Lactate kinetics of rainbow trout during graded exercise: 
Do catheters affect the cost of transport?

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Running head: Lactate kinetics in swimming trout

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SUMMARY

Changes in lactate kinetics as a function of exercise intensity have never been measured in an ectotherm. Continuous infusion of tracer is necessary to quantify rates of lactate appearance (Ra) and disposal (Rd), but it requires double catheterization that could interfere with swimming. Using rainbow trout, our goals were: (1) to determine the potential effects of catheters and blood sampling on metabolic rate (MO2), total cost of transport (TCOT), net cost of transport (NCOT), and critical swimming speed (Ucrit), and (2) to monitor changes in lactate fluxes during prolonged, steady-state swimming or graded swimming from rest to Ucrit. This athletic species maintains high baseline lactate fluxes of 24 µmol kg⁻¹ min⁻¹ that are only increased at intensities >2.4 body lengths per s (BL s⁻¹) or 85% Ucrit. As the fish reaches Ucrit, Ra lactate is more strongly stimulated (+67% to 40.4 µmol kg⁻¹ min⁻¹) than Rd lactate (+41% to 34.7 µmol kg⁻¹ min⁻¹), causing a 4-fold increase in blood lactate concentration. Without this stimulation of Rd during intense swimming, lactate accumulation would double. By contrast, steady-state exercise at 1.7 BL s⁻¹ increases lactate fluxes to ~30 µmol kg⁻¹ min⁻¹, with a trivial mismatch between Ra and Rd that only affects blood concentration minimally. Results also show that the catheterizations and blood sampling needed to measure metabolite kinetics in exercising fish have no significant impact on MO2 or TCOT. However, these experimental procedures affect locomotion energetics by increasing NCOT at high speeds and by decreasing Ucrit.
INTRODUCTION

As glycolytic end-product, oxidative fuel and gluconeogenic substrate, lactate is one of the most dynamic intermediates of cell metabolism (Brooks, 1991; Gladden, 2004; Philp et al., 2005). Animals process lactate at high rates even under resting, normoxic conditions and, in mammals, many studies show that inherently high baseline lactate fluxes are strongly stimulated during exercise (Bergman et al., 1999; Donovan and Brooks, 1983; Issekutz Jr et al., 1976; Stanley et al., 1985; van Hall et al., 2003; Weber et al., 1987). Little is known for fish because adequate methods to quantify lactate fluxes accurately under controlled exercise conditions have only become available recently. Previous studies of fish lactate kinetics report turnover rates that were estimated by bolus injection (Cameron and Cech Jr., 1990; Milligan and McDonald, 1988; Weber, 1991; Weber et al., 1986): an obsolete method with significant limitations (Omlin and Weber, 2010; Wolfe, 1992). They show that the lactate turnover rate of rainbow trout doubles during prolonged, low-intensity swimming (Weber, 1991). Although no measurements have been made at higher swimming speeds, a 3 to 10-fold increase over baseline has been observed during recovery from exhausting exercise for channel catfish (*Ictalurus punctatus*), coho salmon (*Oncorhynchus kisutch*), and starry flounder (*Platichthys stellatus*) (Cameron and Cech Jr., 1990; Milligan and McDonald, 1988). Continuous tracer infusion techniques have been adapted and validated for fish (Haman et al., 1997; Haman and Weber, 1996). They have been used to quantify the rates of metabolite appearance (R_a) and disposal (R_d) accurately under non-steady state conditions. In particular, continuous infusion has been used to characterize the effects of swimming on glucose and lipid kinetics (Bernard et al., 1999; Magnoni et al., 2008; Shanghavi and Weber, 1999). Unfortunately, these fish measurements only deal with prolonged, low-intensity swimming. Therefore, nothing is known about the differential effects of higher intensity exercise on the rates of lactate production and disposal, when significant changes in blood lactate concentration are observed.

Measuring metabolite kinetics by continuous infusion during swimming may be problematic because two catheters exiting from the snout of the fish are necessary (Haman and Weber, 1996). The hydrodynamic drag associated with these catheters may interfere with locomotion, and blood sampling may decrease capacity for oxygen transport. Therefore,
cannulated fish may have a different metabolic rate (MO$_2$), a higher cost of transport [total cost (TCOT) or net cost (NCOT)] (Schmidt-Nielsen, 1972), and a lower critical swimming speed ($U_{crit}$; Farrell, 2008) than non-catheterized animals. The cost of transport is the amount of energy (or oxygen) used to move one unit body mass by one unit distance. TCOT is the total amount of energy needed to power movement, including the cost of sustaining life in resting tissues. By contrast, NCOT only accounts for the cost of locomotion, but it excludes all maintenance costs incurred at rest. To allow meaningful comparisons between fish studies, exercise intensity is traditionally standardized as %$U_{crit}$ or as swimming speed in body lengths/s (BL s$^{-1}$). However, it is unclear whether non-instrumented and catheterized animals swimming at the same %$U_{crit}$ or at the same speed have the same MO$_2$. Therefore, the goals of this study were: (1) to determine whether double catheterization and blood sampling have an effect on MO$_2$, TCOT, NCOT or $U_{crit}$ in rainbow trout, (2) to apply continuous tracer infusion methods to measure the effects of steady swimming on lactate fluxes, and (3) to determine the relationship between exercise intensity and the rates of lactate production and disposal, using a graded swimming protocol.

**METHODS**

**Animals**

Male and female rainbow trout (*Oncorhynchus mykiss*, Walbaum) were purchased from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada)(see Table 1). They were held in a 1300-liter flow-through tank containing dechlorinated, well-oxygenated water at 13°C for at least 2 weeks before experiments. Fish were kept under a 12h:12h light-dark photoperiod and fed commercial floating pellets (Martin Mills, Elmira, Ontario, Canada) 3 times a week until satiation. The effects of exercise were either measured during prolonged, steady-state swimming at 1.7 BLs$^{-1}$ or during graded swimming ($U_{crit}$ protocol). Fish used for graded exercise were randomly divided in 2 sub-groups: control/sham-catheterization (to measure swimming energetics only) and actual catheterization (to measure swimming energetics and lactate kinetics). To avoid training or fatigue effects in the first sub-group, locomotion energetics were measured in random order for the control (no catheters) and sham-catheterized
conditions in the same individuals. The steady-state swimming group was catheterized to
measure lactate kinetics only. All procedures were approved by the animal care committee of
the University of Ottawa and adhered to the guidelines established by the Canadian Council on
Animal Care for the use of animals in research.

Catheterizations

Fish were fasted for at least 24 h before surgery. They were anesthetized with ethyl-N-
aminobenzoate sulfonic acid (MS-222; 60 mg L⁻¹) in well oxygenated water. The animals used
to measure lactate kinetics were doubly cannulated in the dorsal aorta using PE-50 catheters
(Intramedic, Clay-Adams, Sparks, MD, USA), as detailed in (Haman and Weber, 1996). The
catheters were kept patent by flushing with Cortland saline (Wolf, 1963) containing 50 U ml⁻¹
heparin (Sigma-Aldricht, St-Louis, MO, USA). Only animals with a hematocrit >20% after
recovery from surgery were used in tracer experiments. For the sham-catheterized group, the
two catheters were sutured to the palate, but they were not inserted in the dorsal aorta. The aim
of sham-catheterization was to produce the same hydrodynamic drag experienced during actual
tracer experiments, but without affecting the vasculature or drawing blood samples.

Swim tunnel respirometry

All experiments were carried out at 13°C in a 90 l swim tunnel respirometer (Loligo
Systems, Tjele, Denmark) filled with the same quality water as the holding tank. A
“honeycomb” grid was placed before the swimming chamber to promote laminar flow. The
fish always swam in the anterior part of the chamber (kept dark) to avoid the posterior part
(brightly lit). The swim tunnel was calibrated with a flow probe (Global Water Geotech,
Denver, CO, USA) to establish the linear relationship between water velocity (in cm/s) and
motor speed (in RPM). Swimming speeds were corrected for solid blocking as in (Claireaux et
al., 2006). Metabolic rate (MO₂) was measured by intermittent flow respirometry using
galvanic oxygen probes connected to a DAQ-PAC-G1 instrument controlled with AutoResp™
software (ver. 2; Loligo Systems). The oxygen probes were calibrated before measurements
using N₂-saturated water (0% O₂) and air-saturated water (20.9% O₂). Before experiments,
each fish was placed in the swim tunnel overnight for acclimation to the experimental setup.
During this period, water velocity was kept at 0.5 BL s⁻¹, a low speed requiring no swimming
but enabling the fish to rest at the bottom of the respirometer. Critical swimming speed ($U_{\text{crit}}$) and the effects of graded exercise on $MO_2$, cost of transport and lactate kinetics were quantified using a stepwise $U_{\text{crit}}$ protocol (Jain et al., 1997) with velocity increments of 0.2 BL $s^{-1}$ every 20 min. Graded swimming experiments were terminated at exhaustion when the fish was unable to remove itself from the rear grid.

**Lactate kinetics**

The catheters were made accessible through the swim tunnel lid by channeling them through a water-tight port. The rates of lactate appearance ($R_a$) and lactate disposal ($R_d$) were measured by continuous infusion of [U-14C] lactate (New England Nuclear, Boston, MA, USA; 4.84 GBq mmol$^{-1}$). Infusates were freshly prepared immediately before each experiment by drying an aliquot of the solution obtained from the supplier under $N_2$ and resuspending in Cortland saline. Labeled lactate was infused for 1 h in resting fish to quantify baseline lactate kinetics. It was administered at a rate of 2029 ± 227 Bq kg$^{-1}$ min$^{-1}$ (N=15) using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA) at 1 ml h$^{-1}$. Under these conditions, isotopic steady-state is reached in <45 min (Omlin and Weber, 2010). Lactate (labelled+unlabelled) was infused at rates accounting for <0.002% of the endogenous $R_a$ lactate measured in resting fish. Tracer infusion was continued either for 4-5 h to complete a $U_{\text{crit}}$ protocol (graded exercise experiments) or for 2 h at 1.7 BL $s^{-1}$ (steady-state exercise experiments). The water was kept normoxic throughout the measurements (10.64 ± 0.07 mg $O_2 l^{-1}$). Blood samples (100 µl each) were drawn at the end of the initial resting period and at regular intervals during swimming (5 min before each stepwise velocity increment for graded exercise and every 10 min for steady-state exercise). The total amount of blood sampled from each fish accounted for <10% of blood volume. Samples were immediately deproteinized in 200 µl perchloric acid (6% w/w) and centrifuged for 5 min at 16,000 G (Eppendorf 5415C, Brinkmann, Rexdale, Canada). Supernatants were kept frozen at -20°C until analyses.

**Sample analyses**

Blood lactate concentration was measured spectrophotometrically (Bergmeyer, 1985) using a Spectra Max plus 384 (Molecular Devices, Sunnyvale, CA, USA). To measure activity, lactate was separated using ion exchange columns as described in (Omlin and Weber,
Before passing through the columns, each deproteinized blood sample was neutralized with 1 M potassium bicarbonate and diluted with 5 ml deionized H$_2$O. Preliminary experiments with known amounts of labeled lactate showed that 70% of total activity was recovered, and measured lactate activities were corrected accordingly. Radioactivity was measured by scintillation counting (Beckman Coulter LS 6500, Fullerton, CA, USA) in Bio-Safe II scintillation fluid (RPI Corp, Mount Prospect, IL, USA).

**Calculations and statistics**

Critical swimming speed ($U_{\text{crit}}$ in BL s$^{-1}$) was calculated according to (Brett, 1964):

$$U_{\text{crit}} = V_f + [(t_f/t_i) \times V_i]$$

Where $V_f$ was the highest speed at which a full time interval was completed (in BL s$^{-1}$), $V_i$ was the speed increment between intervals (0.2 BL s$^{-1}$), $T_f$ was the time spent swimming during the last interval causing exhaustion (in min), and $T_i$ was the full interval (20 min). The amount of energy needed to transport one unit body mass by one unit distance, or total cost of transport (TCOT) (Schmidt-Nielsen, 1972) was calculated from total MO$_2$ as follows:

$$\text{TCOT} = \frac{\text{total MO}_2}{U}$$

Where TCOT is in μmol O$_2$ kg$^{-1}$ m$^{-1}$, MO$_2$ is the metabolic rate in μmol O$_2$ kg$^{-1}$ min$^{-1}$, and U is the swimming speed in m min$^{-1}$. The net cost of transport (NCOT) was calculated similarly, but from net MO$_2$ defined as swimming MO$_2$ minus resting MO$_2$. Resting MO$_2$ was obtained by averaging the ten lowest values recorded during the night preceding the exercise measurements. The rates of lactate appearance ($R_a$) and disposal ($R_d$) were calculated using the non-steady state equations of Steele (Steele, 1959). Statistical comparisons were performed using one- or two-way repeated measures analysis of variance (RM ANOVA) with Dunnett’s post-hoc test to determine which means were different from baseline, or Holm-Sidak test to compare treatments (SigmaPlot v.12, Systat Software, Inc., San Jose, CA, USA). When the assumptions of normality (Shapiro-Wilk test) or homoscedasticity (Levene test) were not met, Friedman RM ANOVA on ranks was used or the data were normalized by log$_{10}$ transformation.
before parametric analysis. All values presented are means ± s.e.m and P<0.05 was used as level of significance in all tests.

RESULTS

1. Graded swimming

Metabolic rate

Resting rates of oxygen consumption were not different between treatments (P>0.05; Table 1). Metabolic rate increased progressively with swimming speed and was higher than resting MO2 at all speeds (P<0.001; Fig. 1A). The exercise-induced increase in MO2 was not significantly different between groups (P>0.05). The highest MO2 was reached at the end of the graded exercise protocol (Table 1). Two individuals of the control group were able to reach the highest swimming speed of 3.6 BL s⁻¹ (Fig. 1A). Figure 1B shows changes in MO2 as a function of exercise intensity expressed as %Ucrit. The 3 treatments were not different from each other (P>0.05) and the pooled data were fitted with a polynomial regression of second order (P<0.001, r²=0.72): MO2=109.136 – 1.669 (%Ucrit) + 0.0285 (%Ucrit)².

Cost of transport and Ucrit

Across speeds, treatment groups had the same TCOT (P=0.28; Fig. 2A), but a different NCOT (P<0.01; Fig. 2B). Maximum TCOT was measured at the lowest swimming speed of 0.8 BL s⁻¹ for all groups (Fig. 2A). As exercise intensity was increased, TCOT became lower than maximal values for all swimming speeds above 1 BL s⁻¹ (P<0.001). Averaged among groups, TCOT decreased from a maximum of 5.4 µmol O₂ kg⁻¹ m⁻¹ at 0.8 BL s⁻¹ to a minimum of 3.1µmol O₂ kg⁻¹ m⁻¹ at 2.2 BL s⁻¹. NCOT was also affected by exercise intensity (Fig. 2B) and was higher between 2.4 and 3.2 BL s⁻¹ than for the lowest swimming speed (P<0.01). Treatments had a significant effect on Ucrit (P<0.05; Fig. 3) that was highest in controls (3.4 BL s⁻¹), intermediate in sham-catheterized (3.1 BL s⁻¹), and lowest for lactate kinetics (2.8 BL s⁻¹). Minimum TCOT was the same for all treatments (P>0.05), but occurred at different swimming speeds. Minimal NCOT was not different between controls and sham-catheterized (P>0.05), but it was higher for sham-catheterized than for lactate kinetics (P<0.05). Minimal
TCOT was measured at higher swimming speeds (2.0-2.4 BL s^{-1}) than minimal NCOT (1.0-1.2 BL s^{-1}).

**Effects of exercise intensity on lactate metabolism**

Swimming speed was progressively increased over 4 h, following a classic stepwise $U_{crit}$ protocol (Fig. 4A). Both, $R_a$ lactate (P<0.001) and $R_d$ lactate (P<0.01) were strongly stimulated over time as exercise intensity increased (Fig. 4B). Mean $R_a$ lactate values above 2.4 BL s^{-1} (or above 85% $U_{crit}$) were higher than baseline (P<0.05). $R_a$ lactate increased from a baseline level of 24.2 µmol kg^{-1} min^{-1} to a maximum of 40.4 µmol kg^{-1} min^{-1}. $R_d$ lactate increased from 24.6 to a maximum of 34.7 µmol kg^{-1} min^{-1}. Lactate concentration increased from a baseline value of 1.3 mM to a maximum of 5.1 mM with exercise intensity (P<0.001; Fig. 4C). Mean blood lactate concentrations for speeds above 2.0 BL/s were higher than baseline (P<0.05).

### 2. Steady-state swimming

**Metabolic rate**

The first 60 min were monitored at rest to quantify baseline lactate kinetics. The transition from rest to steady-state swimming was made progressively over 30 min before maintaining a constant speed of 1.7 BL s^{-1} for 90 min (Fig. 5A). Metabolic rate increased from resting levels of ~80 µmol O₂ kg^{-1} min^{-1} to a maximum of 126.8 µmol O₂ kg^{-1} min^{-1} after 40 min of exercise (Fig. 5B). MO₂ was maintained above resting values between 40 and 80 min (P<0.05) before declining to 99.9 µmol O₂ kg^{-1} min^{-1} over the last 30 min.

**Steady exercise and lactate metabolism**

Blood lactate concentration increased from a resting value of 0.7 to ~1.4 mM during the first 30 min of steady swimming at 1.7 BL/s, and stayed at that level until the end of the experiment (P<0.05; Fig. 6A). Both, $R_a$ and $R_d$ lactate increased over time (P<0.001; Fig. 6B) from baseline values of 22.4 µmol kg^{-1} min^{-1} ($R_a$) and 23.7 ($R_d$) to maximal levels of 30.9 ($R_a$) and 29.8 ($R_d$). Mean $R_a$ and $R_d$ lactate were higher than baseline between 30 and 50 min of steady-state swimming (P<0.05), but returned to resting values for the last 60 min of exercise (P>0.05).
DISCUSSION

This study is the first to characterize the relationship between exercise intensity and lactate kinetics in an ectotherm. It shows that the lactate fluxes of rainbow trout are stimulated at speeds greater than 2.4 BL s\(^{-1}\) (or \(\sim 85\%\) \(U_{\text{crit}}\)), when lactate production starts diverging from lactate disposal. At these high exercise intensities, the change in \(R_a\) lactate stops being matched by the increase in \(R_d\) lactate, leading to a significant accumulation of glycolytic end product in the circulation. By contrast, steady-state submaximal exercise causes \(R_a\) and \(R_d\) lactate to increase similarly from \(-20\) to \(-30\) \(\mu\)mol kg\(^{-1}\) min\(^{-1}\), with a trivial mismatch between production and disposal that only affects blood concentration minimally (from 0.7 to 1.4 mM).

Earlier measurements by bolus injection had underestimated true lactate fluxes, but the same relative effect of steady, low-intensity swimming was observed (Weber, 1991). Present results show that catheterization has no impact on metabolic rate and cost of transport below \(85\%\) \(U_{\text{crit}}\). At these submaximal speeds, swimming energetics are not affected by the catheters or by sampling blood, and, therefore, all the parameters of metabolite kinetics measured by continuous tracer infusion also apply to non-instrumented fish. Above \(85\%\) \(U_{\text{crit}}\), catheterization increases NCOT, and direct comparisons between intact and instrumented animals swimming at the same speed should be made with caution.

Lactate production during swimming

Below \(85\%\) \(U_{\text{crit}}\), swimming has no effect on \(R_a\) and \(R_d\) lactate of rainbow trout (Fig. 4). At higher speeds, glycolysis is sharply stimulated, causing an increase in lactate production from 24 to 40 \(\mu\)mol kg\(^{-1}\) min\(^{-1}\) (Fig. 4B). This 67% rise in \(R_a\) lactate was measured at the highest speed allowing metabolite flux measurements in a swimming fish. Trout may be able to upregulate \(R_a\) lactate more strongly than reported here because previous studies suggest several-fold changes for flounder, salmon and catfish between rest and recovery from exhausting exercise (Cameron and Cech Jr., 1990; Milligan and McDonald, 1988). However, these fluxes measured post-exercise were estimated by bolus injection and may need to be confirmed with more reliable methods.
The stimulation of lactate flux is stronger in mammals than in trout because submaximal exercise induces a 6-fold increase in dogs (Issekutz Jr et al., 1976), thoroughbred horses (Weber et al., 1987), and humans (Bergman et al., 1999). Moreover, humans can increase lactate production by 22-fold over resting values during a graded exercise protocol similar to what was used here for fish (Stanley et al., 1985). Trout may only be able to show a modest relative increase in flux because their metabolic scope is much smaller than in mammals (Brett, 1972). Also, greater stimulation of lactate fluxes may not be possible for trout because their baseline levels could already be quite high. This notion is supported by the fact that the $R_a$ lactate/MO$_2$ ratios of trout and humans are similar during intense exercise (8.9 for trout vs 6.4 for humans), but much higher in resting trout (19.5) than in resting humans (only 2.9)(Stanley et al., 1985; this study).

**Intense exercise stimulates lactate disposal**

Above 85% $U_{crit}$, $R_d$ lactate increases by 41% (Fig. 4B). Without this response, circulating lactate would reach twice the concentration actually observed at the end of exercise (Fig. 4C). Therefore, increasing the rate of lactate disposal during intense swimming plays an important role in reducing the lactate load on the circulation, a metabolic strategy previously noticed during exposure to hypoxia (Fig. 5 in Omlin and Weber, 2010). Such a response is rather surprising at a time when anaerobic glycolysis is stimulated. As the only two pathways available for lactate clearance, how could gluconeogenesis and/or oxidation contribute to the increase in $R_d$ lactate? The effects of swimming on gluconeogenesis have never been measured directly in fish, but several tracer studies suggest that this pathway is not stimulated by exercise (Moyes and West, 1995). Hepatic glucose production actually decreases during submaximal swimming, but it is unclear whether gluconeogenesis or glycogenolysis are responsible for this decline (Shanghavi and Weber, 1999). Intuitively, stimulating gluconeogenesis during swimming would seem undesirable because glucose synthesis is energetically costly (6 ATP/glucose (Clark et al., 1974)). Overall, current information suggests that the stimulation of lactate disposal reported here is not accounted for by gluconeogenesis, but by an increase in lactate oxidation. Highly aerobic tissues like heart, red muscle, kidney and brain can readily use lactate as an oxidative fuel (Bilinski and Jonas, 1972; Soengas and Aldegunde, 2002) and they are probably responsible for increasing lactate clearance during
exercise. For example, perfused trout heart experiments show that lactate oxidation is
stimulated when cardiac work or lactate availability become elevated (Lanctin et al., 1980;
Milligan and Farrell, 1991). In addition, important physiological roles for various lactate
shuttles have been demonstrated in mammals (Brooks, 1998; Gladden, 2004). Exercising fish
may also rely on lactate shuttles to transport the end product from white muscle to aerobic
tissues for oxidation. In trout, however, intertissue lactate shuttles may be constrained by white
muscle lactate retention: a phenomenon that has intrigued fish biologists for decades (Turner
and Wood, 1983; Wang et al., 1997). We have recently demonstrated that white muscle has a
very limited capacity to export lactate because this tissue shows minimal expression of
monocarboxylate transporters (MCTs), even after exercise (Omlin and Weber, 2013).
Presumably, $R_d$ lactate could be stimulated a lot more than observed here during exercise if
white muscle expressed MCTs at the higher levels prevalent in mammalian glycolytic fibers.
The spatial separation of fish white and red muscles also precludes the intramuscular lactate
shuttle between adjacent glycolytic and oxidative fibers well characterized within mixed
mammalian muscles (Brooks, 1998; van Hall, 2000).

**Previous experiments by bolus injection underestimated lactate fluxes**

Continuous tracer infusion is the preferred method to quantify in vivo metabolite fluxes
accurately in humans and animals (Wolfe, 1992). Its application to fish (this study; Omlin and
Weber, 2010) shows that the lactate fluxes of rainbow trout are ~3 times higher than
previously estimated by bolus injection (Weber, 1991). This is because the bolus injection
method relies on problematic estimates of surface areas to calculate flux ($flux = dose injected /$
surface area under the specific activity decay curve). Overestimation of surface area under the
decay curve can happen for a number of reasons: (1) curve fitting for early sampling points is
ever precise because specific activity changes very rapidly just after the injection of
the bolus, (2) $^{14}$C recycling can artificially increase specific activities in the right hand side of
the decay curve (later sampling times), and (3) a single catheter is used for bolus injection of
the tracer and subsequent blood sampling; therefore, residual bolus activity on the catheter
walls can increase specific activity in sampled blood by contamination. Finally, the bolus
injection technique assumes steady state conditions, and each experiment only yields a single
measurement of flux: two important restrictions that do not apply to continuous tracer infusion.
For all these reasons, bolus injection has been virtually abandoned as a practical tool to quantify metabolite kinetics.

**Effects of catheters and blood sampling on swimming performance**

We have tested whether applying the continuous tracer infusion technique has an impact on key physiological indices of performance: MO2, TCOT, NCOT and U_crit. TCOT and NCOT were quantified separately because they are both helpful, but in different contexts. For example, TCOT is useful to determine the cost of migration. By contrast, NCOT only takes into account the energy used to power movement and excludes maintenance costs incurred by all tissues including muscle. In biomechanics, NCOT is particularly useful to quantify the efficiency of muscle contraction. It has been shown that instrumenting aquatic animals with catheters, tracking systems, or individual markers can affect locomotion energetics (Bannasch et al., 1994; Culik and Wilson, 1991; Gauthier–Clerc et al., 2004). However, these devices only impact locomotion at high speeds because hydrodynamic drag forces increase with the square of velocity (Biewener, 2003). In this study, we have quantified the cost of transport from measured rates of oxygen consumption, but have ignored the contribution of anaerobic metabolism. To estimate the potential error introduced by this approach, we have calculated the relative importance of anaerobic compared to aerobic metabolism at the highest swimming speed for which lactate kinetics could be measured. Assuming that carbohydrate was the only fuel consumed and that R_d lactate was either completely oxidized or not oxidized at all, we have determined a range of potential errors. At 2.8 BL/s, metabolic rate was 202 µmol O2 kg\(^{-1}\) min\(^{-1}\) (Fig. 1A), or 33.7 µmol glucose kg\(^{-1}\) min\(^{-1}\), or 1212 µmol ATP kg\(^{-1}\) min\(^{-1}\). If 100% of R_d lactate was oxidized, anaerobic metabolism would be 5.7 µmol lactate kg\(^{-1}\) min\(^{-1}\) (=net lactate production=R_a lactate - R_d lactate; Fig. 4B) or 11.4 µmol ATP kg\(^{-1}\) min\(^{-1}\), and would only account for <1% of aerobic metabolism (=11.4/1212). If 0% of R_d lactate was oxidized (a very unlikely scenario), anaerobic metabolism would be 40.4 µmol lactate kg\(^{-1}\) min\(^{-1}\) (=R_a lactate) or 80.8 µmol ATP kg\(^{-1}\) min\(^{-1}\), and would account for 6.7% of aerobic metabolism (=80.8/1212). Therefore, cost of transport could have been underestimated by 1 to 6.7% at the highest swimming speed. Results show that two catheters exiting from the snout of the fish do not significantly increase MO2 or TCOT at any swimming speed tested in our study (Figs. 1 and 2). However, catheterized animals have a higher NCOT than non-instrumented controls.
(+21% for sham catheterized and +29% for lactate kinetics) when they travel faster than 2.4
BL s\(^{-1}\), and their \(U_{\text{crit}}\) is reduced (Figs. 2B and 3A). The higher NCOT observed in
instrumented animals is not due to differences in resting MO2 because catheterization has no
effect on this parameter (Table 1), indicating that the stress of surgery is very low. All
metabolite fluxes previously measured by continuous tracer infusion in swimming fish were
not influenced by double catheterization because the experiments were performed at low,
sustainable speeds (<1.5 BL s\(^{-1}\)) and NCOT is only affected above 2.4 BL s\(^{-1}\) (Bernard et al.,
1999; Magnoni et al., 2008; Shanghavi and Weber, 1999).

In rainbow trout, the relationship between cost of transport and swimming speed is U-
shaped (Fig. 2A), as predicted by various models derived exclusively from hydrodynamic
theory (Pettersson and Hedenström, 2000; Wakeman and Wohlschlag, 1981). This finding is
highly consistent with empirical observations made in other fish species including European
sea bass (Claireaux et al., 2006), Atlantic cod (Syme et al., 2008), Pacific bonito (Sepulveda et
al., 2003), several flatfish (Duthie, 1982), and zebrafish (Palstra et al., 2010). Here, optimal
swimming speed (\(U_{\text{opt}} = \text{speed with minimal cost of transport}\) was 2.1 BL s\(^{-1}\) for TCOT (Fig.
2A) and 1.1 BL s\(^{-1}\) for NCOT (Fig. 2B). This interesting difference has been commonly
reported in the fish literature. It indicates that maximal aerobic efficiency (Webb, 1971) is
achieved at ~70% \(U_{\text{crit}}\), whereas maximal muscle efficiency occurs at ~30-40% \(U_{\text{crit}}\) (Luna-
Acosta et al., 2011; Palstra et al., 2008; Palstra et al., 2010).

Critical swimming speed was significantly reduced by the experimental manipulations
necessary to measure metabolite kinetics. On their own, the drag forces elicited by the two
catheters decreased \(U_{\text{crit}}\) by 11%. When the stress of sampling blood was added to this
mechanical interference, \(U_{\text{crit}}\) was further decreased by 6% (-17% compared to non-
instrumented controls)(Fig. 3A), possibly through a reduction in oxygen transport. Another
study reported no effect of cannulation on \(U_{\text{crit}}\) (Butler et al., 1992), but the measurements were
made on brown trout implanted with a single catheter that may have caused less drag than the
two catheters of our experiments. It may also be easier to demonstrate significant effects of
hydrodynamic drag in rainbow trout because they have a better swimming capacity (\(U_{\text{crit}}\) of
2.8-3.4 BL s\(^{-1}\)) than brown trout that are less athletic (\(U_{\text{crit}}\) of ~2.2 BL s\(^{-1}\))(Butler et al., 1992).
Conclusions

This study is the first to show how the lactate kinetics of an ectotherm change with exercise intensity, and quantifies the rates of lactate production and disposal in rainbow trout, from rest to $U_{\text{crit}}$. This aerobic species maintains high baseline lactate fluxes of 24 µmol kg$^{-1}$ min$^{-1}$ that are only increased at speeds greater than 2.4 BL s$^{-1}$ or \approx 85\% $U_{\text{crit}}$. When the fish accelerates to reach $U_{\text{crit}}$, $R_a$ lactate is more strongly stimulated than $R_d$ lactate (+67\% vs +41\%) and this mismatch causes a 4-fold increase in blood lactate concentration. Without this stimulation of $R_d$, the accumulation of end product would double and impose an extra load on the circulation. Increased lactate oxidation by aerobic tissues (red muscle, heart, kidney and brain) is probably responsible for the higher $R_d$ lactate observed during intense swimming.

Results also show that the hydrodynamic drag from double catheterization and blood sampling needed to measure metabolite kinetics in swimming trout have no significant impact on $MO_2$ or TCOT. However, these experimental procedures affect locomotion energetics by increasing NCOT at speeds >2.4 BL s$^{-1}$ and by decreasing $U_{\text{crit}}$. 
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We thank Bill Fletcher and Christopher Lavergne for taking care of the animals.

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References


Figure legends

**Fig. 1.** Effects of graded swimming on the metabolic rate (MO₂) of rainbow trout. Three groups of fish were measured: non-catheterized controls, sham catheterized, and catheterized animals during the measurement of lactate kinetics. Panel A shows MO₂ as a function of swimming speed in BL/s. Values are means ± s.e.m. For speeds < 3 BL s⁻¹, sample sizes were N=6 for controls and sham-catheterized, and N=8 for the lactate kinetics group. Not all fish were able to swim at the higher speeds (where N<6). * indicates significant differences from resting values (P<0.001). Panel B shows MO₂ as a function of exercise intensity expressed as %U₉crit. The 3 treatments were not different from each other (p>0.05). The thick line was fitted by polynomial regression of second order (P<0.001, r²=0.72): MO₂=109.136 – 1.669 (%U₉crit) + 0.0285 (%U₉crit)². Confidence intervals (±99%) are also indicated by thin lines.

**Fig. 2.** Effects of graded swimming on the cost of transport (COT) in 3 groups of rainbow trout (non-catheterized controls, sham catheterized, and catheterized animals during the measurement of lactate kinetics). Only the statistical differences between treatment groups are indicated directly on the graph (but the effects of swimming speed are not). The Total COT (A) was not different between groups, but it was lower at all speeds above 1 BL s⁻¹ than at the lowest speed (0.8. BL s⁻¹) for all groups (P<0.001). Net COT is presented in panel B. Between 2.4 and 3.2 BL s⁻¹, it was lower in controls than in the other groups (P<0.01) and it was higher than at 0.8 BL s⁻¹ across groups (P<0.01).

**Fig. 3.** Critical swimming speed (U₉crit) in 3 groups of exercising rainbow trout (non-catheterized controls, sham catheterized, and catheterized animals during the measurement of lactate kinetics). Values are means ± s.e.m. (N=6 for controls and sham-catheterized, N=7 for lactate kinetics). The 3 treatments are different from each other (P< 0.05).

**Fig. 4.** Effects of graded exercise on lactate metabolism in rainbow trout. (A) Changes in swimming speed, (B) lactate fluxes and (C) blood lactate concentration are indicated. Panel B shows the rates of lactate appearance (Ra lactate in red) and disposal (Rd lactate in green). Exercise was started at time 0 and tracer infusion at -60 min. Values are means ± s.e.m. (for
lactate fluxes, N=7 before 180 min, and N=4 and 2 for the last 2 points). * indicates differences from first value (P<0.05).

**Fig. 5.** (A) Changes in swimming speed and (B) metabolic rate (MO₂) over time for rainbow trout during steady-state exercise experiments. Values are means ± s.e.m. (N=7). * indicates differences from resting MO₂ (P<0.05).

**Fig. 6.** (A) Changes in blood lactate concentration and (B) lactate fluxes in rainbow trout during steady-state swimming at 1.7 BL s⁻¹. Panel B shows the rates of lactate appearance (Rₘ lactate in red) and disposal (Rₐ lactate in green). Values are means ± s.e.m. (N=6). * indicates differences from baseline at time 0 (P<0.05).
Table 1. Morphological characteristics and metabolic rate (MO$_2$) of rainbow trout in the different treatment groups. The same individuals were measured twice in random order for the control and sham-catheterized conditions. Sample size (N) is indicated on the first line, except for the highest MO$_2$ measured where N is in parentheses (bottom line; because not all individuals within each group were able to reach the highest speed). Values are means ± s.e.m.

<table>
<thead>
<tr>
<th></th>
<th>Graded swimming</th>
<th>Steady-state swimming</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Sham</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>338.8±14.7</td>
<td>369.0±10.1</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>32.4±0.7</td>
<td>33.6±0.4</td>
</tr>
<tr>
<td>Resting MO$_2$ (µmol kg$^{-1}$ min$^{-1}$)</td>
<td>64.7±5.8</td>
<td>61.4±4.6</td>
</tr>
<tr>
<td>Highest MO$_2$ (µmol kg$^{-1}$ min$^{-1}$)</td>
<td>247.7±1.2 (2)</td>
<td>224.0±38.7 (4)</td>
</tr>
</tbody>
</table>
Fig. 1

A

Swimming speed (BL s⁻¹)

MO₂ (μmol kg⁻¹ min⁻¹)

* P<0.001

B

Exercise intensity (%Uₗₗ)

MO₂ (μmol kg⁻¹ min⁻¹)

Control
Sham catheters
Lactate kinetics

Fig. 1
Fig. 2

A

Total COT (µmol O₂ kg⁻¹ m⁻¹)

B

Net COT (µmol O₂ kg⁻¹ m⁻¹)

Swimming speed (BL s⁻¹)

- Control
- Sham catheters
- Lactate kinetics

* P<0.01
Fig 3

$U_{\text{crit}}$ (BL s$^{-1}$)

Control | Sham catheters | Lactate kinetics

3.5
3.0
2.5
Fig. 4

A. Graded swimming

B. Lactate flux (µmol kg⁻¹ min⁻¹)

C. Lactate (mM)

Swimming speed (BL s⁻¹)

-20 0 20 40 60 80 100 120 140 160 180 200 220 240

Time (min)

* *
Fig. 5

A

Swimming speed (BL s\(^{-1}\))

Rest

Steady state swimming

B

MO\(_2\) (µmol kg\(^{-1}\) min\(^{-1}\))

* p<0.05
Fig. 6

A

B

Lactate (mM)

Lactate flux (µmol kg⁻¹ min⁻¹)

Time (min)

Rest

Steady state swimming (1.7 BL s⁻¹)

* p<0.05

* * *