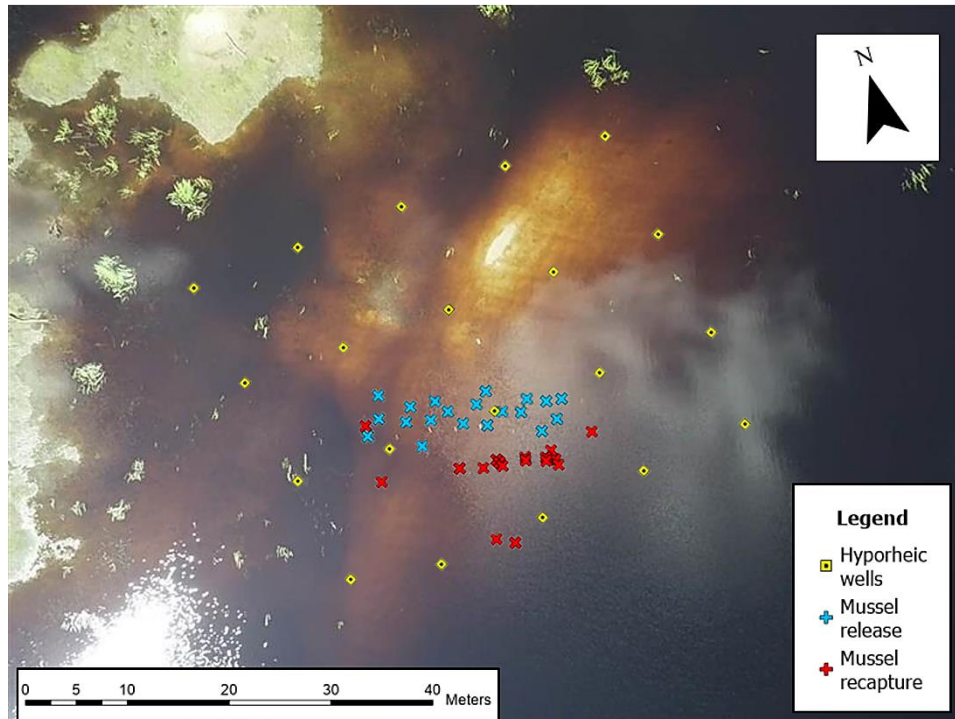


Figure 3-10: Tagged Yellow Lampmussel release versus recapture points at CR01 (top) and SR01 (bottom)

On average, YLM at CR01 were relocated 2.8x (± 3.78 m) the distance from their release points at CR01 versus SR01 (Table 3-3). Mussels at CR01 were also captured moving on the underwater cameras 5.5x as often versus SR01.

Table 3-3: Summary of horizontal movement and camera observations for CR01 and SR01. Camera observations are for both tagged and untagged mussels.

Site	Tagged mussels recovered	Mean Distance (m)	SD (m)	Max Distance (m)	Camera observations (horizontal)
CR01	16	7.53	3.78	15.39	61
SR01	20	2.70	1.60	5.96	11

Onset of horizontal movement temperatures revealed bimodal distributions for both sites, however the median onset temperature differed by ~ 3 °C and 51% of all movement observations occurred at ≥ 25 °C (n=72; Figure 3-11). There was little evidence of horizontal movement at higher water levels at SR01, outside of a single outlier, and both sites showed substantial movement when water levels drop below ~ 40 cm. CR01 showed more variation in onset of horizontal movement water level, with 45% (n=61) of observations occurring when water levels were >40 cm.

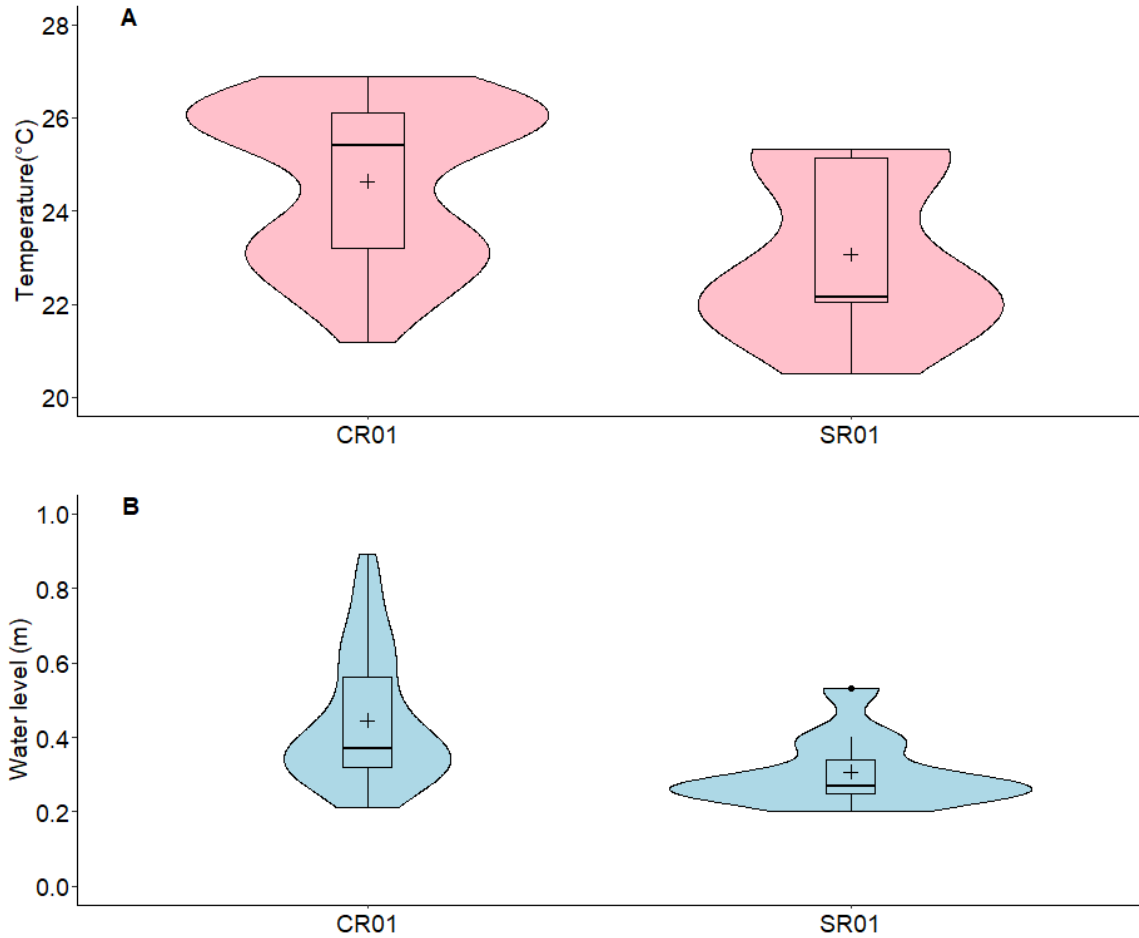


Figure 3-11: Onset surface water temperature (A) and water level (B) of horizontal movement at both CR01 (n=61) and SR01 (n=11). Onset values were taken from sensor 3 readings, at both sites, at the time when movement was first observed on the underwater cameras. Crosses represent mean values.

Drivers of movement behaviour

The multiple binary logistic regression on the onset horizontal movement data showed that water temperature, water level, and site were all statistically significant predictors of the likelihood of a YLM horizontal movement response for the model: movement ~ temperature + depth + site (Table 3-4).

The model outputs show that an increase in water temperature was positively and significantly associated with an 8 - 84% increase in the likelihood of a mussel movement response (Table 3-4). An increase in water level was negatively associated and significantly associated with a 61 to 80% decrease in the likelihood of a YLM movement response. However, the inverse would also be true where a decrease in water level would be associated with an increased likelihood of a movement response of the same magnitude. Site being significant means that YLM at CR01 are predicted to be 88 - 296% more likely to have a movement response. The intercept of the model reveals that the likelihood of a YLM movement response when $x = 0$ is significantly different from zero.

McFadden's pseudo R^2 (based on the log-likelihood kernels for the intercept-only model and the full estimated model) was calculated to be 0.17, meaning that the model is not a strong fit for the data. McFadden's pseudo R^2 values are generally lower than traditional R^2 values where values of 0.2-0.4 indicate a good-excellent fit (Louviere et al., 2010; McFadden, 1974).

Table 3-4: Summary table of the multiple binary logistic regression model: movement ~ water temperature + water level + site. A bolded value indicates a statistically significant result.

Explanatory variable	Estimate	Std error	z-value	p value
Intercept	-5.317	0.238	-22.314	<0.001
Temperature	0.347	0.134	2.590	0.0096
Water level	-1.269	0.172	-7.360	<0.001
Site (Canaan)	0.975	0.188	5.176	<0.001

Explanatory variable	β-value	Chance of response (%)	Lower CI (95%)	Upper CI (95%)
Intercept	0.005	0.50	0.20	0.79
Temperature	1.414	41.40	8.90	84.30
Water level	0.281	-71.90	-80.20	-61.0
Site (Canaan)	2.652	165.20	87.70	296.20

DISCUSSION

YLM displayed substantial differences in their movement behaviour between sites not only in terms of distance, but also in frequency of movement. The results from the multiple binary logistic regression suggest that water temperature, water level, and site have an influence on the likelihood YLM horizontal movement response, matching the prediction. The model outputs and environmental differences between sites suggest that these movement differences are being driven by differences in the temperature and water level regimes between sites. Sediment habit use of tagged mussels was similar between sites, despite differences in environmental regimes, which was unexpected. YLM remained burrowed shallowly (<10 cm) for extended periods of time punctuated by infrequent horizontal migrations.

Movement behaviour

The tagged YLM moved at CR01 travelled further distances and were observed more frequently on the underwater cameras. The increased amount of movement, along with the common directionality towards deeper water, suggests that the YLM at CR01 were being stimulated to move further and more often. The concept of stimuli driving this movement is reinforced by the fact that the most (52%, n=61) of the movement at CR01 happened during the 3-day stretch (6-9 August) when water levels were lowest (Figure 2-15). Likewise, 81% (n=11) of horizontal movement occurred during the same time frame at SR01. This 3-day stretch was also the only time water levels were so low that five of the cameras at CR01 became exposed, albeit only for ~2-3 hours, until the tide began to rise. The mean movement distance observed by YLM is within the range of movement reported by other studies (e.g., Newton et al., 2014; Peck et al., 2014), although horizontal movement tends to vary with species (Newton, 2015; Schwalb & Pusch, 2007).

Mussels have been reported to engage in horizontal migration in response to decreasing water levels (e.g., Galbraith et al., 2015; Newton et al., 2014), which was likely the stimulus responsible for the large number of movements observed during 6-9 August. However, water level alone does not appear to explain movements that occurred frequently (45%; n=61 at CR01) at water levels ≥ 40 cm, which would not be a considerable emersion threat. During these observations, high temperatures and accumulated thermal stress could explain the differences in movement behaviour between sites. Elevated water temperatures (i.e., as low as 25 °C via Ganser et al., 2015) rarely cause mortality, but mussels will experience several sub-lethal stressors (e.g., oxidative

stress, reduced hypoxia tolerance, increased energetic demands; Haney et al., 2020; Ganser et al., 2015). CR01 reported approximately double the number of growing degree days (base: 25 °C) as well as generally higher temperatures. This level of additional thermal stress could incite movement and/or refugia seeking behaviour irrespective of water level. The difference in accumulated thermal stress may also explain why there were differences in movement between sites, especially considering that SR01 had similarly low water levels to CR01, for a substantial portion of the sampling period. Variation in water temperature was similar between sites, however, temperature was generally higher at CR01. Increases in temperature (i.e., ~4-5°) have been shown to influence mortality in juvenile mussels, when they are already thermally stressed (Pandolfo et al., 2010).

Various species of mussels have been reported to horizontally migrate in response to additional stimuli. Sullivan & Woolnough (2021) reported flow variability and shell size, along with water depth, influenced mussel movement behaviour. Measures of flow (e.g., discharge, surface water velocity) were not taken at either of the study sites, but flow variability could have influenced YLM behaviour. CR01 was generally a low flow depositional area, with notable algal growths that were easily disturbed by water movement. This site was also located almost outside of the main flow of the river in the corner of a meander. SR01 was still a depositional area, however it was situated further into the main flow of Salmon River than CR01 of Canaan River. Periods of increased surface velocity were observed at SR01, where sediment was displaced from the vicinity of the underwater cameras. Sullivan & Woolnough (2021) found that mussels were more likely to move when flow variability was low and SR01 experienced at least some

periods of higher water velocity, flow may have influenced movement differences between sites. It is possible that YLM are less likely to move under greater shear stress forces than they may have experienced at SR01. Certain mussel species have been reported to seek out flow refugia (e.g., Strayer, 1999), but given the low mean distance of horizontal movement and relative homogeneity of the riverbed, that is likely not the case at SR01. Another explanation for site differences in movement could be that YLM were missed moving by the underwater cameras at SR01. However, the significantly lower mean distance moved by the tagged mussels at SR01 suggests that this is also unlikely.

YLM likely also migrate for non-stressful reasons (e.g., facilitation of fertilization) in addition to responses to environmental stimuli. There has been evidence supporting that aggregation improves fertilization in Eastern *Elliptio* (*Elliptio complanata*), a species that occurs at both study sites (Downing et al., 1993).

Binary logistic regression model

The multiple binary logistic regression analysis revealed that all three variables (water temperature, water level, and site) had a statistically significant effect on the likelihood of a YLM horizontal movement response. Water level reported the highest z-value (-7.360) among all variables, inferring it had the greatest effect. The relative importance of water level on the likelihood of a movement response is likely due to 1) the number of movement observations when water levels were <40 cm and 2) the high amount of movement observations during the period of lowest water levels (6-9 August). The negative slope and variable importance associated with water level is congruent with other studies (e.g., Galbraith et al., 2015; Gough et al., 2012; Schwalb & Pusch, 2007;

Sullivan & Woolnough, 2021), suggesting that emersion is a significant threat for YLM. Site being significant is a factor of CR01 reporting a greater number of movement observations (~5.5x) and substantial differences in the other significant variables (e.g., more observations of YLM moving at higher temperatures at CR01).

Water temperature was positively and significantly associated with an increase in the likelihood a YLM movement response. Temperature have been demonstrated to affect movement behaviour in freshwater mussels (e.g., Allen & Vaughn, 2009; Block et al., 2013; Schwalb & Pusch, 2007), however the interaction between water temperature and horizontal migration is still poorly understood. Schwalb & Pusch (2007) specifically reported that mean monthly water temperature and mean distance moved horizontally (cm/wk) were linearly related ($R^2 = 0.95$, $p < 0.01$). Given the literature supporting temperature affecting mussel movement in general, along with reporting that temperature can affect horizontal movement directly, it is not surprising that temperature was a significant variable for YLM. Unlike water level, where emersion is a more immediate mortality threat, temperature represents more of a chronic, sub-lethal stressor (Ganser et al., 2015). The accumulation of thermal stress is likely to cause refuge seeking behaviour and could certainly explain the differences in movement between sites, particularly at higher water levels.

Temperature reported the lowest z-value (2.59); however, the overall influence of temperature may have been affected by observation techniques. There was generally a delay of unknown duration between when a given YLM emerged and started moving horizontally and when it was recorded on camera. The mean duration a YLM was recorded moving was 30 (± 18.9 SD) minutes, so it is possible that onset temperatures

could be shifted equally as much, for each observation where the YLM were not witnessed emerging. Temperature would have likely played a larger role in YLM movement if temperatures did not drop suddenly during a cold snap during/after the 9th of August. Temperatures remained below 25 °C for the rest of the season and the remaining movements (20% n=72) were likely driven by low water or potentially reduced thermal tolerance, due to previously stressful conditions (i.e., high temperatures, number of GDD above 25°, and amount of movement just before the cold snap). Reduced thermal stress tolerance after previous stressful events have been reported in other ectotherms (O'Sullivan et. al, 2023).

The absence of statistical support for the logistic regression is likely due to a relatively short sampling duration (~51 days) at a limited number of sites. Increasing the sampling duration would allow for more observations of both model parameters outside of the ranges experienced during the sampling period. YLM presumably engage in horizontal migration less frequently during higher water levels (e.g., >1 m) and lower temperatures (e.g., <16 °C), which would better separate the distribution of movement and no movement data points. There is little evidence in the data at CR01 to show what conditions are conducive to lower movement activity since there were no extended periods of time without any movement observations. Increasing the number of sites would increase the breadth of temperature and water level regimes associated with YLM movement (or lack thereof) and likely improve the model fit. By adding one site, the model fit in this chapter was improved by 5% compared to its previous iteration using CR01 only data (See Chapter 2: Drivers of movement). Along with additional sites and longer sampling duration, the fit of the model would likely be improved by adding other

variables that were not measured but are ecologically/biologically important to freshwater mussels (e.g., discharge or dissolved oxygen).

Mussels have been reported to move more during periods where the flow variability (e.g., Sullivan & Woolnough, 2021) and may have influenced movement differences between CR01 and SR01. Observations of periods of higher flow at SR01, as well as a more direct position further into the flow of Salmon River, may indicate that there were differences in discharge between sites. Differences in flow could indicate differences in dissolved oxygen, however CR01 was regularly (i.e., ~twice per day) inundated with water due to the tidal nature of Canaan River. Water levels generally fluctuated ~30 to 40 cm twice per day and that mixing was likely sufficient to prevent hypoxic conditions. However, it is possible that moderate, non-hypoxic differences and/or variability in dissolved oxygen could influence YLM movement behaviour.

Ultimately, there has yet to be an instance where freshwater mussel movement be explained effectively by a single highly influential variable (Amyot & Downing, 1997; Newton et al., 2014; Schwalb & Pusch 2007, Sullivan & Woolnough 2021). This suggests that movement is likely a result of highly complex interactions between environmental parameters (e.g., temperature, hydraulics, and nutrients) and species-specific tolerances and/or adaptations. The data from this research implies that differences in horizontal movement are likely site specific, depending on environmental stressors/stimuli, at least for YLM in the lower W|SJR system.

Thermal habitat selection

In general, tagged YLM temperatures were slightly cooler than the surface water, while warmer than the 10 cm sediment temperature. Along with observations during tagging, relocation, and camera footage, this data suggests that YLM stay burrowed a few centimeters into the sediment. If YLM were burrowing deeper than a few centimeters, their temperatures would match the 10 cm sediment and/or dips from near surface water temperatures down to those found at 10 cm. If the mussels were consistently on the surface, their temperatures would more consistently match surface water or oscillate between sediment and surface temperatures. The depth of burrowing of observed in YLM is consistent with observations of other species, where they were usually found burrowed <10 cm deep (Balfour & Smock, 1995; Schwalb & Pusch, 2007). The burrowing behaviour observed at both sites is consistent with historical YLM observations in both New Brunswick (e.g., Sabine et al., 2004) and Nova Scotia (COSEWIC, 2004).

By maintaining this sediment position, YLM are experiencing a narrower range in temperature than if they had simply been on the surface. When temperature rises or falls, the thermal inertia within the sediment is greater than at the surface, thus insulating the mussels from extremes. The magnitude of insulation that YLM received was consistent across tagged mussels within sites, but mussels at SR01 received almost double the effect on occasion. The differential in temperatures YLM experienced from being burrowed (i.e., 1-1.75 °C at CR01 and 1-3 °C at SR01, relative to surface water) are similar to temperatures shown in recent studies. Newton et al. (2013) demonstrated that the magnitude of temperature difference within the first 15 cm of sediment of known mussel beds was generally < 3 °C.

While a temperature difference of 1-3 °C may not seem statistically significant, there is evidence to support that it may be biologically significant for mussels. Ganser et al., (2015) and Said & Nassar (2022) both reported that freshwater mussels can experience sub-lethal thermal stress (i.e., respiratory pattern alteration, lowered hypoxia tolerance, oxidative stress, reduction in adenosine triphosphate production) at temperatures as low as 25 °C. Both CR01 and SR01 reported maximum temperatures of ~28 °C, so a differential of 1-3 °C could bring YLM from a very stressful temperature down to one that is more tolerable. Pandolfo et al., (2010) also found that the mean difference in lethal temperature for 5% of juvenile mussels versus 50% was ~5 °C. If a change of 5 °C can cause an order of magnitude increase in mortality, it is likely that a 1-3 °C differential is biologically significant for adult YLM. Maintaining their sediment position would ultimately provide a measure of sub-lethal thermal stress relief, especially during extremes in temperature.

YLM burrowing behaviour would also provide a reduction of energetic demand (relative to being on the surface of the sediment) due to the increased metabolic activity of higher temperatures on ectothermic organisms (Haney et al., 2020). Increased energetic demand from higher temperatures, particularly if there are not sufficient nutrients to compensate, has been shown to be a stressor, but its effects are species dependent (Haney et al., 2020).

Between the temperature data and field/camera observations, it is apparent that YLM spend most of their time burrowed into the sediment. These observations only apply to these sites and the conditions (i.e., temperature and depth regimes) present during the monitoring period. YLM within other rivers/sites may behave differently, although, the

burrowing behaviour exhibited within this study matches historical accounts for this species (COSEWIC, 2004; Sabine et al., 2004). The patterns observed (i.e., YLM consistently being burrowed) were consistent across sites, however the degree of thermal insulation from doing so appeared to be different. The greater temperature differential between surface water and sediment at SR01 may have influenced YLM horizontal migration. The mussels at SR01 may have received sufficient insulation/refuge that allowed them to remain where they were released, as opposed to selecting for new habitat like at CR01.

The seasonal ecology of YLM is still poorly understood and relatively unstudied. These mussels may spend more time at the sediment surface during relatively cooler periods (e.g., May-June, September) or at greater depths than were observed. While YLM were buffered from extremes in temperature, there are other reasons that they could be burrowing such as predator avoidance, photosensitivity, or reduced shear stress.

Future work

Overall, this work suggests that river/site specific differences in temperature and water level can drive movement differences in YLM. Due to the openness of the systems at SR01 and CR01, there are numerous confounding factors that were not measured or accounted for. Future research would include monitoring for key environmental variables that have been shown to influence mussel ecology (e.g., discharge, dissolved oxygen). Measuring additional variables would allow for a greater inference into the mechanistic driver(s) of YLM movement and improve predictive modelling efforts. Additional monitoring outside of the observed conditions (i.e., different seasons, temperatures, and

water levels) would further the understanding of how temperature and water level influence migration in YLM.

Monitoring additional rivers could also provide a gradient of variation in temperature/water level regimes. It would also be insightful to look at a comparison of temperature and water level regimes at sites that have YLM and those that do not. Certain thresholds or extremes in water level or temperature may limit YLM distribution or survival. Studying additional species would allow for comparison of the impact of temperature and water level across native W|SJR mussels. The movement response would likely differ among species and would allow for the identification of species more vulnerable to either stressor. Different species may also be stressed by different environment variables, so monitoring variables such as discharge and dissolved oxygen would be very informative. Overall, improving the understanding of fundamental YLM ecology will be beneficial to its long-term conservation and management, within the lower W|SJR catchment.

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Chapter 4 : GENERAL DISCUSSION

Under the Federal Fisheries Act, when any human activity that may alter an aquatic environment is proposed (e.g., bridge construction or culvert installations), Fisheries and Oceans Canada requires the proponent to mitigate impacts for all fish (Government of Canada Fisheries Act, 1985) with added emphasis for the care of species-at-risk (SAR) within the construction impact zone (Government of Canada Fisheries Act, 1985). If the proposed infrastructure activity is within a SAR-mapped critical habitat, additional work (e.g., mussel relocation) is required to mitigate effects to prevent direct harm to any present SAR during in-water works. Proponents can experience significant delays to the start of construction since the permit approval is dependent on the mitigation of impacts on SAR.

To aid in the identification of sites that would require SAR consideration, and the expedition of the permitting process, the New Brunswick Department of Transportation and Infrastructure (NB DTI) has partnered with researchers from the Canadian Rivers Institute (CRI). They are working to develop a science-based, weight-of-evidence (WOE) risk assessment framework to establish the presence/absence of SAR aquatic species. The WOE framework will be developed using SAR habitat, behaviour, and distribution research done at three spatial scales (i.e., micro, meso, and macro).

My study focused on the micro-scale aspect of the WOE framework, where I examined the summer (i.e., July-September) thermal habitat selection and movement behaviour of the Yellow Lampmussel (*Lampsilis cariosa*; YLM). I set out to accomplish the following objectives outlined in Chapter 1:

- 1) Determine the thermal habitat uses and depth of sediment burrowing behaviour in YLM.
- 2) Compare YLM horizontal movement behaviour between sites/ivers and explore the influence of temperature and water level on movement.

Objective 1

I addressed the first objective in Chapter 2 by illustrating that tagged YLM experienced temperatures that were moderately cooler than surface water, while still being warmer than 10 cm sediment temperatures. Inferring that YLM generally stays burrowed within the first few centimeters of the riverbed sediment. The temperature data was confirmed using underwater camera observations that captured YLM consistently in the burrowed position, for weeks at a time. The regularity of burrowing in YLM also matches historical accounts from both NB (e.g., Sabine et al., 2004) and Nova Scotia (COSEWIC, 2004). Additionally, the cameras also showed that immediately upon cessation of horizontal migration, burrowing occurred, providing further evidence that this is a regular or 'default' position for YLM, at least during the summer. In Chapter 3, I compared vertical habitat selection based upon temperature between sites/ivers and found all tagged YLM behaved in a similar fashion.

The thermal insulation of YLM burrowing behaviour was initially described in Chapter 2. I demonstrated that the tagged YLM do receive a measure of thermal insulation from surface water by burrowing into the sediment, particularly during

extremes in temperature. The tagged YLM at CR01 received 1-1.75 °C insulation numerous times throughout the summer. When the insulation effect was compared to SR01 in Chapter 3, I illustrated that not only was this effect consistent within sites, but also that YLM at SR01 received almost twice the insulation of CR01, on occasion. The magnitude of thermal insulation provided by riverbed sediments was site/river specific and was consistent with findings from similar studies (i.e., <3 °C within 15 cm under known mussel beds; Newton et al., 2014).

Objective 2

In Chapter 2 I described how tagged YLM at CR01 moved considerable distances (i.e., mean 7.53 [\pm 3.78 SD] m, max 15.39 m), for relatively sessile organisms. I argued that this behaviour was unlikely due to the tags themselves, since 1) there were no substantial differences in movement (i.e., frequency or duration) between tagged and untagged mussels and 2) the tag-to-bodyweight ratio used in this study was less than half that of artificial shell structures used in other studies, where no significant effects were reported (e.g., Levine et al., 2014). In the same chapter, I presented logistic regression models that showed that both water level and temperature had a significant effect on the likelihood of YLM engaging in horizontal migration.

When compared to SR01 in Chapter 3, it became apparent that YLM moved greater distances and moved more frequently at CR01. I attributed differences in movement to differences in environmental regimes/stimuli between sites. Water levels varied substantially more and reached lower minimums at CR01, to the point where several underwater cameras became emersed. In addition, >50% of the movement observations at

CR01 were during a 3-day stretch of the lowest water levels. CR01 surface water maintained a higher temperature than SR01. CR01 YLM experienced more cumulative thermal stress, accumulating 10 additional growing degree days above 25 °C, a temperature shown to cause thermal stress in other mussel species (Ganser et al., 2015). My modelling efforts in this chapter revealed that site was also significant, along with temperature and water level. YLM at CR01 were calculated to be 87.7 – 296.2% more likely to have a horizontal movement response, most likely driven by the differences in environmental regimes/stimuli between sites. I concluded that my data strongly suggests that site/river specific environmental regimes can result in substantially different movement behaviour in YLM.

Seeking stability: the selection for stable environments

In general, we know that freshwater mussels prefer stability in ecologically significant abiotic variables such as riverbed substrate (e.g., Allen & Vaughn, 2010; Morales et al., 2006; Randklev et al., 2019; Strayer, 1993) and flow (e.g., Strayer, 1993; Sullivan & Woolnough, 2021). Presumably then, it is likely that instability (i.e., greater variation or extremes) in other important abiotic variables could elicit a movement response or refuge seeking behaviour. There are certain thresholds in both water temperature (e.g., ~25 °C in Ganser et al, 2015) and water level (e.g., water level that results in emersion or near emersion; Galbraith et al., 2015; Newton et al., 2014) that have been shown to elicit movement in mussels, however their effects likely vary depending on species.

With YLM in the lower W|SJR, I have observed substantial differences in movement behaviour between sites/ivers. I argue that this movement likely is not being caused by the tags on the mussels and that the distance moved does not appear to be determined by mussel sex or length. The substrate grain/particle size, which could impede or affect movement, was virtually identical between sites and unlikely to have played a role in movement differences. The differences in movement between sites can be feasibly explained by differences in both water temperature and water level. Canaan River is highly influenced by tidal forces where water levels can fluctuate 30-40 cm twice daily. YLM that were in comfortably deep water can find themselves threatened by emersion only a few hours later. The water temperature in CR01 is generally higher than SR01 and the YLM residing there were exposed to higher stressful temperatures (i.e., ≥ 25 °C) for longer. There were simply greater acute (water level) and chronic (water temperature) stressors at CR01; it was a more dynamic, less stable site than SR01. YLM at CR01 were observed to move greater distances and move more often, which I conceptualize were undertaken to find more stable environments. For each type of observed movement behaviours (i.e., vertical positioning and horizontal migration), YLM have selected for more stable environments.

YLM at both sites burrowed frequently and remained burrowed essentially whenever they were not moving horizontally. Burrowing provides greater stability due to the increased thermal inertia of the sediment versus the surface water. By remaining within the sediment, the YLM are exposed to a narrower range of temperatures, thus providing a measure of insulation from extremes. At SR01, the insulation effect was almost double that at CR01, which may have reduced the impetus to move horizontally. Additionally,

burrowing is a response some mussel species use to avoid/mitigate emersion (e.g., Newton et al., 2014), so this behaviour may provide a measure of stability in against extremes in water level as well. If vertical migration can mitigate receding water levels, why engage in horizontal migration at all? Why not just burrow deeper? For example, Schwalb & Pusch (2007) reported *Unio tumidus* burrowing as deep as 20 cm into the substrate during the summer months. It is likely that there is an energetic and/or respiratory tradeoff between burrowing deeper into the substrate and the thermal insulation YLM receives. While YLM would receive greater thermal and emersion protection from burrowing deeper, dissolved oxygen and food availability (i.e., not being able to filter effectively while buried) would be lower. YLM likely need to maintain contact with the surface water to access enough oxygen and/or sustenance, which would deplete as they burrowed deeper into the sediment.

At CR01, most of the YLM horizontally migrated from shallower water to deeper water. The near uniformity of directional habitat selection towards deeper water, nearer to the center of the channel, suggests a strong ecological impetus to move to that area. By selecting for deeper water, they were likely selecting for greater stability and a less extreme environment. Movement towards deeper water would mitigate the risk of emersion by raising the minimum water level, while also (depending on water depth) offering greater thermal inertia and thus, a more stable environment.

Temperature and water level are both ecologically important factors to YLM, so it is not surprising for them to both be calculated as statistically significant in the logistic regression. However, I cannot confirm with any confidence whether either variable is directly responsible for causing the movement differences between sites. It is likely the

complex interaction of both variables, as well as numerous confounding factors, that are driving the movement behaviours observed at my sites. What variables are directly causing the movement is not clear, but it is apparent that their movement ecology is more complex than a simple cause-effect relationship. Overall, the movement differences between my sites are likely a result of the instability and/or extremes in environmental parameters – measured or not. CR01 was a much more dynamic site, one prone to greater fluctuations in both ecologically and biologically important environmental parameters.

Research significance and implications

My research will ultimately add to the growing body of knowledge of freshwater mussels and their ecology. Along with its meso and macro counterparts, my work will help inform the WOE risk assessment framework so that it can better establish presence/absence of YLM and assist in the expedition of NB DTI construction projects (e.g., culvert installation, bridge construction/repair) where these SAR are unlikely to be found.

This thesis is the first work to examine the relationship between temperature, water level and YLM movement behaviour in the lower W|SJR. As such, it is shedding some new light on the basic ecology of YLM, a knowledge gap that will be useful for long-term management and conservation of this species, especially in a changing climate.

During this study, I used a novel approach of attaching temperature sensors directly to YLM to track their sediment positioning as well as thermal history during the summer months. Insights gained throughout my research (e.g., endobenthic behaviour, tagging techniques, high abundance site locations, site/river specific plasticity in movement

behaviour) will be useful to managers, aquatic scientists, and regulators that are stakeholders in the future conservation of YLM.

Future Work

Ultimately, this work suggests that site/river specific differences in environmental parameters have the potential to cause movement differences in YLM. Due to the openness of the systems that were studied, there are certainly confounding factors that were not measured or accounted for. Future research would include monitoring for additional environmental variables that have been shown to influence mussel ecology (e.g., a measure of flow and/or flow variability, dissolved oxygen). There have been some developments looking at complex hydraulic variables (e.g., relative shear stress, Froude numbers, and Reynolds numbers) that have shown more predictive power for mussel abundance and richness than simpler variables (Allen & Vaughn, 2010). Measuring additional variables would allow for a greater inference into the mechanistic driver(s) of YLM movement and likely improve predictive modelling efforts.

Monitoring additional rivers/sites could also provide a gradient of variation in temperature/water level regimes. This data could isolate certain thresholds that may be limiting to their survival and/or distribution. However, we are currently limited by the relatively few abundant (i.e., measurable density) YLM sites known to exist within the lower W|SJR.

Fully comprehending the influence of temperature (i.e., thermal maximums, tolerances, and sub-lethal stressors) on YLM will only grow in importance as the global climate continues to change and river temperatures continue to rise. Improving our

understanding of fundamental YLM ecology will be crucial to its long-term conservation and management within the W|SJR.

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Appendix

The following tables are the results from the hyporheic grid monitoring at both CR01 and SR01. Summary metrics were calculated for the duration of deployment for each grid: 12 July to 12 September for CR01, and 20 July 20th to 1 September for SR01.

Table A-1: Summary metrics for hyporheic grid monitoring temperatures (10 cm depth). Well numbers 1-20 were deployed at CR01, and wells number 21-40 were deployed at SR01. N/a values represent corrupted data from that logger. Max = Maximum temperature, Min = Minimum temperature, SD = standard deviation, CV = coefficient of variation.

Well #	Min (°C)	Max (°C)	Range (°C)	Mean (°C)	Median (°C)	SD (°C)	CV (%)
1	20.42	25.9	5.48	22.73	22.33	1.29	5.68
2	19.95	24.45	4.5	21.97	21.66	1.02	4.66
3	20.42	27.08	6.65	23.24	22.91	1.55	6.67
4	20.71	26.29	5.58	23.04	22.62	1.37	5.95
5	20.62	26.2	5.58	22.98	22.53	1.39	6.05
6	20.42	26.1	5.67	22.88	22.43	1.36	5.93
7	20.42	25.81	5.38	22.73	22.24	1.32	5.82
8	20.62	26.78	6.17	23.31	22.81	1.5	6.43
9	20.42	26.59	6.16	23.11	22.62	1.49	6.46
10	20.62	26.59	5.97	23.2	22.72	1.47	6.34
11	20.42	26.29	5.87	22.96	22.53	1.41	6.15
12	20.42	26.29	5.87	22.95	22.43	1.42	6.18
13	20.52	26.49	5.97	23.13	22.62	1.46	6.32
14	20.23	25.9	5.67	22.66	22.14	1.37	6.05
15	20.33	26.39	6.06	23.04	22.53	1.48	6.43
16	20.42	26	5.58	22.7	22.33	1.31	5.78
17	20.42	25.71	5.28	22.58	22.24	1.23	5.46
18	20.52	26	5.48	22.79	22.33	1.35	5.91
19	20.42	25.81	5.38	22.68	22.24	1.33	5.88
20	n/a	n/a	n/a	n/a	n/a	n/a	n/a
21	19.47	26.20	6.72	21.82	21.47	1.50	6.86
22	19.47	26.00	6.53	21.80	21.38	1.51	6.92
23	19.47	25.71	6.24	21.76	21.38	1.52	6.98
24	19.47	25.61	6.14	21.71	21.28	1.49	6.88
25	19.57	25.71	6.14	21.74	21.28	1.53	7.02
26	19.57	25.71	6.14	21.76	21.38	1.48	6.81
27	19.47	25.51	6.04	21.67	21.28	1.48	6.84
28	19.38	25.61	6.23	21.67	21.28	1.51	6.96
29	19.57	25.51	5.95	21.73	21.28	1.47	6.77
30	19.38	25.71	6.33	21.67	21.28	1.54	7.12
31	19.57	25.51	5.95	21.70	21.28	1.46	6.73
32	19.57	25.42	5.85	21.62	21.19	1.45	6.73
33	19.47	26.10	6.63	21.85	21.47	1.62	7.41
34	19.38	25.90	6.53	21.75	21.38	1.57	7.23
35	19.47	25.51	6.04	21.61	21.19	1.48	6.86

36	19.38	26.20	6.82	21.86	21.47	1.66	7.62
37	19.47	25.61	6.14	21.65	21.19	1.51	6.99
38	19.47	25.71	6.24	21.72	21.28	1.53	7.04
39	n/a	n/a	n/a	n/a	n/a	n/a	n/a
40	19.19	26.10	6.91	21.78	21.47	1.62	7.42

Table A-2: Summary metrics for hyporheic grid monitoring temperatures (20 cm depth). Well numbers 1-20 were deployed at CR01, and wells number 21-40 were deployed at SR01. N/a values represent corrupted data from that logger. Max = Maximum temperature, Min = Minimum temperature, SD = standard deviation, CV = coefficient of variation.

Well #	Min (°C)	Max (°C)	Range (°C)	Mean (°C)	Median (°C)	SD (°C)	CV (%)
1	20.33	24.93	4.6	22.28	21.86	1.11	4.98
2	20.42	25.22	4.8	22.53	22.14	1.17	5.21
3	20.52	26.39	5.87	22.99	22.53	1.42	6.16
4	20.52	25.61	5.09	22.61	22.14	1.25	5.52
5	20.62	25.71	5.09	22.74	22.24	1.29	5.67
6	20.52	25.32	4.8	22.51	22.05	1.19	5.28
7	20.42	25.32	4.9	22.48	22.05	1.22	5.41
8	20.71	26	5.29	22.95	22.43	1.35	5.86
9	20.52	25.61	5.09	22.67	22.14	1.3	5.72
10	20.62	25.71	5.09	22.74	22.24	1.31	5.78
11	20.52	25.61	5.09	22.61	22.14	1.26	5.59
12	20.52	25.51	4.99	22.58	22.05	1.27	5.62
13	20.62	25.71	5.09	22.73	22.24	1.3	5.73
14	20.23	24.93	4.7	22.22	21.76	1.2	5.4
15	20.52	25.61	5.09	22.66	22.14	1.28	5.67
16	20.42	25.51	5.09	22.51	22.14	1.2	5.35
17	20.23	24.84	4.6	22.16	21.76	1.09	4.91
18	20.33	25.03	4.7	22.33	21.86	1.18	5.29
19	20.23	24.93	4.7	22.22	21.76	1.18	5.3
20	20.42	25.42	4.99	22.52	22.05	1.26	5.58
21	19.57	24.74	5.17	21.34	20.90	1.24	5.82
22	19.57	24.55	4.98	21.30	20.81	1.23	5.78
23	19.57	24.84	5.27	21.42	20.90	1.32	6.17
24	19.57	24.45	4.88	21.25	20.81	1.24	5.86
25	19.66	25.13	5.46	21.55	21.09	1.38	6.40
26	19.57	25.71	6.14	21.76	21.38	1.48	6.81
27	19.57	24.55	4.98	21.26	20.81	1.23	5.78
28	19.57	24.93	5.36	21.45	21.00	1.34	6.25
29	19.47	24.55	5.07	21.23	20.71	1.26	5.95
30	19.57	24.74	5.17	21.39	20.90	1.30	6.08
31	19.57	24.45	4.88	21.24	20.71	1.22	5.76
32	19.47	23.97	4.49	21.02	20.52	1.11	5.29
33	19.57	25.03	5.46	21.44	21.00	1.37	6.40
34	19.57	24.93	5.36	21.38	20.90	1.34	6.28
35	19.47	24.55	5.07	21.24	20.71	1.26	5.95

36	19.57	25.61	6.04	21.71	21.28	1.49	6.84
37	19.57	25.13	5.56	21.49	21.00	1.37	6.39
38	19.57	24.93	5.36	21.44	21.00	1.35	6.28
39	19.57	24.84	5.27	21.38	20.90	1.30	6.09
40	19.28	24.93	5.65	21.35	21.00	1.38	6.49

Table A-3: Summary metrics for hyporheic grid monitoring temperatures (40 cm depth). Well numbers 1-20 were deployed at CR01, and wells number 21-40 were deployed at SR01. N/a values represent corrupted data from that logger. Max = Maximum temperature, Min = Minimum temperature, SD = standard deviation, CV = coefficient of variation.

Well #	Min (°C)	Max (°C)	Range (°C)	Mean (°C)	Median (°C)	SD (°C)	CV (%)
1	19.95	24.45	4.5	21.97	21.66	1.02	4.66
2	19.76	24.16	4.4	21.9	21.57	1.02	4.67
3	20.62	25.81	5.19	22.76	22.33	1.29	5.66
4	20.33	25.13	4.8	22.4	22.05	1.16	5.17
5	20.33	25.13	4.8	22.4	21.95	1.2	5.36
6	20.04	24.64	4.6	22.14	21.76	1.1	4.96
7	20.23	24.93	4.7	22.32	21.95	1.13	5.06
8	19.95	24.55	4.6	22.1	21.66	1.12	5.07
9	20.33	25.13	4.8	22.41	21.95	1.19	5.3
10	20.33	25.13	4.8	22.39	21.86	1.21	5.41
11	20.23	25.03	4.79	22.28	21.86	1.17	5.26
12	20.23	24.93	4.7	22.31	21.86	1.17	5.25
13	20.04	24.64	4.6	22.09	21.66	1.14	5.16
14	19.47	23.87	4.4	21.55	21.19	1.02	4.72
15	20.52	25.61	5.09	22.66	22.14	1.28	5.67
16	19.76	24.16	4.4	21.78	21.47	1	4.58
17	19.57	23.87	4.3	21.6	21.28	0.97	4.47
18	19.57	23.97	4.4	21.7	21.38	1	4.59
19	19.19	23.48	4.3	21.33	21	0.96	4.5
20	19.57	23.97	4.4	21.64	21.28	1.03	4.75
21	19.57	24.06	4.50	21.13	20.71	1.10	5.19
22	19.57	23.77	4.21	20.99	20.52	1.06	5.06
23	19.47	23.68	4.21	20.92	20.42	1.06	5.08
24	19.66	24.06	4.40	21.13	20.62	1.11	5.24
25	19.66	24.55	4.88	21.32	20.81	1.25	5.85
26	19.57	23.87	4.30	21.07	20.62	1.07	5.06
27	19.47	23.68	4.21	20.88	20.42	1.04	4.99
28	19.57	24.26	4.69	21.17	20.71	1.20	5.66
29	19.38	23.77	4.40	20.89	20.42	1.10	5.27
30	19.47	23.87	4.40	20.99	20.52	1.11	5.29
31	19.57	23.68	4.11	20.93	20.52	1.03	4.92
32	19.28	22.53	3.24	20.38	20.04	0.81	3.96
33	19.57	24.35	4.78	21.18	20.71	1.22	5.74
34	n/a	n/a	n/a	n/a	n/a	n/a	n/a
35	19.38	23.39	4.01	20.71	20.23	1.02	4.94

36	19.47	24.74	5.27	21.32	20.81	1.33	6.26
37	19.66	24.55	4.88	21.31	20.81	1.24	5.81
38	19.57	24.35	4.78	21.16	20.62	1.20	5.69
39	19.38	23.97	4.59	20.93	20.42	1.14	5.46
40	19.38	23.77	4.40	20.84	20.42	1.11	5.33

Curriculum Vitae

Candidate's full name: Darren Michael Andrew Greeley

Universities attended:

University of New Brunswick, 2021, Bachelor of Science in Environment and Natural Resources

Conference Presentations:

Gray, M.A., S.A. Cusack, D.M. Greeley, and E.B. Noël. 2021. An integrative framework to predict the presence of species-at-risk mussels in New Brunswick. 4th Biennial Canadian Freshwater Mollusc Research Meeting. 7-8 December, Burlington, ON.

Greeley D. M., Gray M.A., O'Sullivan A.M., and Wegscheider B. 2023. Thermal habitat selection and movement behaviour in Yellow Lampmussel (*Lampsilis cariosa*) in the lower Wolastoq | Saint John River catchment, New Brunswick. Science Atlantic Environment Conference. 25 March, Corner Brook, NFLD.

Greeley D. M., Gray M.A., O'Sullivan A.M., and Wegscheider B. 2023. Thermal habitat selection and movement behaviour in Yellow Lampmussel (*Lampsilis cariosa*) in the lower Wolastoq | Saint John River catchment, New Brunswick. Inaugural Society of Canadian Aquatic Sciences. 25-27 February, Montreal, QC.

Greeley D. M., Gray M.A., O'Sullivan A.M., and Wegscheider B. 2023. Movement behaviour of Yellow Lampmussel (*Lampsilis cariosa*) in the lower Wolastoq | Saint John River catchment, New Brunswick. Yellow Lampmussel Working Group. 24 March, Fredericton, NB.