Development and testing of a simple field-based intermittent-flow respirometry system for riverine fishes

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By understanding range-wide intraspecific variation in metabolic rate we can better understand how organisms have adapted to their environment. However, methods to quantify metabolic rate of fishes from remote areas or those that cannot be brought back to the laboratory because of imperilment status are lacking. Consequently, practical and reliable field-based methods are needed. To address this need, we developed a simple yet robust intermittent-flow respirometry system, adapted from a design commonly used in the laboratory that is readily suited for field use. Standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS) estimates were obtained from juvenile lake trout (Salvelinus namaycush) and brook trout (Salvelinus fontinalis) using both field- and laboratory-based systems. Whole-fish SMR, MMR and AS estimates from the field and laboratory methods did not differ from one another (ANCOVA and LMM: all P > 0.05) for either species and were comparable to estimates previously reported. Our field setup is a simpler system than the conventional laboratory-based system that requires less power and equipment to operate, yet still offers users the ability to: (1) acclimate fish to the respirometry chamber; (2) measure oxygen consumption during a shorter period (1 h), which yield metabolic rate estimates comparable to systems that take measurements over longer periods; and (3) take repeated oxygen consumption measurements with manual user-defined flush and measurement phase routines. Developing practical and reliable field respirometry methods, as demonstrated here, is important if we wish to improve our ability to predict how imperiled species will respond to changes in their environment. Such knowledge is critical for informing conservation strategies.

Key words: Brook trout, field respirometry, metabolic rate

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Introduction

A long-standing and common method of understanding the physiological capacity of fish is to estimate their metabolic rate—the rate at which an organism assimilates, transforms and expends energy (Fry and Hart, 1948; Brett, 1962; Beamish, 1964). Metabolic rate is regarded as the fundamental biological process that governs an organism’s survival (Brown et al., 2004) and is often estimated in fish using whole-animal respirometry (Svendsen et al., 2016). This method uses the oxygen consumption (MO2) of an organism over time in an enclosed vessel as an indirect measure of metabolic rate. Although MO2 has previously been measured in the field using respirometry, these systems are often very similar to those used in laboratory situations—i.e. the laboratory is brought to the field and measurements are taken (Farrell et al. 2003; Rodnick et al., 2004). These methods may be feasible for locations with road access (Farrell et al. 2003; Rodnick et al., 2004); however, in remote locations where logistical constraints (e.g. helicopter-only access) limit the overall size and weight of systems that can be used, a simpler, smaller and less energy consumptive system is required.

Recently, laboratory-based respirometry has been used to examine intraspecific variability in aerobic scope (AS) of salmonids (Farrell et al., 2008; Eliason et al., 2011, 2013), where AS is defined as the capacity of a fish to deliver additional oxygen to organs to complete activities beyond those required for existence (Farrell et al., 2009). One calculates AS by subtracting the metabolic rate of a non-digesting, quiescent fish (i.e. standard metabolic rate; SMR), from the maximum metabolic rate (MMR)—typically taken from fish that are exhausted after a prolonged swimming bout (Clarke et al., 2013). These studies provided a mechanistic understanding of how Pacific salmon populations may respond to different thermal regimes. Unfortunately, many populations that have adapted to novel environments are found at range margins (Sexton et al., 2009), often in remote areas (e.g. Arctic), which precludes use of conventional laboratory-based respirometry. In these situations a reliable field respirometry system would improve our capacity to conduct similar studies.

Here, we describe a field-based intermittent-flow respirometry system that is a simplification of a common design used in the laboratory. The aim was to develop a practical system that works within the constraints often encountered in remote field conditions, yet yields reliable and consistent measurements comparable to a commonly used laboratory system. The design of the respirometer is described and its use is demonstrated with juvenile lake trout (Salvelinus namaycush) and brook trout (Salvelinus fontinalis) in a controlled laboratory setting. Using a two-phased approach, we first tested the field prototype on lake trout to assess the feasibility of this method, and then refined the system by adding a mixing device during the measurement period and conducted a second experiment to test the new field system on brook trout. This approach was employed to show multi-species applicability of the system while improving the precision of metabolic rate estimates based on results of the first phase of the experiment. In both experiments we compared estimates of metabolic rate (SMR, MMR and AS) derived from the standard laboratory-based method to those obtained using the field-based method.

Methods

Collection and husbandry

Lake trout were reared from gametes collected from adult fish captured in Clearwater Lake, Manitoba (54.0500°N, 101.0500°W) through a project conducted by the Fisheries Branch of Manitoba Sustainable Development. For further information on rearing conditions see Kissinger et al. (2017). Juvenile brook trout were obtained from the Whiteshell Fish Hatchery, Whiteshell, Manitoba, which is managed by the Aquaculture Branch of Manitoba Sustainable Development. Both groups of fish were placed on a moderate growth feeding regime (i.e. slightly above maintenance) and reared for 18 months before conducting respirometry. All procedures were approved by the University of Manitoba Animal Care Committee (Animal use protocol #FF13-029) and the Freshwater Institute Animal Care Committee (Animal use protocol# FWI-ACC-2016-03).

Experimental design

Experiments were performed using a two-phased approach. An initial experiment was conducted on lake trout using a simple field-based respirometry design to determine if this method was feasible for obtaining reliable metabolic rate estimates. Results of this experiment were analyzed and the setup and experimental design of the field system was further improved to determine if higher precision estimates for metabolic rates could be obtained. The second experimental series also expanded the scope of applicability by using a second species, brook trout. In both experiments, the field-based respirometry system was compared to the same standard laboratory system.

Juvenile lake trout and brook trout were initially placed in round flow-through acclimation tanks (62 cm in diameter and 61 cm high) with a capacity of 736 l. The initial water temperature was −8°C and the water temperature was raised gradually by 1°C d−1 until it reached 10°C. This temperature was selected, as it is within both species’ optimal temperature range for growth (Christie and Regier, 1988; Xu et al., 2010; McDermid et al., 2013). After 4 weeks of thermal acclimation, MO2 was calculated individually for a total of 19 lake trout and 17 brook trout using oxygen depletion slopes obtained using intermittent-flow respirometry (Beauregard et al., 2013). Since we were using oxygen consumption rates as an indirect estimate of minimum and maximum aerobic metabolic rates, hereafter, these will be referred to as SMR and MMR, respectively (Clark et al., 2013). The AS was calculated by taking the difference between SMR and MMR (Clark et al., 2013). For the lake trout experiment, all fish came from the same population; however, eight fish (mean
body mass = $26.28 \pm 1.88$ SE) were used for the laboratory-based respirometry experiment in June 2015 and 10 weeks later 11 different, but similar sized fish (mean body mass = $21.94 \pm 1.78$ SE), were randomly selected from the general population for the field-based respirometry experiment. A paired design was used for the brook trout experiment where 17 fish were tested using the field method and then the same fish were tested using the lab method.

All fish were fed a daily maintenance ration (1% of body weight) between the laboratory- and field-based treatments and the mass of fish used for laboratory and field treatments did not differ for either experiment (lake trout $t$-test: $t = 1.72, P = 0.10$; brook trout $t$-test: $t = -0.818, P = 0.43$). In the lake trout experiment (Field-system 1), the two methods were not run concurrently and the sample sizes were unequal because the fish used in the lab-based treatment were part of a control group for another experiment (Kissinger et al., 2017). For the brook trout experiment (Field-system 2) sample sizes were equal and fish were split into two groups; half were placed in the field units and half in the laboratory units. After the initial run through the respirometry systems (i.e. laboratory or field units) fish were placed in tanks for 10 days to recover and then were subjected to the opposite method during the second run. This was done to determine if the order in which the fish went into each treatment had an effect on metabolic rate estimates. During this recovery period fish were offered food as follows: *ad libitum* for the first 2 days and 1% of body weight for the next 8 days. All fish consumed their entire ration by the third day of the recovery period.

**Respirometry design**

**Laboratory-based system**

Oxygen consumption of juvenile lake trout and brook trout was quantified using an intermittent-flow respirometry system.
as described in AutoRespTM 2.2.0 user manual (Loligo® Systems Tjele, Denmark). Briefly, four plexiglass cylindrical chambers (62 mm in diameter × 160 mm in length, 483 ml in volume) from Loligo Systems (Tjele, Denmark) were submerged in a temperature controlled water bath (10.0°C ± 0.2°C) that was maintained with a cooling coil and temperature regulator (Fig. 1A). Oxygen saturation was kept at 100% through injection of air using multiple air stones. Each unit had two Eheim pumps (Deizisau, Germany) with a flow rate of 5.0 l min⁻¹ connected by non-toxic, polyvinyl chloride (PVC) tubing, which was used to reduce oxygen diffusion/exchange during the experiment. One pump re-circulated water throughout the system and across an adjacent oxygen probe chamber during the closed measurement phase and the other pump was used to bring oxygenated water from the water bath back to the chamber to restore oxygen content during the flush phase. Oxygen concentration was measured using a multi-channel oxygen meter (OXY-4 mini, PreSens, Regensburg, Germany—Experiment 1; Witrox 4, Loligo Systems, Viborg, Denmark—Experiment 2) coupled to four optical sensor dipping probes (DP-PSt3-L2.5-ST10-YOP; precision ± 0.05 mg O₂ l⁻¹, PreSens, Regensburg, Germany). AutoResp software (version 2.01; Loligo Systems, Tjele, Denmark) was used to control the pumps during the experiment and to collect the oxygen depletion data during each interval.

Field-based system

To assess the effectiveness of our field-based system and control for as many confounding factors as possible, we tested it in a controlled laboratory setting. An initial prototype was built and tested on lake trout (Experiment 1) and then a second system was refined to address deficiencies of the first and tested on brook trout (Experiment 2).

Field-system 1

The laboratory-based system described in the previous section was simplified to accommodate constraints associated with application in remote locations. First, the recirculation and flow-through pumps were removed so that flow could be achieved using the stream gradient. Since the field system was tested in the laboratory, the stream gradient was replicated by positioning a 201 aquarium tank above the chambers allowing water to be siphoned through the system (Fig. 1B). This was meant to simulate a pool-riffle-run gradient that naturally occurs in rivers (Fig. 1B and C). Ball valves were attached to the inflow tubing so users could manually close the system during the measurement phase and then re-establish flow from the siphon during the flush phase (Fig. 2A and B). The intent was to reduce both the complexity and the power requirements of the system by using the natural stream gradient and mechanical valves to control water flow. The recirculation pump was not replaced with an alternate mixing method, with the understanding that incomplete mixing during the measurement phase may affect the variability and consistency of MO₂ measurements (Clark et al., 2013; Svendsen et al., 2016; Rodgers et al., 2016). The chamber volume was 735 ml, which included the water in the PVC tubing, and was selected to accommodate the average length of fish used in the experiment. It is expected that matching appropriate chamber size to particular fishes will be required to develop intrinsic mixing by ventilation and spontaneous activity. Second, the adjacent oxygen sensing chamber was removed and the oxygen dipping probe was mounted directly to the back end of a custom built respirometry chamber (Loligo Systems, Tjele, Denmark). The baffle on the back end of the
chamber was removed so that the oxygen probe could be inserted far enough into the chamber to measure oxygen uptake but not interfere with the fish (Figs. 1B and 2). In addition, the probe was positioned at the back of the chamber to try and reduce the effects of incomplete mixing (Svendsen et al., 2016), as it was postulated that a more highly variable oxygen decline may take place near the head of the fish (Fig. 1B). To account for this, fish were initially oriented with their head towards the front of the chamber at the baffle. Fish were checked at the beginning and end of each measurement phase to confirm that they maintained this orientation and were in a quiescent state; we assumed each fish remained in this position and a relatively low activity state for the entire measurement period. The baffle at the front of the chamber near the inflow port was kept to displace water and prevent swimming activity during the flush phase (Fig. 1B). The cooling coil in the water bath was removed, therefore, water temperature fluctuations were slightly higher (10.0°C ± 1.0°C), as this pattern is expected to be more consistent with hourly temperature regimes in a stream.

Field-system 2

Results from the first prototype suggested that incomplete mixing may have influenced the variability and consistency of MO2 measurements. Therefore, a small-battery powered mixing device was introduced and this necessitated use of an in-line oxygen chamber so that the oxygen probe could record MO2 during the closed phase (Fig. 1C). The chamber volume was 610 ml, which included the water in the PVC tubing, and was selected to accommodate the average length of fish used in the experiment.

Respirometry protocol

Individual fish were removed from the acclimation tank and fasted for 24 h, then placed into the respirometry chamber for an additional 24 h acclimation/fasting period; after which oxygen consumption was measured—specific measurement periods are described in detail below for each system. During the acclimation period and for subsequent oxygen concentration measurements, the water bath was covered with an opaque plastic sheet to darken the environment and obscure movements of observers around the experimental area when the fish were in the respirometry chambers. Water temperature was maintained at 10°C for all experiments and both laboratory and field treatments were done in climate controlled rooms where ambient air temperature was held at 14°C. Lights followed a 12 h on/off cycle to simulate equal day and night conditions when fish were held in the chambers. The experiments were conducted between 10:00 and 12:00 because this period is thought to be the least logistically challenging time to conduct similar experiments in remote field settings. Oxygen concentration measurements without any fish in the chamber were conducted before and after the experiment to quantify biological oxygen demand (BOD)—i.e. bacterial consumption of oxygen when no fish are in the chamber. Although these levels were found to be extremely low even after two experiments, the system was cleaned with 5% hypochlorite solution and rinsed with freshwater for 24 h after every other experiment to minimize the level of BOD in the system. Dissolved oxygen sensors were calibrated daily using a two-point calibration in an anoxic solution of sodium sulfite (0% oxygen; 1 g Na2SO3·100 ml of water) and in water vapor-saturated air in an enclosed vessel.

SMR using the laboratory-based system

An intermittent-flow respirometry system was used to measure MO2 during a series of open and closed water circulation phases over a 24 h period. During the open phase, fully oxygenated water was circulated through the system into the chambers using a flush pump. During the closed phase, water was circulated throughout the system using a recirculation pump, but no additional water or oxygen entered the system. During this phase, MO2 was measured in each chamber and logged by the oxygen meter every second (OXY-4 mini, PreSens, Regensburg, Germany/Witrox 4, Loligo Systems, Viborg, Denmark). The closed period lasted 140 s; however, measurements were not taken during the first 20 s to allow the chamber to achieve homogenous mixing based on observed oxygen depletion curves. The closed phase was followed by a 180 s flush period (open phase) where fully oxygenated water was pumped back through the system. The closed and open phase intervals were determined based on preliminary experiments, which demonstrated that sufficient oxygen depletion slopes were obtained but also that the oxygen content in chambers never approached concentrations that could be harmful to the fish (<9.0 mg O2 l−1; Graham, 1949; Spoor, 1990; Evans, 2007).

SMR using the field-based system

Field-system 1. Unlike the laboratory system, during the closed phase water was not re-circulated throughout the system using a pump. Therefore, the mixing period after the closed phase was longer (180 s) to allow for mixing via fish movement in the chamber once water flow had stopped. This interval was determined based on preliminary inspection of oxygen depletion slopes showing negligible decline over this period followed by relatively constant rates of decline. This resulted in a 180 s flush phase, 180 s of mixing during the closed phase, and 420 s for the closed measurement phase. Only the closed measurement phase was used to estimate MO2 rates. A total of six measurements were collected during a 78 min period in the chamber.

Field-system 2. The wait, measurement, and flush periods using this system were 20, 140, 180 s, respectively, and were similar to the periods used in the laboratory system during the first experiment. There were minor deviations in phase times because data from the first experiment showed that a shorter flush phase could be used to bring oxygen content in the chamber back to pre-measurement levels and shorter measurement periods could be used if water was re-
circulated during this phase. The same time intervals were used for both the field and laboratory systems in the second experiment because the flush and recirculating regimes were identical. The closed measurement phase was used to estimate MO$_2$ rates; however, unlike the first system, a total of 20 measurements were collected over a 113 min period in the chamber. This was done to see if metabolic rate estimates obtained during a 56 min period were similar to those obtained over a 113 min period.

**MMR using both systems.** MMR was estimated for both respirometry methods using the same chase protocol—an exhaustive chase bout in flowing water (Norin and Malte, 2011). Although a different observer implemented the chase protocol for each treatment in the first experiment, time to exhaustion did not differ between observers (t-test: $t = -1.789, P = 0.09$). After MO$_2$ measurements were taken for SMR estimates, individual fish were placed in a circular tank with flowing water that was $10^\circ C$, and chased using a small net until exhaustion. Fish were encouraged to burst forward into flowing water through repeated tapping on the side of the tank with the net and this resulted in multiple burst swim bouts, followed by slower swimming, and then exhaustion where fish struggled to maintain their position. In the first experiment, the exhaustion end point was reached when the fish could be easily netted three consecutive times without fleeing, whereas, in the second experiment this end point was reached when fish did not respond to a tail pinch (Roche et al., 2013). In the second experiment only one person conducted the chase protocol. Once exhaustion was reached each fish was quickly (~20–30 s) placed back into the respirometry chamber for three measurement cycles. The flush, mixing, and closed time intervals for the lab- and field-based systems were similar; however, the mixing phase in the initial field-based system was slightly longer lasting 30 s, whereas it was only 20 s in the second experiment because mixing occurred during this phase (Table 1). The very first flush phase was skipped at the beginning of each measurement series to ensure recovery by the fish did not impact MO$_2$ measurements. Following this initial measurement all subsequent cycles included a 140–180 s flush to ensure low oxygen concentrations did not occur in the chambers (Table 1).

**Data analyses**

MO$_2$ (mg O$_2$ h$^{-1}$) during the closed measurement intervals for SMR and MMR protocols (described above) was calculated as:

$$\text{MO}_2 = (\Delta \text{O}_2 - \text{BOD}) \cdot (V_R - V_F)$$  

(1)

where $\Delta \text{O}_2$ (mg O$_2$ l$^{-1}$ h$^{-1}$) is the average rate of decrease in oxygen concentration in the respirometry chamber during the closed phase, BOD (mg O$_2$ l$^{-1}$ h$^{-1}$) is the average rate of decrease in oxygen concentration measured in the respirometry chamber during six runs without fish—three before and three after each experiment, $V_R$ (l) is the volume of the respirometry chamber, and $V_F$ (l) is the assumed volume of the fish based on mass. The average value of BOD of each experiment was subtracted from all measurements. SMR was calculated as the value representing the lower 20th percentile of all MO$_2$ rates calculated from the SMR protocol (Chabot et al., 2016). MMR was estimated as the highest of the three MO$_2$ rates calculated using the MMR protocol (described above). In the first experiment, SMR and MMR were calculated from a subset of the MO$_2$ dataset where slopes of oxygen depletion had an $r^2 > 0.50$ (Fig. 3A and B), whereas only slopes with $r^2 > 0.80$ were used for the second experiment. AS was calculated as the difference between SMR and MMR for each fish (Clark et al., 2013).

**Experiment 1—Lake trout**

Analysis of covariance (ANCOVA, Type III) was used to test for the effects of MO$_2$ estimation method and fish body mass (g) on values of SMR, MMR and AS. In each ANCOVA, body mass was set as a continuous covariate and MO$_2$ estimation method (levels = laboratory or field) was set as a categorical variable (hereafter denoted ‘treatment’). To meet the assumptions of ANCOVA, body mass and response variables (SMR, MMR and AS) were log$_{10}$ transformed for analysis and the full model was specified as:

$$\log_{10}\text{SMR, MMR, or AS} = \log_{10}(M_b) \times \text{Treatment} + \log_{10}(M_b) + \text{Treatment}$$

(2)

where $M_b$ is the body mass of the fish expressed in grams (g).

The ANCOVA procedure, which included first testing for a significant log$_{10}(M_b)$× Treatment interaction and then testing for the effect of treatment, was used for model selection.

**Experiment 2—Brook trout**

A linear mixed effects model (LMM) was used to examine the relationship between MO$_2$ estimation method, fish body mass (g), and treatment order on values of SMR, MMR, and AS. In each LMM, the fixed effects were body mass, MO$_2$ estimation method (levels = laboratory or field), and treatment order—i.e. the sequence that fish were exposed to each treatment. Body mass was set as a continuous covariate and MO$_2$ estimation method (laboratory or field) and order (field/lab or lab/field) were set as categorical variables. Individual fish were specified as a random intercept on metabolic rate metrics. To meet the assumptions of LMM, body mass and response variables (SMR, MMR and AS) were log$_{10}$ transformed for analysis and the full model was specified as:

$$\log_{10}\text{SMR, MMR, or AS} = \log_{10}(M_b) + \text{Treatment} + \text{Order} + \text{(random = Fishid)}$$

(3)

where $M_b$ is the body mass of the fish expressed in grams (g).

All analyses were performed in R, version 3.1.3 (R Core Team, 2015) and we considered $P < 0.05$ as statistically significant.
Table 1: Comparison of closed and intermittent-flow respirometry systems used to estimate metabolic rate of salmonids in the field

| Reference                | Sample size | Mixing—closed phase | Measure period (h) | Water temperature (°C) | Fast period (h) | Acclimation period (h) | $r^2$ reported | $M_{O2\min}$—measurement phase (s) | $M_{O2\max}$—measurement phase (s) |
|--------------------------|-------------|---------------------|-------------------|------------------------|----------------|------------------------|----------------|-----------------------------------|-----------------------------------|
| This study               |             |                     |                   |                        |                |                        |                | Flush | Wait | Measure | Flush | Wait | Measure |
| Lake trout               |             |                     |                   |                        |                |                        |                |        |       |         |        |       |         |
| Laboratory               | 8           | Yes                 | 12                | 10 ± 0.02             | 24             | 24                     | Yes            | 180   | 20   | 120     | 180   | 20   | 120     |
| Field                    | 11          | No                  | 1                 | 10 ± 1.0              | 24             | 24                     | Yes            | 180   | 180  | 420     | 180   | 30   | 120     |
| Field studies            |             |                     |                   |                        |                |                        |                |        |       |         |        |       |         |
| Rasmussen et al. (2012)  |             |                     |                   |                        |                |                        |                |        |       |         |        |       |         |
| 98 (~7/treat)            | No          | 0.5                 | Ambient-river     | 0.5–1.0               | No             | –                      | –              | 1800  | NA   | NA      | NA    | NA   | NA      |
| Hartman and Jensen (2016)|             |                     |                   |                        |                |                        |                |        |       |         |        |       |         |
| 29 (15, 14)              | No          | 24                  | Ambient-river: range = 1.1 ± 0.2 | NS | 24 | No | – | – | Not specified—measured until O$_2$ in chamber depleted by 1.0 mg l$^{-1}$ | NA | NA | NA |
| Warnock and Rasmussen (2014) |             |                     |                   |                        |                |                        |                |        |       |         |        |       |         |
| 46 (23, 23)              | No          | 1                   | Ambient-river     | 0.5                    | 0              | No | – | – | 3600 | NA | NA | NA |
| Gamperl et al. (2002)$^b$|             |                     |                   |                        |                |                        |                |        |       |         |        |       |         |
| 33 (17, 16)              | Yes         | NS                  | 12, 24            | ~12                    | No             | – | – | 360–600 | – | – | 360–600 |
| Rodnick et al. (2004)$^b$|             |                     |                   |                        |                |                        |                |        |       |         |        |       |         |
| 31 (9, 8, 7, 7)          | Yes         | NS                  | 12, 24            | ~12                    | No             | – | – | 360–600 | – | – | 360–600 |

Note: NS = not specified; NA = not applicable; $M_{O2\min}$ = minimum oxygen consumption; $M_{O2\max}$ = maximum oxygen consumption.

$^a$Total sample size with individual number/treatment in parentheses.

$^b$This study used a swim tunnel to measure $M_{O2\min}$ and $M_{O2\max}$ and fasting periods were in enclosures in the river but drift was still accessible.
Results

Lake trout experiment

The mean and standard deviation of SMR, MMR and AS was 66.8 (SD = 26.3), 256.2 (SD = 42.8) and 189.4 (SD = 53.1), respectively, for the laboratory treatment, and 59.1 (SD = 22.4), 278.4 (SD = 143.3) and 219.4 (SD = 140.0), respectively, for the field treatment.

The slope of the relationship between the calculated metabolic rate metrics (SMR, MMR and AS) and fish body mass did not differ between treatments (SMR-Mass x Treatment: $F_{1,15} = 0.343, P = 0.566$; MMR-Mass x Treatment: $F_{1,15} = 1.04, P = 0.324$; AS-Mass x Treatment: $F_{1,14} = 0.901, P = 0.358$) and the intercept of SMR, MMR and AS values estimated by each treatment also did not differ (SMR-Treatment: $F_{1,16} = 0.979, P = 0.337$; MMR-Treatment: $F_{1,16} = 0.007, P = 0.931$; AS- Treatment: $F_{1,16} = 0.048, P = 0.829$) (Fig. 4A and B). Together, these results indicate that the metabolic rate metrics did not differ among respirometry methods. As expected, SMR, MMR and AS increased as a function of body mass ($\log_{10}(\text{SMR}) = -1.37 + 0.762 \times \log_{10}(M_b), n = 19, r^2 = 0.20, P = 0.05$; $\log_{10}(\text{MMR}) = -2.74 + 1.21 \times \log_{10}(M_b), n = 19, r^2 = 0.39, P = 0.004$; $\log_{10}(\text{AS}) = -2.94 + 1.41 \times \log_{10}(M_b), n = 19, r^2 = 0.32, P = 0.012$) (Fig. 5A–C).

Brook trout experiment

The mean and standard deviation of SMR, MMR and AS was 58.0 (SD = 10.4), 504.0 (SD = 119.2) and 447.0 (SD = 77.2), respectively, for the laboratory treatment, and 65.5 (SD = 12.8), 504.0 (SD = 119.2) and 440.5 (SD = 117.0), respectively, for the field treatment.

The order (field/lab vs lab/field) that fish went into each system did not have a significant effect on SMR, MMR or
AS between treatments (SMR-Order: $F_{1,15} = 0.319, P = 0.581$; MMR-Order: $F_{1,15} = 0.166, P = 0.680$; AS-Order: $F_{1,15} = 0.232, P = 0.637$) (Table 2). Similarly, SMR, MMR and AS estimates did not differ between treatments (SMR-Treatment: $F_{1,15} = 1.75, P = 0.205$; MMR-Treatment: $F_{1,15} = 0.125, P = 0.730$; AS-Treatment: $F_{1,15} = 0.430, P = 0.523$) (Table 2; Fig. 4C and D). Together, these results indicate that the metabolic rate metrics did not differ among respirometry methods or our experimental design (i.e. order of treatments). However, SMR and MMR, did increase as a function of body mass \([\log_{10}(\text{SMR}) = -1.54 + 1.24 \times \log_{10}(M_b), n = 17, r^2 = 0.52, P = 0.0004; \log_{10}(\text{MMR}) = 0.176 + 0.667 \times \log_{10}(M_b), n = 17, r^2 = 0.26, P = 0.02]\) (Fig. 5D and E). Although AS did increase as a function of body mass, this relationship was not significant at the 0.05 level \([\log_{10}(\text{AS}) = 0.25 + 0.550 \times \log_{10}(M_b), n = 17, r^2 = 0.17, P = 0.07]\) (Fig. 5F).

Because each metabolic rate metric increased linearly with mass on a \(\log_{10}-\log_{10}\) scale for both species, we standardized each value to the mean size of fish used in each experiment (lake trout = 23.7 g; brook trout = 28.3 g) by centering the residuals of the \(\log_{10}-\log_{10}\) relationship of whole body SMR, MMR and AS to each value predicted for a 23.7 g lake trout and 28.3 g brook trout using the regression equations above.

**Discussion**

This field-based intermittent-flow respirometry system is a promising option for measuring metabolic rates of fishes in remote areas. It offers users a smaller and simpler apparatus with fewer power requirements than conventional lab-based systems and some other field-based systems (Farrell *et al.* 2003). For instance, our system differs from the one used by Farrell *et al.* (2003) because it can be transported more easily (e.g. backpack or using a small boat or helicopter); does not require the same level of power to run (i.e. all requirements can be met by simple 12 V DC power sources that can be recharged using solar panels); and uses a static respirometry chamber as opposed to a swim tunnel, which in Farrell *et al.* (2003) included a 7.5 hp, 208 V, motor.

Using our simplified system we demonstrate that the mean SMR, MMR and AS estimates obtained from both juvenile lake trout and brook trout using both of our field
systems are similar to those of the common laboratory-based setup and are within the range of values reported in the literature (Graham, 1949; Evans, 2007). For our SMR estimates in particular, agreement between field and laboratory methods is strong, and the level of variability among fish using both methods is relatively low when compared to similar studies (Evans, 2007; Kelly et al., 2014). In addition, our second experiment demonstrates that adding a simple mixing device and minor changes to experimental design can yield more precise estimates using this field system. Furthermore,
getting similar results with brook trout to those of lake trout suggest that this system is well suited for interspecific comparisons of metabolic rate of freshwater fishes.

Although mean MMR values from the field and laboratory methods were not significantly different in the first experiment, the range of estimates obtained using the field method was higher and yielded greater variances. However, other studies have also found that MMR estimates can be more variable than SMR estimates (Reidy et al., 1995; Norin and Malte, 2011; Roche et al., 2013). We propose several reasons for this result. First, the end point used after the exhaustive chase protocol in this study may have increased variability in these estimates between treatments, given that two different individuals conducted each experiment. The ability of any given observer to consistently determine the end point used in our first experiment—i.e. that an individual fish can be easily netted three times—did vary within treatments, however, there were no differences in time until exhaustion between treatments/observers. In a recent experiment with this lake trout population where a physical stimulation end point was used (i.e. non-response to caudal pinch), less variable exhaustion times were observed (Matthew Guzzo, unpublished data). The physical stimulation end point is different from our method, as fish are deemed to be exhausted when they fail to elicit any response to a physical stimulus, such as a tail pinch (Roche et al., 2013). This latter method, while more invasive to the animal, has been shown to provide more precise results in other studies, and therefore was adopted for use in the second experiment (Norin and Malte, 2011; Roche et al., 2013; Kelly et al., 2014). Second, mixing during the closed phase has been shown to influence the quality of results from intermittent-flow respirometry (Clarke et al., 2013; Svendsen et al., 2016; Rodgers et al., 2016). In the first field system, where no mixing occurred during the closed phase, it is possible that oxygen stratification occurred within the chamber and probably influenced oxygen mixing characteristics leading to more variability in oxygen depletion slopes. The results from our second experiment, which used a mixing device, were much less variable and demonstrate noticeable improvements in the quality of our results (Fig. 3). Third, the fish selected for the first experiment were on a maintenance feeding regime for a longer period (10 weeks), which may have reduced growth slightly and could have led to slightly lower SMR estimates and greater variation in MMR during the experiment. This was addressed in the design of the second experiment.

High variability observed in MMR values produced by the first field system could be problematic when interpreting AS estimates between treatments. In situations where less pronounced differences in AS exist between populations, highly variable MMR measurements could mask these differences. Therefore, we suggest that potential users consider using the second field respirometer despite it being less compact as the first system.

The field-based system that we tested builds upon similar systems that others have used in the field (Farrell et al., 2003; Rasmussen et al., 2012; Warnock and Rasmussen, 2014; Hartman and Jensen, 2016) and provides population-level estimates of SMR, MMR and AS similar to the commonly used laboratory-based system. We see several key

Table 2: Results of linear mixed effect models testing for the influence of respirometry treatment and mass on standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS) estimates obtained from brook trout

| Response | Fixed factors | $F$ | $n_{df}$, $d_{df}$ | $P$ | Parameter | est | se | $df$ | $t$ | $P$ | $R^2_{m}$ |
|----------|---------------|----|-------------------|----|-----------|-----|----|-------|-----|-----|-----------|
| SMR      | Treatment-Lab | 1.74 | 1, 15 | 0.21 | Treatment-Lab | −0.04 | 0.03 | 15 | −1.32 | 0.21 | 0.52 |
|          | Mass          | 29.0 | 1, 15 | <0.001 | Mass | 1.24 | 0.23 | 15 | 5.38 | <0.001 |
|          | Order-Lab/Field | 0.31 | 1, 15 | 0.58 | Order-Lab/Field | −0.02 | 0.03 | 15 | −0.56 | 0.58 |       |
|          | Intercept     | 21.64 | 1, 15 | 0.0003 | Intercept | −1.54 | 0.33 | 15 | −4.65 | 0.0003 |       |
| MMR      | Treatment-Lab | 0.13 | 1, 15 | 0.73 | Treatment-Lab | 0.01 | 0.02 | 15 | 0.35 | 0.73 | 0.26 |
|          | Mass          | 6.38 | 1, 15 | 0.02 | Mass | 0.67 | 0.26 | 15 | 2.53 | 0.02 |       |
|          | Order-Lab/Field | 0.17 | 1, 15 | 0.68 | Order-Lab/Field | 0.02 | 0.04 | 15 | 0.41 | 0.68 |       |
|          | Intercept     | 0.25 | 1, 15 | 0.62 | Intercept | 0.18 | 0.37 | 15 | 0.50 | 0.62 |       |
| AS       | Treatment-Lab | 0.43 | 1, 15 | 0.52 | Treatment-Lab | 0.02 | 0.02 | 15 | 0.65 | 0.52 | 0.17 |
|          | Mass          | 3.71 | 1, 15 | 0.07 | Mass | 0.55 | 0.28 | 15 | 1.92 | 0.07 |       |
|          | Order-Lab/Field | 0.23 | 1, 15 | 0.64 | Order-Lab/Field | 0.02 | 0.04 | 15 | 0.48 | 0.64 |       |
|          | Intercept     | 0.44 | 1, 15 | 0.52 | Intercept | 0.25 | 0.41 | 15 | 0.66 | 0.52 |       |

Individuals were treated as a random factor in each model and accounted for 5.0%, 42% and 42% of the variation in SMR, MMR and AS, respectively. $R^2_{m}$ are marginal and represent the proportion of variance in each response variable accounted for by the fixed factors. The structure of each model was: Response = Treatment + Mass + Order + (random intercept = FishID).
advances with our field-based system: (1) the ability to acclimate fish to the respirometry chamber over extended periods; (2) users can gather MO2 measurements during a shorter period (1 h), which yield metabolic rate estimates comparable to systems that take measurements over a 24 h period; (3) ability to take repeated measures using a similar flush and measurement phase routine, typically used in most laboratory-based systems; and (4) a simpler system requiring less power and equipment to operate, yet allows for separation between the chamber and the researcher (Table 1; Fig. 1B–D).

The results of this study show that this field-based intermittent-respirometry system should yield reliable metabolic rate estimates in field settings. Potential users of this system would benefit from additional laboratory experiments examining how different fasting/acclimation periods may influence SMR estimates, as shorter acclimation periods would be more practical in the field and could help users increase their sample size. Developing practical and reliable field tools to acquire physiological data will help improve our understanding of the adaptive capacity of organisms occupying a broad range of environmental conditions. Moreover, gathering metabolic rate estimates for imperiled fishes (e.g. bull trout, *Salvelinus confluentus*) that cannot be transferred to laboratory settings will be a valuable step in understanding range-wide intraspecific variation in the physiological capacity of these fishes. This knowledge can be used to develop a mechanistic understanding of how populations may respond to changes in their environment and can be explicitly integrated into mechanistic niche models (Kearney and Porter, 2009) to inform conservation planning.

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