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 lifeline for future generations, but also as a valuable food source for a thriving community. However, these juvenile animals are particularly vulnerable to both biotic and abiotic changes in the ecosystem. Understanding the juvenile life-stage of fish is challenging, as juveniles tend to be small and hard to track, therefore making it hard to gather information about them. As new acoustic tags become increasingly smaller, we are finally able to shed light on this understudied life-stage. But tagging such small animals can introduce sub-lethal effects that alter their physiology and behaviour, ultimately biasing the collected data, any subsequent analyses and the conclusions drawn. Here, we tested if tagging juvenile brook trout (*Salvelinus fontinalis*) of two size classes (10-12 cm and 13-16 cm fork length) with a LOTEK JSATS PinTag induced noticeable changes in their oxygen consumption rates ($\dot{M}O_2$) immediately after tagging and over a period of two days. We observed a sublethal effect of small magnitude on SMR across the treatment groups. The small magnitude of this effect, taken together with the absence of significant effects on MMR, aerobic scope, and post-exercise recovery, suggests that the JSAT PinTag does not impose a substantial burden on fish of this size. By confirming that these tags do not exert a great impact on aerobic metabolic rates even shortly after tagging, our findings suggest that the data collected using this technology may be used to explore questions surrounding juvenile salmonid movement patterns.

Keywords: salmonids, acoustic telemetry, aquatic respirometry, sub-lethal 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 (Matley et al., 2022, 2024). Traditionally, telemetry studies focused on larger animals, because the size of the tag components limited the implantation of tags into smaller animals. This, in turn, creates an important gap in knowledge regarding the behaviour, needs, and vulnerabilities of smaller fish. In recent years, developments in technology are allowing the construction of increasingly miniature 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 short- and long-term stress induced by the procedure, which can in turn affect the animals' behaviour, fitness, and survival (Haas et al., 2023; Heim et al., 2024; Robertson et al., 2003). This is of particular concern for smaller, lighter animals, where even small tags represent a potentially large 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). Matley et al. (2024) explored 112 tagging effects studies and found that the median experimental duration was 33 days for lab studies, 153 days for tracking studies, and 196 days for recapture studies. Among those experiments, salmonids were identified as the principal focal group, with extensive investigation into the tagging effects across multiple life stages. For example, a study by Geist et al. (2018) found that juvenile Chinook salmon (*Oncorhynchus tshawytscha*) over 100mm tagged with the Lotek JSATS pin tag (3.4mm diameter, 15mm length, 0.22g in air) experienced 77-92% survival rate and no tag expulsion when subjected to conditions mimicking rapid

decompression and shear forces from hydropower turbine passage.

Short-term sub-lethal effects of tagging are particularly relevant because they may alter the behaviour of the fish. Alterations to behaviour would make tagged animals unrepresentative of wild counterparts, leading to misguided management decisions. These short-term effects may arise directly from the tag burden, but also indirectly from the surgery procedure and materials used. For example, Moore et al. (1990) found that tagging led to higher opercular rates in Atlantic salmon (*Salmo salar*) during the first 70 minutes after tagging, but noted no other immediate behavioural changes. Similarly, the physiological response to wound closure methods can influence recovery time, as seen in studies comparing suture types. A study of the longevity and healing response of absorbable sutures linked wound recovery to suture type, with monofilament suture absorbing more slowly than multi-braided suture and thus resulting in a longer recovery time (Gilliland, 1994). Together, both tag burden and the choice of surgery materials may cause discomfort and introduce stress (Jepsen et al., 2001; Lower et al., 2005), which in turn increases the oxygen consumption ($\dot{M}O_2$) of the animal (Davis & and Schreck, 1997).

Here, we used intermittent-flow respirometry to determine the immediate effects of tagging and handling on the oxygen consumption rates of juvenile brook trout (*Salvelinus fontinalis*), and tested wound recovery (measured as wound healing and suture retention) following surgery using two types of suture (braided or monofilament). 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, and 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 (approx. 6 months old) of two size classes (10-12 cm and 13-16 cm fork length, n = 50 of each) 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 $CaCO_3$), which was 6°C at the time of procurement.

The juvenile trout were transported to the Aquatron facility at Dalhousie University (Halifax, Nova Scotia), where they were housed in a temperature-controlled recirculating aquaculture system under constant supervision and a diurnal photoperiod. The specimens were divided among six, 145 litre circular containers and brought from 6 to 10°C over a span of two days. Fish were given one week to acclimate to the new temperature and conditions prior to the start of experiments. The fish were separated by size class to avoid competitive dominance. The fish were fed a salmonid pellet diet (mixture of 2 mm floating and sinking pellets, approx. 1% body-weight daily).

2.2 Experimental Design

2.2.1 Study groups:

This study ran two simultaneous experiments between two different study groups; metabolic rate determination of tagged fish using intermittent-flow respirometry (n = 40 small and 40 large animals) and post-tagging recovery analysis of two different suture types (n = 10 small and 10 large animals). Animals were used for one single experiment, to avoid habituation effects.

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

The 20 animals used for the wound healing experiment were all tagged. On half (5 small and 5 large), the surgery incision was closed with a monofilament suture (Ethicon™

5-0 Monocryl™ Violet Monofilament Absorbable Suture, P-3 13mm 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 13mm 3/8 cutting needle). Each suture group was housed separately in 145 litre 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-hour starvation 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 MS-222 (Tricaine Methanesulfonate; Syndel) buffered with sodium bicarbonate (1:2 ratio, Syndel) for 3-5 minutes, until the opercular rate became slow and irregular. Once sedated, fish were measured (nearest mm) and weighed (nearest 0.1g) before surgery. The acoustic transmitter (Lotek JSATS pin tag, 3.4mm diameter, 15mm length, 0.22g in air) was inserted into the body cavity through a 4-5 mm incision slightly to the side of the mid-ventral line, posterior to the pelvic girdle. For surgery-group animals, 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 a combination of monofilament and braided for the wound healing experiment). The surgery procedure lasted on average 1m40s (range 1m to 3m20s), and the animals 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.

2.2.3 Metabolic rate experiment:

$\dot{M}O_2$ was measured using intermittent-flow respirometry. The respirometry system consisted of 8 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 = 5.2cm, small chamber

length: 16cm, large chamber length: 22cm), leading to volumes of 360 ml and 483 ml including tubing for the small and the large respirometers, respectively. Each chamber was covered with an opaque plastic sleeve to reduce visual stimulus during the experimental period.

Oxygen was measured every second through an O_2 probe (OXFLOW-HS; PyroScience GmbH, Aachen, Germany) installed on the recirculation loop of the respirometer. Additionally, one temperature probe (TDIP15; PyroScience GmbH, Aachen, Germany) was also installed to the recirculation loop of one of the small 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, Aachen, Germany). The readings were relayed in realtime to an R-based integration system developed by Dr. Flávio, 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 mentioned before. The lowest oxygen concentration reached per run averaged 81% dissolved oxygen. Flush phases lasted on average 80 seconds, and measurement phases lasted on average 165 seconds. The first 20 seconds 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 (5-6 cycles on each side), 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 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 × experimental group were tested. The group-chamber combinations were rotated every trial, to avoid equipment bias. Animals were placed in the chambers immediately following anesthesia/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 hours, 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 to exhaustion over a 5 minute period. After each chasing period, the subjects were immediately returned to the chambers, and their oxygen consumption was measured for the following 4 hours. At the end of this period, animals were euthanized with an overdose of MS-222 buffered with sodium bicarbonate.

2.2.4 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 7^{th}, 14^{th}, and 21^{st} day after surgery. During the first two wound checks, animals were transferred into a transparent container and photographed from below. On the 21^{st} day, animals were euthanized with an overdose of MS-222 buffered with sodium bicarbonate.

2.3 Calculations, statistics and data analysis

2.3.1 $\dot{M}O_2$ calculations

$\dot{M}O_2$ was calculated in R v4.4.3 (R Core Team, 2025) using the R package pyroresp v0.1.1 (Flávio, 2025). Pre-background averaged 2.5% of SMR (max. 10.7%) and post-background averaged 4.4% of SMR (max. 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 O2/g/h) by taking into account the corrected volume of the respirometer and the mass of the animal, as follows: $MO_2 = S \times V \times M^{-1}$, where: S = corrected oxygen decrease slope (µmol O_2/L/h), V = respirometer volume (corrected for the mass of the animal, assuming a 1 g:1 mL animal density), and M = mass of the animal (g).

2.3.2 SMR, MMR, and aerobic scope calculations and modelling

Standard metabolic rate (SMR) was calculated as the quantile 0.2 of the pre-chasing measurements (Chabot et al., 2016). Maximum metabolic rate (MMR) was determined as the highest $\dot{M}O_2$ measurement recorded after chasing. 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, the four experimental groups were coded as a continuous degree of invasiveness variable (control = 1, anesthesia = 2, surgery = 3, tagged = 4). Further, given slight but significant differences between the masses of the different groups (Table 2), mass (in grams) was included as a continuous variable. As such, the effects of degree of invasiveness (continuous) and mass (continuous) on each of the four calculated metrics (SMR, MMR, AAS, and FAS) were tested using generalized linear models (GLM) with 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). Significance of the tested variables was assessed through ANOVA (type III) testing using the R package car v3.1.3 (Fox & Weisberg, 2019).

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

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

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 hours had passed. A GLM with Gamma distribution and log link was used to determine the effects of degree of invasiveness (continuous) and mass (continuous) on EPOC.

2.3.4 Wound healing scores

At the three designated periods (1, 2, and 3 weeks after tagging), wound healing rates were scored from 0-2 (0 = wound healed over, 1 = wound closed but not healed over, 2 = wound held in proximity but not closed). Suture retention was scored from 0-2 (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 two weeks, but started falling off during the third week. As such, a GLM with Bernoulli distribution (logit link) was applied to test for differences in suture retention by week 3 between the two types of suture (factorial, two levels).

3 Results

3.1 Metabolic rate experiment

Pre-experiment background respiration was 2.52 percent of SMR (SD = 2.74) with post-experiment values only slightly greater having a mean of 4.36 percent (SD = 2.56) across all runs. Background respiration was not considered to significantly affect the interpretation of results.

3.1.1 SMR, MMR, and aerobic scope

The smaller brook-trout showed significantly higher SMR than the larger individuals (Table 1 Figure 1A). Further, the degree of invasiveness was shown to have a significant effect on SMR (Table 1), causing a significant decrease in SMR as degree of invasiveness increases (Figure 1A). MMR, on the other hand, was not significantly affected by degree of invasiveness nor mass (Table 1, Figure 1B). Finally, both absolute and factorial aerobic

scopes were significantly affected by mass, but not by degree of invasiveness (Table 1, Figure 1D). A summary of the average values for each metric, divided by experimental and size group, is provided in Table 2.

3.1.2 Post-exercise oxygen consumption

Recovery trajectory was significantly affected by the mass of the animal (GAM, N = 80, F = 15.710, p-value = 7.48×10^{-5}), but not by the different groups (GAM, N = 80, F = 0.556, p-value = 0.64). Post-chasing oxygen consumption quickly decreased towards SMR values in the first 30 min post-chasing (Figure 2). After the initial 30 minutes, recovery trajectory switched drastically, with $\Delta\dot{M}O_2$ remaining slightly elevated throughout the remainder of the four hour period.

Excess post-exercise oxygen consumption (EPOC) was significantly affected by mass, with smaller fish having lower EPOC (Table 1, Figure 2). Degree of invasiveness did not have a significant effect on EPOC (Table 1). Additionally, metabolic rates remained elevated past four hours after exercise (Table 2). Fold over SMR after four hours was not significantly influenced by degree of invasiveness (GLM, N = 80, χ^2 = 0.226, p-value = 0.63) nor mass (GLM, N = 80, χ^2 = 1.68, p-value = 0.19).

3.2 Wound healing experiment:

During the first week, the two suture types presented similar results (Table 3, Figure 3, upper panels), with wound sides holding in close proximity and sutures remaining tight in position. The only exception to this is one braided suture animal which 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 animals was noted to have fungus growing on the suture. Finally, at the third week check, most of the braided sutures had dropped, while the monofilament sutures were noted to be becoming loose, but were still mostly present (Table 3, Figure 3, lower panels). Fungus presence, while still scarce, was now visible on the remaining monofilament sutures. By week three, the difference in the number of sutures that had dropped from the animals was already significant (Figure 4; GLM, N = 20, χ^2 = 5.3, p-value = 0.021).

Table 1: Summary of the significance of the effects of degree of invasiveness and mass on the various respirometry metrics calculated.

		SMR	MMR	AAS	FAS	EPOC
Degree of invasiveness	χ2	6.017	1.101	0.695	0.064	0.89
	p-value	**0.014**	0.294	0.404	0.8	0.346
Mass	χ2	87.94	2.116	5.178	35.08	19.14
	p-value	**$< 2\times10^{-16}$**	0.146	**0.023**	**3.2×10^{-9}**	**1.2×10^{-5}**

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Table 2: Summary metrics across treatment groups and sizes. Data shown as mean ± SEM. Fold after 4 h indicates how elevated MO2 was four hours after chasing in comparison to the animals' previously recorded SMR. Groups: C = Control, A = Anesthesia, S = Surgery, T = Tagged. Sizes: S = Small, L = Large.

Group	Size class	n	Mass (g)	Fork length (cm)	SMR (µmol/g/h)	MMR (µmol/g/h)	AAS (µmol/g/h)	FAS (fold)	4h EPOC (µmol/g)	Fold after 4h
C	S	10	13.65 (±0.72)	11.1 (±0.17)	2.82 (±0.06)	13.59 (±0.66)	10.77 (±0.65)	4.83 (±0.23)	8.10 (±0.80)	1.92 (±0.23)
C	L	10	30.41 (±1.31)	14.4 (±0.18)	2.46 (±0.07)	15.79 (±0.87)	13.34 (±0.87)	6.48 (±0.40)	12.88 (±1.76)	1.45 (±0.15)
A	S	10	14.91 (±0.45)	11.3 (±0.13)	2.74 (±0.07)	14.23 (±0.89)	11.49 (±0.88)	5.22 (±0.34)	8.47 (±1.05)	1.68 (±0.19)
A	L	10	33.13 (±1.52)	14.4 (±0.19)	2.40 (±0.05)	14.65 (±0.86)	12.26 (±0.87)	6.16 (±0.43)	10.62 (±1.40)	1.71 (±0.28)
S	S	10	14.98 (±0.93)	11.1 (±0.19)	2.80 (±0.07)	13.90 (±0.92)	11.10 (±0.92)	5.00 (±0.36)	8.58 (±0.65)	1.70 (±0.18)
S	L	10	36.49 (±1.80)	15.1 (±0.20)	2.22 (±0.11)	14.82 (±0.93)	12.59 (±0.90)	6.75 (±0.50)	11.52 (±0.87)	1.86 (±0.24)
T	S	10	15.21 (±0.59)	11.2 (±0.13)	2.58 (±0.10)	13.28 (±0.70)	10.71 (±0.71)	5.23 (±0.33)	7.26 (±0.41)	1.33 (±0.09)
T	L	10	35.28 (±1.38)	14.8 (±0.17)	2.19 (±0.10)	14.60 (±0.83)	12.40 (±0.78)	6.70 (±0.35)	12.49 (±1.83)	2.39 (±0.43)

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Table 3: Summary of the wound healing metrics recorded for each week. Numbers represent the number of animals ranked at each level of the respective scale (Note: fungus presence is not exclusive in relation to the remaining suture ranks).

		Week 1		Week 2		Week 3	
		Monofil.	Braided	Monofil.	Braided	Monofil.	Braided
Wound	Held in proximity	10	10	9	9	1	0
	Closed	0	0	1	1	9	10
	Healed over	0	0	0	0	0	0
Suture	In place	10	9	10	7	4	1
	Loose	0	0	0	2	3	1
	Absent	0	1	0	1	3	8
	Fungus noted	0	0	0	1	2	0

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Figure 1: Distribution of metabolic rate metrics calculated for each animal in each treatment group. A) Standard metabolic rate, B) Maximum metabolic rate), C) Absolute aerobic scope, D) Factorial aerobic scope. Predicted lines of fit and respective confidence intervals for each group are displayed in all plots.

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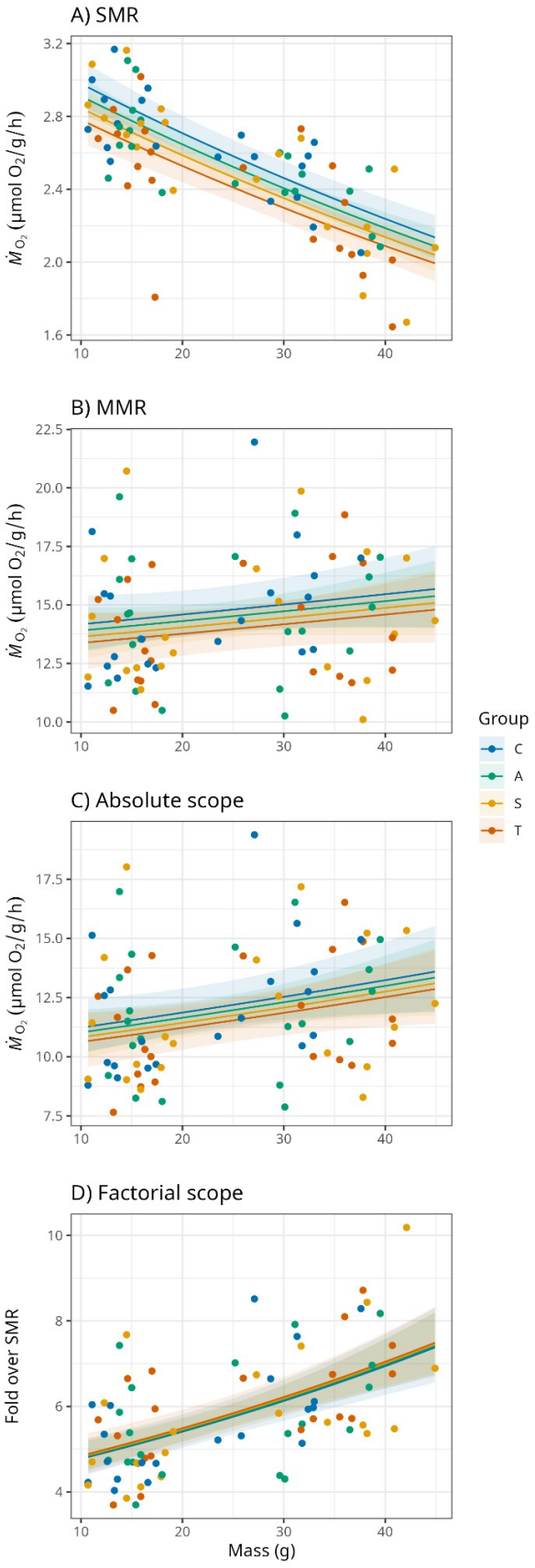

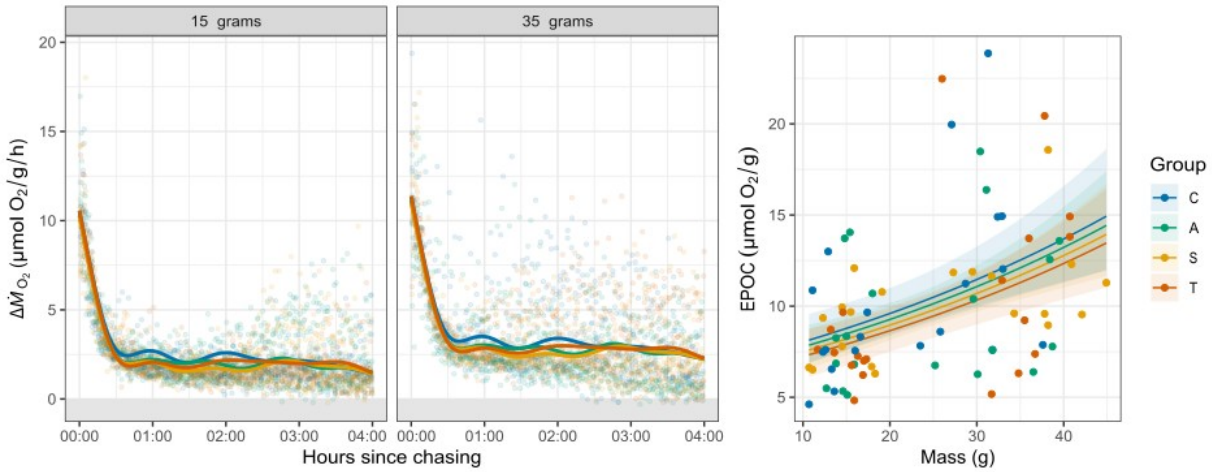

Figure 2: Post exercise recovery. The left and centre panels show the predicted recovery trajectory for an animal weighing 15 grams vs an animal weighing 35 grams (slightly higher). The points are grouped to the closest reference mass (i.e. points for animals below 25 grams are shown on the left panel, and points for animals above 25 grams are shown on the centre panel). The total oxygen consumed above baseline (i.e. the EPOC) is shown on the right panel.

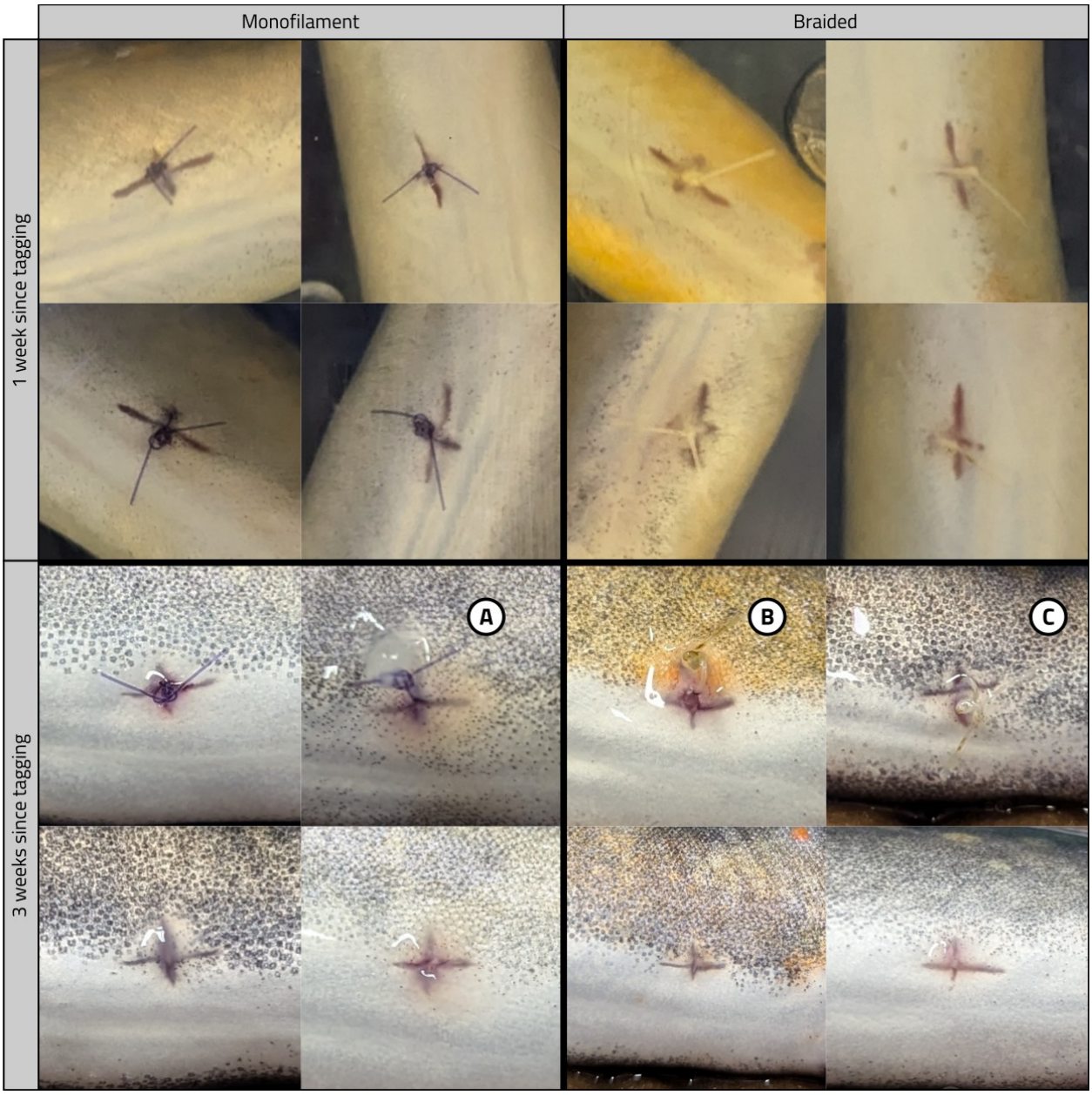

Figure 3: Wound evolution examples one and three weeks after surgery, for wounds closed with monofilament and braided suture. (A) denotes an example where fungus growth is visible around the suture. (B) denotes skin irritation around a loosely attached suture. (C) denotes a loosely attached suture with no apparent irritation.

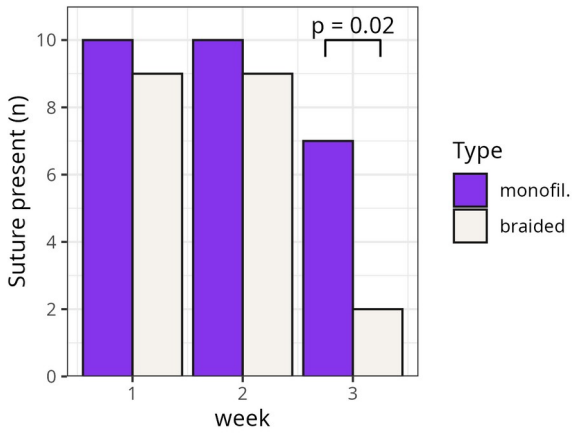

Figure 4: Number of animals that retained the suture up to one, two, and three weeks post-tagging. By the third week, a significantly lower number of animals sutured with a braided wire still retained the suture.

4 Discussion

The degree of invasiveness of each treatment had a modest, negative effect on the SMR of the animals. This finding contradicts our expectation that SMR would be highest among individuals subject to the most invasive procedures. Previous research has found variable effects of tag implantation on physiological traits such as SMR or swimming performance. For example, Darcy et al. (2019) found that tagging did not affect the SMR of lake trout (*Salvelinus namaycush*), but led to a slight increase in the SMR of rainbow trout (*Oncorhynchus mykiss*), which was attributed to chronic stress following the procedure. Hove and Moss (1997) did not find an effect of MS-222 anesthesia on the SMR of skate (*Raja erinacea*), though the study did not look into recovery effects. Reemeyer et al. (2019) found that gulf killifish (*Fundulus grandis*) express elevated cortisol levels two hours after tagging, but only tested SMR one week after tagging, at which point no changes were noticeable. Given these examples, and the well-documented increase in oxygen consumption caused by short term stress induction (Barton & Schreck, 1987; Morgan & Iwama, 1996), our results are unexpected. However, Makaras and Stankevičiūtė (2024) found that juvenile brown trout (*Salmo trutta*) which had been anaesthetised showed reduced swimming activity for one hour, while juveniles that underwent surgery and tagging showed reduced activity for 24 hours. It could be that the lower SMR found in our

study derives from a generally lower level of activity. Future research should take complementary measurements of oxygen consumption and activity to further explore this possibility (see Reeve et al., 2022).

Despite the effect on SMR, the degree of invasiveness did not impair the animals' capacity for instantaneous increases in metabolic rate (i.e. no effect on MMR nor aerobic scope), nor did it impair 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 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 increasing degree of invasiveness 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 on how populations are able to respond to a changing climate (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). As such, future research aiming to better disentangle the effect of individual variation from the effects of surgery and tagging could incorporate repeated MMR measurements before and after the procedure.

The animals did not recover fully from vigorous exercise within the four hours of post-chase monitoring, with larger animals requiring more oxygen and taking longer to recover. The increased rate of oxygen consumption post-exercise fuels elevated metabolic rates that work to restore homeostasis following strenuous activity. This is 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). The lack of full recovery within four hours is in line 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 hours, preceding a plateau and slow phase collectively lasting around

12 hours. Further, our animals took between 1 and 11 hours to enter an SMR state after initial placement in the chamber, indicating that even sub-exhaustive disturbances can lead to prolonged periods of elevated oxygen consumption. Fold-over SMR measured after four hours (1.45 - 2.39) agreed with previous findings corresponding to the 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 groups, indicating that the tagging procedure does not 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 three week monitoring period. Ivasauskas et al. (2012) tagged rainbow trout (100-264g) with 2-0 sutures and found suture retention to go beyond 21 days for both monofilament and braided silk sutures. However, the larger animal size and thicker suture lines could help explain the longer retention time. Despite a faster suture expulsion, the larger surface area of braided sutures could represent an increased risk in the wild, where conditions are markedly non-sterile and infection risk likely increases. Additional concerns emerge when considering inflammation at wound sites, as 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 71g) tagged with braided sutures tended to show a better wound healing score when recaptured after ~5 months than those with a monofilament suture. 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 animals 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 animals used here. Hatchery rearing practices have been proven to alter the brain phenotype of salmonids, resulting in behavioral 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 subjects. 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 animals used here may show slightly different oxygen consumption profiles than their wild counterparts, we would still expect them to respond in a similar manner to the various degrees of invasiveness. Ultimately, a more focused future study could look specifically into oxygen consumption differences between control and tagged animals, 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. We observed a sublethal effect of small magnitude on SMR across the treatment groups. The unexpected direction of the effect on SMR (i.e. lower SMR as the degree of invasiveness increases) warrants future research into the underlying causes for the noted difference. Regardless, the small magnitude of the effect on SMR, taken together with the absence of significant effects on MMR, aerobic scope, and post-exercise recovery, suggests that the JSAT PinTag does not impose a substantial burden on fish of this size. By confirming that these tags do not exert a great impact on aerobic metabolic rates even shortly after tagging, our findings suggest that the data collected using this technology may be used to explore questions surrounding juvenile salmonid movement patterns. This step toward addressing knowledge gaps in the movement, behavior, and habitat use of young fish is

critical to understanding the ecology of animals at this life stage. Additionally, our comparison of suture types identified the potential for surgical refinements to enhance post-tagging survivability. The faster expulsion of braided sutures suggests they may facilitate faster healing, though their increased potential for inflammation warrants further investigation. Future research should focus both on immediate post-tagging behavioural changes (e.g. lower activity levels), and on monitoring the healing process for a longer period, and in a more natural setting.

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Author contributions

RL and HF conceived the experiments. RL obtained funding for the experiments. HF led the experiments, with contributions from ONG, PM, and AB. HF and ONG designed and constructed the respirometry equipment for this study. All authors contributed to the data analysis. ONG led the manuscript writing, with contributions from all authors.

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