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Assessing metabolic rate and post-tagging recovery in juvenile fish

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Abstract

Juvenile fish play a crucial role in the health of aquatic ecosystems, serving both as the foundation for future adult populations and as a valuable food source. Studying the juvenile life stage of fish using acoustic telemetry is inherently challenging due to their small size and associated difficulties in tracking and data collection. Recent advances in telemetry, including the miniaturization of tags, have enabled researchers to investigate previously understudied size classes of fish. Tagging fish can introduce sublethal effects that alter their physiology and behaviour, ultimately biasing the collected data; this effect is exacerbated in smaller-bodied fish, which are generally more sensitive to tagging procedures. Our study tested whether tagging juvenile brook trout (*Salvelinus fontinalis*) of two size classes (10–12 and 13–16 cm fork length, $n = 80$) with a LOTEK JSATS PinTag (0.22 g, mean tag burden 1.11% of body mass, range 0.54–2.00%) impacted their oxygen consumption rates ($\dot{M}_{O_2}$) over the 2 days following tagging. Handling and tagging caused no mortalities, although we observed a nearly significant effect ($p = 0.06$) of the experimental group (control, anaesthesia, surgery, tagged) on standard metabolic rate (SMR). This was driven by a 7% decrease in the SMR of the tagged animals, which were significantly different from the control group when compared directly ($p = 0.02$). This marginal effect in standard metabolic rate, combined with the absence of significant effects on maximum metabolic rate, aerobic scope and post-exercise recovery, suggests that the JSATS PinTag does not impair the aerobic metabolic capacity of fish of this size. Our findings indicate that neither the surgery procedure nor the presence of the tag limit the brook trout's capacity for burst movement, which is highly relevant for predator-evasion responses after release in the wild. In addition, we evaluated wound recovery and suture retention over a 3-week period using either braided or monofilament suture material. While braided sutures caused more immediate irritation and were more prone to fungal growth, their quicker expulsion may reduce long-term discomfort compared to monofilament sutures. Determining the optimal suture type may ultimately depend on study duration and objectives, and longer-term monitoring could clarify which material best supports overall healing.

KEYWORDS
acoustic telemetry, aquatic respirometry, salmonids, sublethal effects

1 | INTRODUCTION

Acoustic telemetry is a central tool in exploring migratory patterns, home ranges and physiological or behavioural parameters of aquatic animals (Crossin et al., 2009; Jacoby & Piper, 2023; Welch et al., 2024). This approach has been especially valuable for salmonids, which hold global ecological, economic and cultural significance yet continue to face population declines (Chaput, 2012; Gibson, 2017) despite extensive conservation efforts (Dauwalter et al., 2020; Muhlfeld et al., 2019; Solomon et al., 2003). Traditionally, telemetry studies focused on larger fish because the size of the tag components limited the implantation of tags into smaller-bodied animals. This, in turn, creates an important knowledge gap regarding the behaviour, ecological requirements and vulnerabilities of smaller fish. In recent years, developments in technology have enabled the construction of increasingly miniaturized tags, opening new research opportunities for previously inaccessible size classes of fish (Cooke et al., 2013; Lennox et al., 2025). The ability to study younger life stages is crucial for a comprehensive understanding of the environmental requirements for any given species and may lead to the development of more integrative management options in the future.

When handling fish and performing tagging operations, an important consideration is the immediate and long-term stress induced by the procedure, which can affect the fish's behaviour, fitness and survival (Haas et al., 2023; Heim et al., 2024; Robertson et al., 2003). This is of particular concern for smaller, lighter fish, where even small tags represent a potentially great burden. The long-term effects of acoustic tag implantation have been extensively studied, with research often focusing on growth, tag retention and survival (Matley et al., 2024). Short-term sublethal effects of tagging are particularly relevant because they may alter the behaviour of the fish, making tagged fish unrepresentative of wild counterparts, leading to biased data and potentially resulting in misguided management decisions. These short-term effects may arise directly from the tag burden, but also indirectly from the surgery procedure and materials used.

Stress is likely to induce changes in energy use (Wendelaar Bonga, 1997). For instance, Moore et al. (1990) found that tagging led to higher opercular beat rates in Atlantic salmon (*Salmo salar*) during the first 70 min after tagging, but noted no other immediate behavioural changes. These changes in energy use are reflected in the mass-specific oxygen consumption $\left(\dot{M}_{O_2}\right)$ of the fish (Davis & Schreck, 1997). Intermittent-flow respirometry is a practical approach to quantify $\dot{M}_{O_2}$, allowing for the determination of short-term effects of tagging, short-term post-tagging recovery and the ability of the subject to survive in the immediate hours/days after tagging. We used a customized intermittent-flow respirometry system to determine the immediate effects of tagging and handling on the $\dot{M}_{O_2}$ rates of two size classes of juvenile fish, using brook trout (*Salvelinus fontinalis*) as a model salmonid species. We hypothesize that tagging will lead to a short-term stress response in juvenile fish, which will be reflected by an increase in their $\dot{M}_{O_2}$ following the tagging procedure. Further, we hypothesize that the magnitude of this impact will be dependent on the size of the juvenile fish.

Similarly, the physiological response to wound closure materials can influence post-tagging recovery. Together, both tag burden and the choice of surgery materials may cause discomfort and introduce stress in recently tagged animals (Jepsen et al., 2001; Lower et al., 2005). Suture type in particular contributes to wound recovery time, infection, inflammation and tag retention (Ivasauskas et al., 2012). Sutures are generally either monofilament or braided multifilament material; these differences in composition affect multiple dimensions of wound recovery on a species-level and environment-specific basis (Cooke et al., 2003; Hurty et al., 2002). Braided sutures are considered less cumbersome to work with, which may enable shorter surgeries (Wagner & Cooke, 2005). However, the braided structure of these sutures facilitates bacterial wicking (Wagner et al., 2000). In contrast, monofilament sutures are less likely to induce bacteria-related inflammation due to their non-capillary structure (Wagner et al., 2000), but may be retained after the wound is healed. Gilliland (1994) linked wound recovery to suture type, with monofilament suture absorbing more slowly than braided suture, resulting in a longer recovery time. Here, we tested wound recovery (measured as wound healing and suture retention) following surgery using two types of absorbable suture (braided or monofilament) for a period of 3 weeks. We hypothesize that braided sutures will more quickly be expelled, leading to lesser irritation of the tissue surrounding the incision.

2 | METHODS

2.1 | Experimental animals

Juvenile brook trout (approximately 6 months old) were procured from MacGowan Lake Hatchery (Kejimkujik National Park, Nova Scotia) in November 2024. At the hatchery, the fish were housed in outdoor troughs fed by lakewater from MacGowan Lake (pH 5–6, alkalinity around 10 mg/L $CaCO_3$, 6°C at the time of procurement).

Two size classes were obtained to allow testing for size-dependent effects: 50 smaller fish with 10–12 cm fork length ($\bar{x} = 11.1$ cm) weighing 10.7–19.1 g ($\bar{x} = 14.4$ cm), and 50 larger fish with 13–16 cm fork length ($\bar{x} = 14.7$ cm) weighing 23.5–44.9 g ($\bar{x} = 34$ g). The juvenile brook trout were transported to the Aquatron facility at Dalhousie University (Halifax, Nova Scotia), where they were housed in a temperature-controlled recirculating aquaculture system with automatic partial replacement and a diurnal photoperiod. The recirculation system was equipped with physical, UV and biological filtration. The fish were divided among six 145-L circular containers. Oxygen and temperature were monitored twice daily. The fish were brought from 6 to 10°C over a span of 2 days and were given 1 week to acclimate to 10°C and to the new holding conditions prior to the start of experiments. The two size classes of fish specified above were housed separately to avoid competitive dominance, which could inhibit equal access to food during the study period. The fish were fed a salmonid pellet diet (mixture of 2 mm floating and sinking pellets, ~1% body weight daily).

2.2 | Experimental design

2.2.1 | Study groups

This study ran two simultaneous experiments: (1) metabolic rate determination of tagged fish using intermittent-flow respirometry ($n = 40$ small and 40 large fish) and (2) post-tagging recovery analysis of two different suture types ($n = 10$ small and 10 large fish). Each fish was used for one single experiment to avoid habituation effects.

The brook trout used for the respirometry experiments were further divided into four test groups: control, anaesthesia, surgery and tagged. Control fish were netted from the holding tanks and moved directly into the respirometers. Anaesthetised fish were fully sedated before being placed in the respirometers. Fish from the surgery group were anaesthetised and operated on in a sham tagging procedure where the tag was partially inserted and immediately removed before the fish was sutured. Fish from the tagged group were sedated, operated on and implanted with a tag for the duration of the study (more details on the tagging procedure below).

The 20 brook trout used for the wound healing experiment were all tagged. On half (five small and five large), the surgery incision was closed with a monofilament suture (Ethicon™ 5–0 Monocryl™ Violet Monofilament Absorbable Suture, P-3 13 mm 3/8c reverse cutting needle), while on the other half, the surgery incision was closed with a braided suture (Ethicon™ 5–0 Vicryl Rapide™ Undyed Braided Synthetic Absorbable Suture, P-3 13 mm 3/8 cutting needle). Each suture group was housed separately in 145-L containers identical to their previous holding container, under the same conditions for the duration of their recovery.

2.2.2 | Surgery procedure

Fish awaiting surgery underwent a 48-h fasting period in a separate container to minimize the effect of digestion on metabolic activity prior to the trial. Surgery procedures were identical between experiments; each fish was placed in a 100–125 mg/L solution of tricaine methanesulfonate (MS-222; Syndel) buffered with sodium bicarbonate (1:2 ratio; Syndel) for 3–5 min until the opercular rate became slow and irregular. Once sedated, fish were measured (to the nearest millimetre) and weighed (to the nearest 0.1 g) before surgery. The acoustic transmitter (Lotek JSATS PinTag, 3.4 mm diameter, 15 mm length, 0.22 g in air) was inserted into the body cavity through a 4–5-mm incision to the side of the mid-ventral line, posterior to the pelvic girdle. Tag burden ranged from 1.27% to 2% ($\bar{x} = 1.57\%$) for the small tagged fish, and from 0.54% to 0.85% ($\bar{x} = 0.64\%$) for the large tagged fish. For surgery-group brook trout, the tag was partially inserted then removed to mimic the stress on the body wall induced by the tag. The incision was closed with one single absorbable suture (monofilament for the respirometry experiment and either monofilament or braided for the wound healing experiment). The surgery procedure lasted on average 1 min and 40 s (range 1 min to 3 min and 20 s), and the brook trout quickly regained consciousness once placed back in clean water. Fish in the control group were measured and weighed at the end of the respirometry trial to avoid unnecessary disturbance prior to the data collection.

2.3 | Metabolic rate experiment

Oxygen consumption ($\dot{M}_{O_2}$) was measured using intermittent-flow respirometry. The respirometry system consisted of eight custom-built chambers constructed from transparent PVC pipe, acrylic caps and PVC tubing. Chambers of two different lengths were constructed to comfortably accommodate each size class of fish in the study (inner diameter 5.2 cm, small chamber length 16 cm, large chamber length 22 cm), leading to volumes of 360 and 483 mL (including tubing) for the small and large respirometers, respectively. The respirometers were leak-proofed both by confirming watertightness when emersed and by running dye tests. Each chamber was covered with an opaque plastic sleeve to reduce visual stimuli during the experimental period. A schematic and photograph of the system are provided in Supplementary Material 1.

Oxygen was measured every second through an O_2 probe (OXFLOW-HS; PyroScience GmbH) installed on the recirculation loop of the respirometer. Additionally, one temperature probe (TDIP15; PyroScience GmbH) was also installed to the recirculation loop of one of the small chambers and one of the large chambers to monitor temperature within the chambers. The probes were connected to two PyroScience Firesting Pros (FSPRO-4; PyroScience GmbH). The readings were relayed in real time to a custom-made R-based integration system, which monitored oxygen levels and unexpected changes in temperature. The integration system calculated oxygen consumption slopes and controlled the intermittent-flow phases based on R^2 thresholds and minimum acceptable oxygen levels (i.e. dynamic cycling). Each chamber was equipped with a recirculation and a flush pump (models AD20P-0510A and H20632-NQC6TX, respectively). The flush pumps were connected to a custom-built flush controller, which received instructions from the R-based integration system. The lowest oxygen concentration reached per run averaged 81% dissolved oxygen. Flush phases lasted on average 80 s and measurement phases lasted on average 165 s. The first 20 s of the measurement phase were discarded (wait phase). The lowest recorded dissolved oxygen per cycle averaged 91% for both before and after-chase readings (lowest recorded: 75.5% during the pre-chase period and 80.2% post-chase). Background oxygen consumption was recorded both before and after the experiments (five to six cycles respectively) to account for any microbial oxygen consumption that may have occurred throughout the duration of the experiment. The entire system was cleaned and disinfected using 70% ethanol when background levels reached 10% of the animal's standard metabolic rate (SMR).

Prior to each experimental trial, the O_2 probes were calibrated with an 100% O_2 solution. An initial check of the factory's 0% calibration point confirmed that the probe's 0% point was well calibrated. No further 0% calibrations were performed. In each trial, one animal of each size and experimental group were tested. The group–chamber combinations were rotated every trial, to avoid equipment bias. Fish were placed in the chambers immediately following anaesthesia/surgery and monitored until they regained equilibrium. Once the animal recovered, the chamber was covered with an opaque plastic sheath to limit external visual stimuli. The subjects remained inside the chambers undisturbed for 37 to 44 h, during which time their resting oxygen consumption was quantified. After this period, the fish were individually removed and placed inside a 5-gallon bucket where they were chased for 5 min (determined to be enough to cause loss of equilibrium during preliminary testing). After 5 min of chasing, the subjects were immediately returned to the chambers (~10 s between the end of the chase and closing the chamber). Oxygen consumption measurements were resumed immediately and continued for the following 4 h. At the end of this period, fish were euthanized with an overdose of MS-222 buffered with sodium bicarbonate.

2.4 | Wound-healing experiment

All surgeries for the wound-healing experiment took place on the same day, with each tagged group undergoing the same procedure under identical conditions. Braided and monofilament fish were housed in separate containers after tagging. Recovery assessment took place on the 7th, 14th and 21st days after surgery. During the first two wound checks, fish were transferred into a transparent container and photographed from below. On the 21st day, fish were euthanized with an overdose of MS-222 buffered with sodium bicarbonate.

2.5 | Calculations, statistics and data analysis

2.5.1 | $\dot{M}_{O_2}$ calculations

$\dot{M}_{O_2}$ was calculated in R v4.5.1 (R Core Team, 2025) using the R package pyroresp v0.1.1 (Flávio, 2025). Pre-background averaged 2.5% of SMR (maximum 10.7%) and post-background averaged 4.4% of SMR (maximum 12.5%). Recorded O_2 values (hPa) were converted to μmol O_2/L/h using the R package respirometry v2.0.1 (Birk, 2024). Changes in background respiration were linearly modelled over time using the pre- and post-background readings to estimate background at the time of each experimental cycle. The estimated background oxygen slopes were then subtracted from the oxygen consumption slopes calculated for each experimental cycle. Cycles with an R^2 of or above 0.9 were considered valid for $\dot{M}_{O_2}$ determination. The respective slopes were converted into $\dot{M}_{O_2}$ (μmol O_2/g/h) by taking into account the corrected volume of the respirometer and the mass of the animal, as follows: $\dot{M}_{O_2} = S \times V \times M^{-1}$, where S is the corrected oxygen decrease slope (μmol O_2/L/h), V is the respirometer volume (corrected for the mass of the animal, assuming a 1 g:1 mL animal density) and M is the mass of the animal (g). The $\dot{M}_{O_2}$ values calculated for each cycle are available in Supplementary Material 2.

2.5.2 | SMR, MMR and aerobic scope calculations and modelling

The SMR, i.e. the metabolic rate of the animal at rest, was calculated as the quantile 0.2 of the pre-chasing measurements (Chabot et al., 2016). The maximum metabolic rate (MMR), or the animal's energy usage in an excitatory or exhausted state, was determined as the highest $\dot{M}_{O_2}$ recorded during a full measurement period after chasing. It was noted that during the 2 days pre-chase, 14 out of 80 fish showed spikes in oxygen consumption that were 1.1 times or higher than the recorded MMR. For methodological consistency, these values were not considered for MMR quantification, i.e. all MMR values were obtained post-chase. Absolute aerobic scope (AAS) was calculated by subtracting SMR from MMR, and factorial aerobic scope (FAS) was calculated by dividing MMR by SMR. For the statistical analyses, given a significant difference between the masses of the different groups (Table 2), mass (in grams) was included as a continuous variable. As such, the effects of experimental group (factorial, four levels) and mass (continuous) and their interaction on each of the four calculated metrics (SMR, MMR, AAS and FAS) were tested using generalized linear models (GLMs) with a gamma distribution (log link). The quality of the models was verified by inspecting Q–Q and residual plots using the R package DHARMa v0.4.7 (Hartig, 2024). The significance of the tested variables was assessed through ANOVA (type III) testing using the R package car v3.1.3 (Fox & Weisberg, 2019). Because the interaction terms were not significant, the models were simplified by removing the interaction term. A near-significant effect of the experimental group on SMR (detailed in the results) was further investigated by directly comparing the SMR of control and tagged fish.

2.5.3 | EPOC calculations and modelling

Post-chase $\dot{M}_{O_2}$ values were converted to $\Delta\dot{M}_{O_2}$ by subtracting the respective SMR for each animal. These $\Delta\dot{M}_{O_2}$ values were then used to assess differences in recovery trajectory between groups (factorial,

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four levels) through the use of a generalized additive model (GAM), as follows:

$$\Delta\dot{M}_{O_2} \sim s(\text{time}, \text{by} = \text{group}, k = 10) + s(\text{id}, \text{bs} = \text{"re"}) + \text{mass} + \text{group}$$

Excess post-exercise oxygen consumption (EPOC) was quantified as the area between post-exercise $\dot{M}_{O_2}$ and SMR (in μmol/g) until $\dot{M}_{O_2}$ reached 1.1 times SMR or 4 h had passed. A GLM with gamma distribution (log link) was used to determine the effects of the experimental group (factorial, four levels) and mass (continuous) and their interaction on EPOC. Similarly to above, the interaction term was removed once shown that it was not significant.

2.5.4 | Wound-healing scores

At the three designated periods (1, 2 and 3 weeks after tagging), wound-healing rates were scored from 0 to 6 following the criteria presented by Miller et al. (2014; see Table 1 within), where 0 represents full healing and 6 represents a fully open wound. Suture retention was scored from 0 to 2 (0 = suture absent, 1 = suture loosely attached, 2 = suture in place and functional). Finally, the apparent presence of fungus around the exposed suture was noted. Sutures held in place for the first 2 weeks, but started falling off during the third week. A GLM with Bernoulli distribution (logit link) was used to test for differences in suture retention (retained or dropped) by week 3 between the two types of suture (monofilament or braided). We did not run models for weeks 1 and 2 given the clear similarity between the two suture types on those weeks.

3 | RESULTS

3.1 | Metabolic rate experiment

3.1.1 | SMR, MMR and aerobic scope

None of the response variables were found to be affected by an interaction between experimental group and mass (Table 1). The smaller brook trout showed significantly higher SMR than the larger individuals (~0.02 μmol/g/h decrease per gram; Table 1 and Figure 1a). The experimental group variable had a nearly significant effect on SMR (Table 1 and Figure 1a), which was noted to be driven by the difference between tagged and control animals. Further investigation revealed that control and tagged fish were significantly different from each other when compared directly (Table 1), with tagged fish showing a 7% reduction in SMR when compared to the control group. MMR was not significantly affected by the experimental group nor mass (Table 1 and Figure 1b). Both absolute and factorial aerobic scopes increased significantly with mass (~0.06 μmol/g/h increase per gram for AAS and ~0.07 fold increase per gram for FAS), but were not significantly different among experimental groups (Table 1 and Figure 1c,d). A summary of the average values for each metric by experimental group and size group is provided in Table 2. No mortalities occurred as a result of the tagging procedure, tag burden or confinement within the respirometers.

3.1.2 | Post-exercise oxygen consumption

The recovery trajectory was significantly affected by the mass of the animal, with larger animals consuming more excess oxygen post-chase (GAM, $n = 80$, $F = 15.606$, $p = 7.9 \times 10^{-5}$), but not by the different experimental groups (GAM, $n = 80$, $F = 0.575$, $p = 0.63$). Post-chasing oxygen consumption quickly decreased towards SMR values in the first 30 min post-chasing (Figure 2). After the initial 30 min, the recovery trajectory switched drastically, with $\Delta\dot{M}_{O_2}$ remaining elevated throughout the remainder of the 4-h period.

EPOC was significantly affected by mass, with smaller fish having lower EPOC (~0.2 μmol/g increase per gram; Table 1 and Figure 2). The experimental groups did not have a significant effect on EPOC (Table 1). Additionally, metabolic rates remained elevated past 4 h after exercise (Table 2). Fold-over SMR after 4 h was not significantly influenced by experimental group (GLM, $n = 80$, $\chi^2 = 0.177$, $p = 0.98$) or mass (GLM, $n = 80$, $\chi^2 = 3.051$, $p = 0.08$).

TABLE 1 Summary of the significance of the effects of degree of invasiveness and mass (g) on several metabolic rate metrics of juvenile brook trout tagged with JSATS PinTags, from generalized linear models.

	Group × mass interaction		Experimental group		Mass (g)	
Metric	χ^2	p	χ^2	p	χ^2	p
SMR	2.44	0.49	7.35	0.06	88.22	**< 2 × 10^{-16}**
SMR (Control vs. Tagged)	–	–	5.37	**0.02**	33.22	**8.2 × 10^{-9}**
MMR	1.34	0.72	1.95	0.58	1.57	0.21
AAS	1.15	0.76	1.63	0.65	4.28	**0.04**
FAS	0.87	0.84	1.66	0.65	32.70	**1.1 × 10^{-8}**
EPOC	3.16	0.37	1.31	0.73	19.64	**9.3 × 10^{-6}**

Note: Bold p values indicate statistical significance ($\alpha = 0.05$). Data collected using an intermittent-flow respirometry system.
Abbreviations: AAS, absolute aerobic scope; EPOC, excess post-exercise oxygen consumption; FAS, factorial aerobic scope; MMR, maximum metabolic rate; SMR, standard metabolic rate.

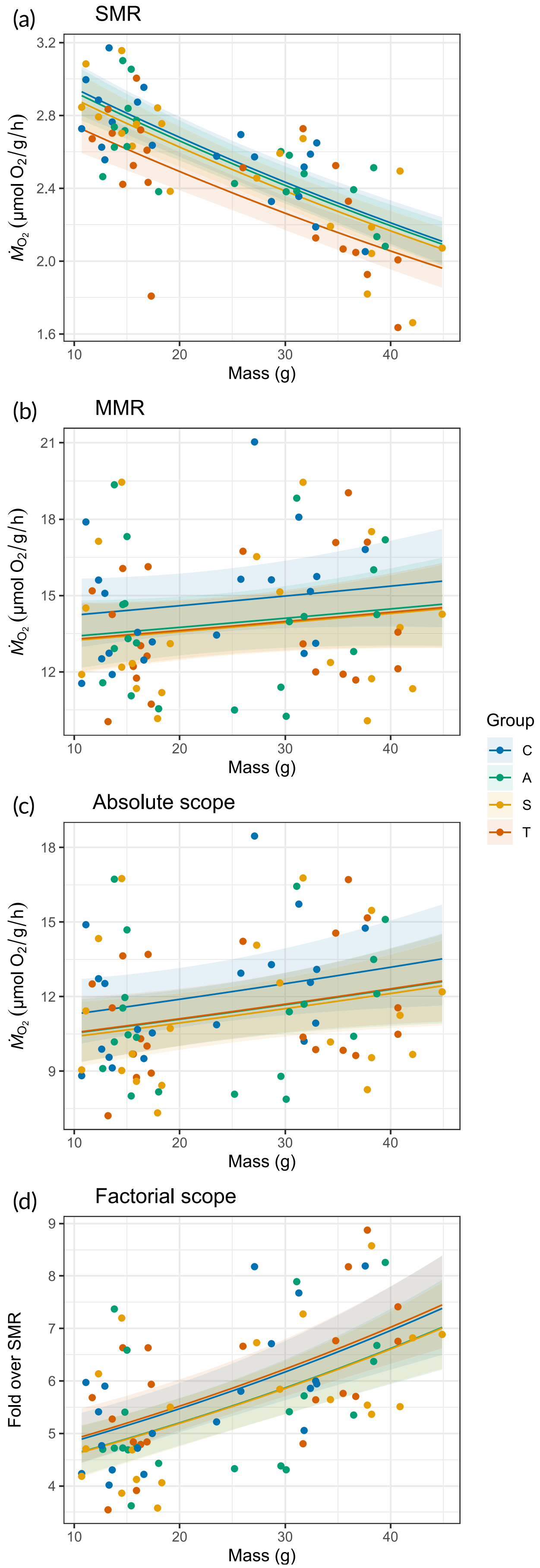

FIGURE 1 Distribution of metabolic rate metrics in relation to mass (g) of juvenile brook trout tagged with JSATS PinTags in an intermittent-flow respirometry system. Generalized linear models (gamma distribution, log link) were used to analyse the effect of mass and degree of invasiveness on (a) standard metabolic rate (SMR), (b) maximum metabolic rate (MMR), (c) absolute aerobic scope and (d) factorial aerobic scope. The respective predicted lines of fit and confidence intervals are shown for each group. C, control; A, anaesthesia; S, surgery; T, tagged.

3.2 | Wound-healing experiment

During the first week, the two suture types presented similar results (Table 3 and Figure 3, upper panels), with wound sides holding in close proximity and sutures remaining tight in position, with the exception of one braided suture animal that lost the suture during the first week. During the second week check, two braided sutures were noted to be loose, while the monofilament sutures held tight. One of the braided fish was noted to have fungus growing on the suture. By the third week, most of the braided sutures had dropped, while the monofilament sutures were beginning to loosen, but were still mostly present (Table 3 and Figure 3, lower panels). Fungus presence, while still scarce, was now visible on some of the remaining monofilament sutures. By week three, significantly fewer braided sutures were retained compared to monofilament sutures (Figure 4; GLM, $n = 20$, $\chi^2 = 5.3$, $p = 0.021$).

4 | DISCUSSION

Assessing the physiological stress responses associated with tagging procedures is essential for evaluating any potential downstream effects on behaviour and for ensuring confidence in movement data. Using intermittent-flow respirometry, we investigated the effects of micro-tag implantation on the oxygen consumption of juvenile brook trout as a proxy for tagging-induced physiological responses. We only found a 7% reduction in SMR in tagged animals that was only significant when compared directly to controls, indicating that neither the tagging procedure nor the tag burden should limit the fish's capacity for aerobic metabolism. As such, our findings do not support our original hypothesis that $\dot{M}_{O_2}$ would be elevated after tagging, nor that there would be a size-dependent effect. Related studies have indicated variable effects of tag implantation on physiological parameters, including SMR. For instance, Darcy et al. (2019) noted that tagging did not affect the SMR of lake trout (*Salvelinus namaycush*), but led to an increase in the SMR of rainbow trout (*Oncorhynchus mykiss*), which was attributed to chronic stress following the procedure. In contrast, Hove and Moss (1997) did not find an effect of MS-222 anaesthesia on the SMR of skate (*Raja erinacea*), although the study did not assess recovery effects. Reemeyer et al. (2019) found that gulf killifish (*Fundulus grandis*) express elevated cortisol levels 2 h after tagging, but only tested SMR 1 week after tagging, at which point no changes were noticeable. Given these previous reports, and the well-

TABLE 2 Summary metrics across treatment groups and sizes of juvenile brook trout tagged with JSATS PinTags in an intermittent-flow respirometry system.

Group	Size class	*n*	Fork length (cm)	Mass (g)	Tag burden (%)	SMR (μmol/g/h)	MMR (μmol/g/h)	AAS (μmol/g/h)	FAS (fold)	4 h EPOC (μmol/g)	Fold after 4 h
C	S	10	11.1 (±0.2)	13.7 (±0.7)	-	2.8 (±0.06)	13.6 (±0.6)	10.8 (±0.6)	4.9 (±0.2)	8.0 (±0.8)	1.8 (±0.2)
C	L	10	14.4 (±0.2)	30.4 (±1.3)	-	2.5 (±0.07)	15.7 (±0.8)	13.3 (±0.8)	6.5 (±0.4)	13.1 (±1.7)	1.6 (±0.2)
A	S	10	11.3 (±0.2)	14.9 (±0.5)	-	2.7 (±0.07)	13.9 (±0.9)	11.1 (±0.9)	5.1 (±0.3)	8.8 (±1.1)	1.7 (±0.2)
A	L	10	14.4 (±0.2)	33.1 (±1.5)	-	2.4 (±0.05)	13.9 (±0.9)	11.5 (±0.9)	5.9 (±0.5)	11.1 (±1.3)	1.9 (±0.3)
S	S	10	11.1 (±0.2)	15.0 (±0.9)	-	2.8 (±0.07)	13.3 (±0.9)	10.5 (±0.9)	4.8 (±0.4)	8.4 (±0.7)	1.6 (±0.2)
S	L	10	15.1 (±0.2)	36.5 (±1.8)	-	2.2 (±0.11)	14.2 (±0.9)	12.0 (±0.9)	6.4 (±0.3)	11.3 (±1.0)	1.8 (±0.2)
T	S	10	11.2 (±0.1)	15.2 (±0.6)	1.5 (±0.06)	2.6 (±0.10)	13.2 (±0.7)	10.6 (±0.7)	5.2 (±0.3)	7.1 (±0.4)	1.3 (±0.1)
T	L	10	14.8 (±0.17)	35.3 (±1.4)	0.6 (±0.03)	2.2 (±0.10)	14.4 (±0.9)	12.2 (±0.8)	6.7 (±0.4)	13.2 (±1.6)	2.3 (±0.4)

Note: Data are shown as mean ± SEM. Fold after 4 h indicates how elevated $\dot{M}_{O_2}$ was 4 h after chasing in comparison to the fish's previously recorded SMR. Groups: C, control; A, anaesthesia; S, surgery; T, tagged. Sizes: S, small; L, large.
Abbreviations: AAS, absolute aerobic scope; EPOC, excess post-exercise oxygen consumption; FAS, factorial aerobic scope; MMR, maximum metabolic rate; SMR, standard metabolic rate.

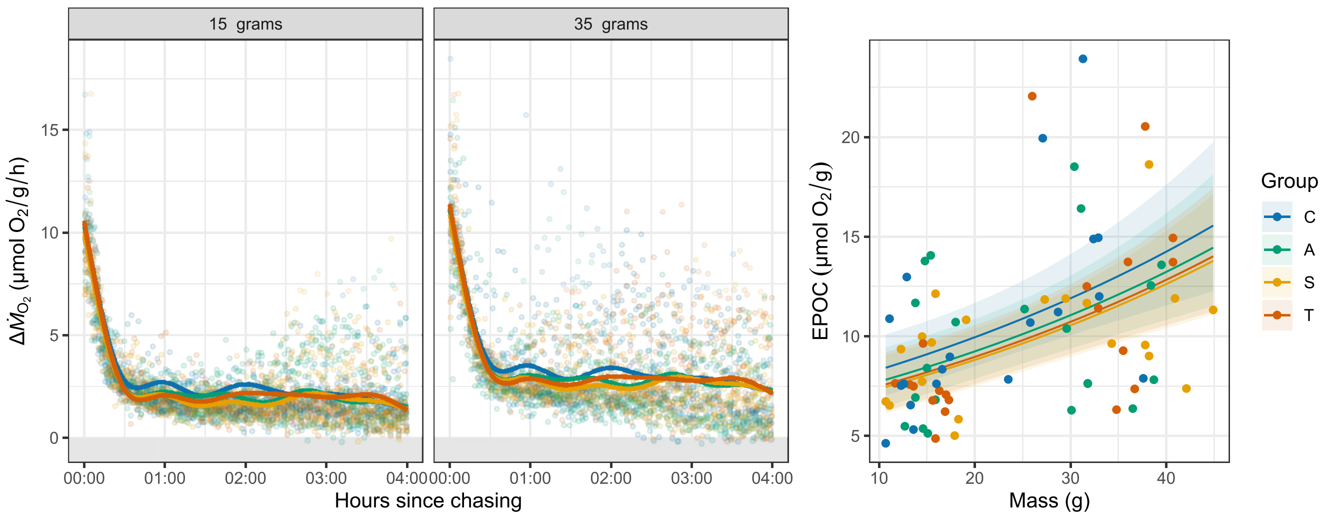

FIGURE 2 The left and centre panels show the after-chasing generalized additive model (GAM)-predicted recovery trajectory (i.e. $\Delta\,\dot{M}_{O_2}$ as a function of time since chasing) for juvenile brook trout tagged with JSATS PinTags for each group (C, control; A, anaesthesia; S, surgery; T, tagged) weighing a reference mass of 15 or 35 g. Raw data points are assigned to the closest reference mass, i.e. points for fish below 25 g are shown on the left panel (15 g) and points for fish above 25 g are shown on the centre panel (35 g). The right panel shows the total oxygen consumed above baseline (i.e. the EPOC) by mass (g) for each group until recovery or until 4 h had elapsed.

TABLE 3 Number of juvenile brook trout tagged with JSATS PinTags recorded within each wound healing and suture retention metric per week following surgery for monofilament and braided sutures.

		Week 1		Week 2		Week 3	
Metric	Value	Monofilament	Braided	Monofilament	Braided	Monofilament	Braided
Wound	2 Held in proximity	10	10	9	9	1	0
	1 Closed	0	0	1	1	9	10
Suture	2 In place	10	9	10	7	4	1
	1 Loose	0	0	0	2	3	1
	0 Absent	0	1	0	1	3	8
	Fungus noted	0	0	0	1	2	0

Note: Wound ranks follow the criteria provided by Miller et al. (2014). Ranks that were not observed were omitted.

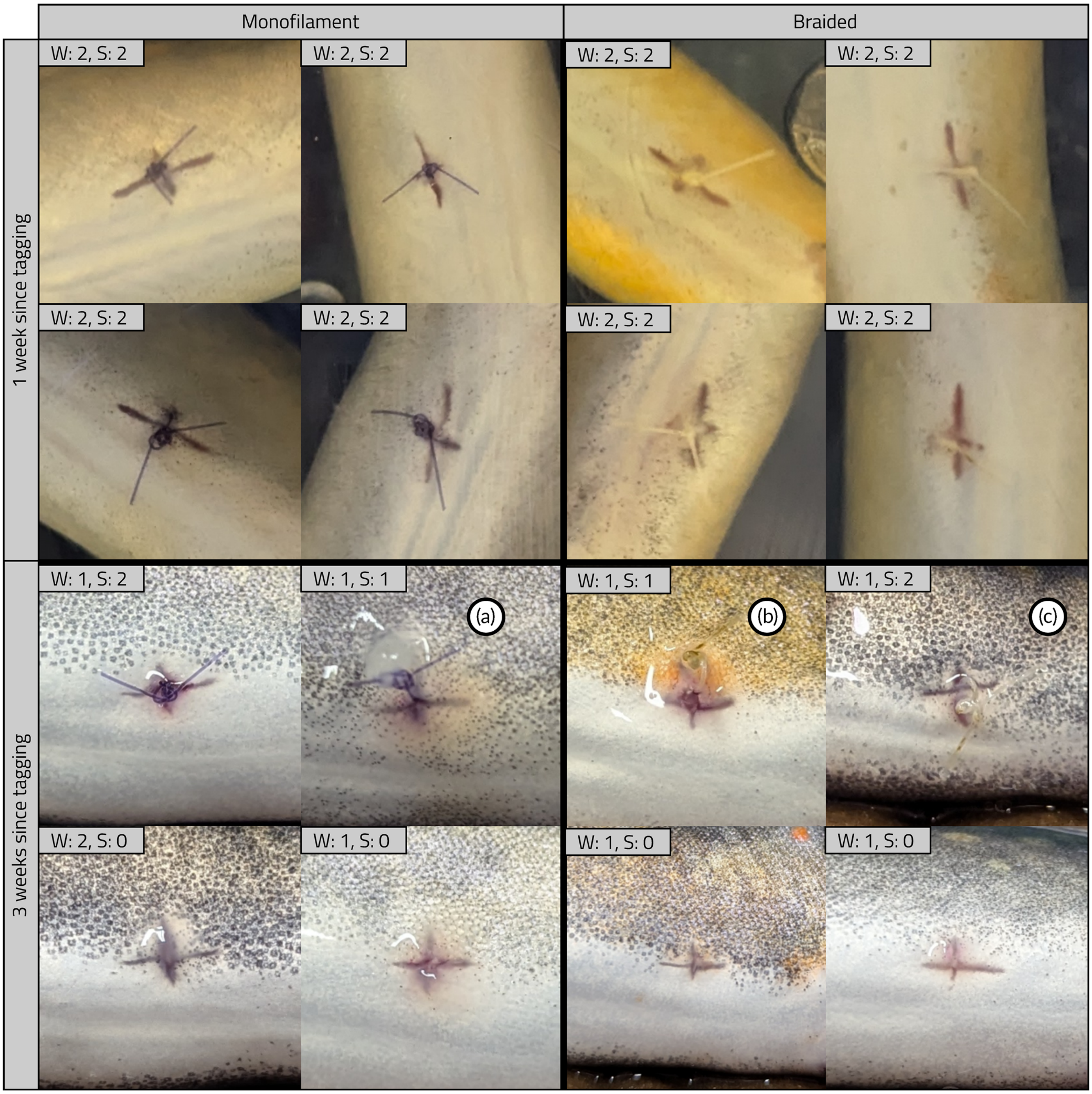

FIGURE 3 Examples of wound evolution 1 and 3 weeks after tagging juvenile brook trout with JSATS PinTags for wounds closed with monofilament and braided suture. (a) An example where fungus growth is visible around the suture. (b) Skin irritation around a loosely attached suture. (c) A loosely attached suture with no apparent irritation. Wound (W) and suture (S) scores are displayed in the top left corner of each photograph.

documented increase in oxygen consumption caused by short-term stress induction (Barton & Schreck, 1987; Morgan & Iwama, 1996), our results pointing towards a potential reduction in SMR are unexpected. However, ultimately the effect we found was very subtle and is unlikely to have an impact on newly tagged juvenile fish released to the wild.

None of the experimental procedures impaired the fish's capacity for instantaneous increases in metabolic rate (i.e. no effect on MMR nor aerobic scope) or the capacity to recover from vigorous exercise (i.e. no effect on EPOC). This indicates that neither the surgery procedure nor the presence of the tag should limit the brook trout's capacity for burst movement, which is highly relevant for predator-evasion responses after release in the wild. Alternatively, any effect of the experimental procedures may be masked by natural variation in individual MMRs. The metabolic rate of fishes can vary greatly among individuals of the same species (Burton et al., 2011; Metcalfe et al., 2016), potentially playing an important role in how populations are able to respond to environmental stressors, e.g. a changing climate

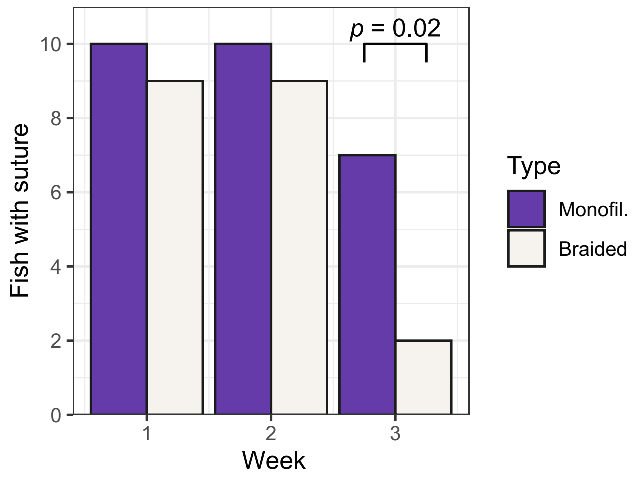

FIGURE 4 The number of juvenile brook trout tagged with JSATS PinTags that retained a monofilament or braided suture up to 1–3 weeks post-tagging. By the third week, a significantly lower number of fish sutured with a braided wire still retained the suture compared to the monofilament suture. The difference in suture retention between the two suture types at week 3 was deemed significant (GLM, Bernoulli distribution, logit link). No statistic tests were conducted for weeks 1 and 2.

(Norin & Metcalfe, 2019). However, measurements of the metabolic rate of a given individual are repeatable, if taken within an adequate time frame (Norin & Malte, 2011). Future research could use an increased sample size and/or incorporate repeated MMR measurements before and after the procedure to disentangle the effect of individual variation from the effects of surgery and tagging.

The fish did not recover fully from exhaustion within the 4 h of post-chase monitoring, with larger fish requiring more oxygen and taking longer to recover. We observed an increased rate of oxygen consumption post-exercise due to elevated metabolic rates that work to restore homeostasis following strenuous activity. Our observations are consistent with previous findings of greater oxygen debt in larger fishes following exhaustive exercise (Clark et al., 2012). Allometric effects on exercise recovery proposed for largemouth bass (*Micropterus salmoides*) include a greater reliance on aerobic metabolism during burst swimming in smaller fishes, leading to a quicker recovery with an accelerated ability to clear metabolites (Gingerich & Suski, 2012). Our noted lack of full recovery within 4 h is aligned with previous research; Zhang et al. (2018) found similar EPOC trajectories following exhaustion in Atlantic salmon parr, dividing recovery into an initial rapid phase lasting approximately 0.7 h, preceding a plateau and slow phase collectively lasting around 12 h. Further, our fish took on average 4 h to recover to SMR levels after initial placement in the chamber (no significant differences between groups; see Data S3), indicating that even subexhaustive disturbances can lead to prolonged periods of elevated stress and oxygen consumption. Fold-over SMR measured after 4 h (1.45–2.39) matched previous findings corresponding to this plateau phase (Zhang et al., 2018). Some of the variance observed after the fast recovery phase may indicate a return to routine $\dot{M}_{O_2}$ rarely used as SMR reference (Zhang et al., 2018). Despite the absence of full recovery, the tracked recovery trajectory did not indicate any difference between the four experimental groups, indicating that neither the tagging procedure nor the tag burden impair post-exercise recovery.

Suture type had a notable effect on suture retention, with braided sutures being expelled faster than monofilament sutures by the end of the 3-week monitoring period. Ivasauskas et al. (2012) tagged rainbow trout (100–264 g) with 2–0 monofilament and braided silk sutures and found suture retention beyond 21 days for both sutures. However, the larger animals in their study and thicker sutures could explain the longer retention time compared to our investigation. Despite a faster suture expulsion, the greater surface area of braided sutures could represent an increased risk to fish in the wild, where conditions are markedly non-sterile and infection risk likely increases. Additional concerns emerge when considering inflammation at wound sites because braided sutures have been documented to cause more inflammation than their monofilament counterparts (Ivasauskas et al., 2012; Thorstad et al., 2013; Wagner et al., 2000). However, Jepsen et al. (2008) found that juvenile brown trout (average mass 71 g) tagged with braided sutures tended to show a better wound-healing score than those with a monofilament suture when recaptured after approximately 5 months. Given these varying results, it remains uncertain whether the faster suture expulsion rate of braided sutures offers a sufficient advantage to outweigh the increased risks of inflammation, tag expulsion and infection in the wild. Mesocosm experiments, where tagged fish can experience a more natural environment but researchers are still able to monitor wound evolution, could be of great benefit for such comparisons, particularly in the context of long-term survival.

When interpreting the results of this study, it is important to consider the hatchery origin of the fish used here. Hatchery rearing practices have been proven to alter the brain phenotype of salmonids, resulting in behavioural and developmental deviations from wild fish, as has been demonstrated in rainbow trout (Marchetti & Nevitt, 2003). In the absence of interspecific competition and predation, the baseline for stress in hatchery reared fish may differ from wild animals. Hatchery-reared parr have demonstrated more delayed reactions to simulated predation events as well as shorter residence times within refuge spaces, suggesting that they are less perturbed by stress-inducing stimuli (Fleming & Einum, 1997). These concerns are especially relevant to our study because our brook trout adapted to artificial habitat parameters may have exhibited different energy expenditures compared to their wild counterparts. However, while the fish used here may show different oxygen consumption profiles than their wild counterparts, we would still expect wild fish to respond in a similar manner to the various experimental procedures. Ultimately, a more focused future study could look specifically into oxygen consumption differences between wild and domesticated fish to confirm that the results found here are also applicable to wild juvenile brook trout.

5 | CONCLUSION

In this study, we provide insight into the physiological effects of micro-acoustic tagging on juvenile brook trout, specifically assessing metabolic responses and suture retention. The absence of significant impacts of experimental group on SMR, MMR, aerobic scope and post-exercise recovery suggests that the JSATS PinTag does not impose a metabolic burden on juvenile salmonids as small as 10 cm. Our comparison of suture types identified the potential for surgical refinements that could enhance post-tagging survivability. The faster expulsion of braided sutures suggests they may facilitate faster healing, although their increased potential for inflammation highlights the need for further research. Future investigations should focus on both immediate post-tagging behavioural changes (e.g. reduced activity) and longer-term healing outcomes, ideally in more natural conditions. Ultimately, our study could not determine a cause for concern in the implantation of JSATS PinTags for studying small juvenile salmonids and identified avenues for improving surgical protocols. These steps towards addressing knowledge gaps in the physiology of young fish will provide insights into broader questions about the movement, behaviour and habitat use of these size classes, which is critical for understanding their ecology at early life stages.

AUTHOR CONTRIBUTIONS

R.L. and H.F. conceived the experiments. R.L. obtained funding for the experiments. H.F. led the experiments, with contributions from O.N.G., P.M. and A.B. H.F. and O.N.G. designed and constructed the respirometry equipment for this study. All authors contributed to the data analysis. O.N.G. led the manuscript writing, with contributions from all authors.

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DATA AVAILABILITY STATEMENT

The data collected for this study and the respective R analysis scripts will be made available as a Zenodo repository (www.doi.org/10.5281/zenodo.15283342) upon publication.

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