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RESEARCH

The first direct measurements of ventilatory flow and oxygen utilization after exhaustive exercise and voluntary feeding in a teleost fish, *Oncorhynchus mykiss*

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Abstract A new "less invasive" device incorporating an ultrasonic flow probe and a divided chamber, but no stitching of membranes to the fish, was employed to make the first direct measurements of ventilatory flow rate (Vw) and % O_2 utilization (%U) in juvenile rainbow trout (37 g, 8ºC) after exhaustive exercise (10-min chasing) and voluntary feeding (2.72% body mass ration). Under resting conditions, the allometrically scaled Vw (300 ml kg^{-1} min^{-1} for a 37-g trout = 147 ml kg^{-1} min^{-1} for a 236-g trout exhibiting the same mass-specific O_2 consumption rate, $\dot{M}O_2$) and the convection requirement for O_2 (CR = 4.13 L $mmol^{-1}$) were considerably lower, and the %U (67%) was considerably higher than in previous studies using surgically attached masks or the Fick principle. After exhaustive exercise, Vw and $\dot{M}O_2$ approximately doubled whereas frequency (fr) and %U barely changed, so increased ventilatory stroke volume (Vsv) was the most important contributor to increased $\dot{M}O_2$. CR declined slightly. Values gradually returned to control conditions after 2–3 h. After voluntary feeding, short-term increases in Vw, Vsv and $\dot{M}O_2$ were comparable to those after exercise, and fr again did not change. However, %U increased so CR declined even more. The initial peaks in Vw, Vsv and $\dot{M}O_2$, similar to those after exercise, were likely influenced by the excitement and exercise component of voluntary feeding. However, in contrast to post-exercise fish, post-prandial fish exhibited second peaks in these same parameters at 1–3 h after feeding, and %U increased further, surpassing 85%, reflecting the true "specific dynamic action" response. We conclude that respiration in trout is much more efficient than previously believed.

Keywords Rainbow trout · Ventilation · Convection requirement for oxygen · Oxygen consumption rate · Specific dynamic action

Abbreviations

α_{O2}	Oxygen solubility coefficient
ANOVA	Analysis of variance
BPL	Elastic balloon piece layer
CR	Convection requirement for oxygen
EPOC	Excess post-exercise oxygen consumption
FP	Ultrasonic blood flow probe
fr	Ventilatory frequency
ID	Internal diameter
IU	International units
$\dot{M}O_2$	Oxygen consumption rate
P_EO_2	Partial pressure of oxygen in expired water
P_IO_2	Partial pressure of oxygen in inspired water
PVC	Polyvinyl chloride
SDA	Specific dynamic action
Vsv	Ventilatory stroke volume

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Vw	Ventilatory flow rate of water
RS	Rubber stopper
%U	Percentage oxygen utilization from the water

Introduction

Exercise and feeding are routine events in the daily lives of fish, and both are known to elevate oxygen consumption rate ($\dot{M}O_2$) (Jobling 1981; Alsop and Wood 1997; Thorarensen and Farrell 2006; Eliason et al. 2008). $\dot{M}O_2$ is one of the most commonly made measurements in fish physiology, but the ventilatory water flow over the gills ("ventilation volume", Vw) that facilitates it is rarely measured directly, because Vw is technically difficult to quantify. Vw represents the total amount of water pumped per unit time by the fish across its respiratory surfaces, and is the product of ventilatory frequency (fr) and ventilatory stroke volume (Vsv). Relative to air, water is a dense, viscous, relatively O_2-poor medium (Dejours 1988), so Vw is thought to have a considerable metabolic cost. While estimates vary, the workload of breathing is generally thought to account for 10–30% of the resting $\dot{M}O_2$ of fish (Schumann and Piiper 1966; Cameron and Cech 1970; Edwards 1971; Jones and Schwarzfeld 1974; Steffensen 1985; Farrell and Steffensen 1987), and the absolute cost undoubtedly increases when Vw and $\dot{M}O_2$ are elevated. Yet, Vw is not measured in most respiratory studies, or else is estimated by proxy indices. These may include breathing frequency (fr) alone, or ventilatory index (the product of fr x some indicator of Vsv—e.g. buccal pressure or opercular position excursions, Vulesevic et al. 2006; Eom et al. 2020), or dye dilution methods (e.g. Jones et al. 1990) or Fick equation calculations based on multiple inflowing and outflowing PO_2 determinations (e.g. Stevens and Randall 1967a, b; Hughes and Saunders 1970; Wood and Munger 1994). Nevertheless, techniques for direct Vw measurements have existed for almost a century since the pioneering devices of Hall (1931) and van Dam (1938). Pan and Perry (2023) have provided a historical perspective.

Van Dam's original device was custom-fitted to the fish's head rather than attached by suturing or gluing, but virtually all subsequent "masks" have been sutured or glued to the fish (e.g. those listed in Table 1; Watters and Smith 1973; Wilkes et al. 1981; Takeda 1990). This necessitates lengthy anaesthesia, surgery, recovery, and stress to the fish. Subsequent refinements have replaced constant-level overflow devices with electronic flow probes connected to attached masks so as to provide Vw, fr, and Vsv recordings in real time (Lomholt and Johansen 1979; Glass et al. 1990). However, the fact remains that the fish cannot be put quickly into these measurement systems after performing the two daily activities, feeding and exercise, that are probably the major daily influences on its routine $\dot{M}O_2$. Anaesthesia and surgery would cause delay and stress. Even if the masks had been attached earlier, they would severely interfere with both swimming and ingestion.

Recently, we have developed a less invasive method for directly measuring Vw (Eom and Wood 2020) that employs an ultrasonic flow probe and allows easy insertion of the fish without anaesthesia. There is no stitching or gluing. The device also allows placement of probes to measure inspired (P_IO_2) and mixed expired oxygen tensions (P_EO_2), so it can provide simultaneous measurements of Vw, fr, Vsv, $\dot{M}O_2$, and percent utilization of O_2 (%U). In the present study on juvenile rainbow trout (*Oncorhynchus mykiss*), we have employed this method to make the first direct measurements Vw, Vsv, and %U for 4 h after either enforced exercise or voluntary feeding. Both of these treatments have been relatively well studied for their effects on $\dot{M}O_2$ and internal metabolism (exhaustive exercise – reviewed by Wood 1991; Kieffer 2000; Kieffer 2010; feeding – reviewed by McCue 2006; Chabot et al. 2016), but there has been only a small amount of work on the ventilatory changes that facilitate the increases in MO_2 that follow these treatments. Based on several previous studies (Altimiras and Larsen 2000; Millidine et al. 2008), it is likely Vw that goes up in accord with increased $\dot{M}O_2$ and increased fr after both of these treatments. However, we are aware of no direct measurements of Vw, Vsv, or %U after feeding, and only indirect measures of these parameters after exercise (Stevens and Randall 1967a, b; Kiceniuk and Jones 1977; Jones et al. 1990; Wood and Munger 1994). Two recent reviews on breathing in fish (Perry et al. 2023; Pan and Perry 2023) do not address these topics.

Our overall hypotheses were (i) that breathing would be more efficient (i.e. lower Vw, higher %U) under both resting and active conditions than

Table 1 A comparison of ventilatory and respiratory parameters in rainbow trout measured with the less invasive system in the present study (data in bold) with those previously reported for the same species, as measured with other systems (Mask = mask attached to the fish by gluing or sewing; Van Dam = the original system of Van Dam (1938) where the mask was form-fitted to the trout's head but not attached by gluing or sewing; Fick = use of the Fick equation with opercular sampling catheters)

Mass (g)	Temp. (°C)	$\dot{M}O_2$ (μmol $kg^{-1}h^{-1}$)	$\dot{V}W$ (ml $kg^{-1}min^{-1}$)	CR (L $mmol^{-1}$)	Vsv (ml kg^{-1})	fr (min^{-1})	%U	Method	Treatment	Reference
37 ± 2	**8**	**4363**	**300**	**4.13**	**3.33**	**90**	**67**	Less invasive	Control	Present Study
		9142	**570**	**3.74**	**6.33**	**90**	**73**	Less invasive	Post-exercise	Present Study
		9061	**550**	**3.64**	**5.70**	**93**	**75**	Less invasive	Post-feeding	Present Study
260–501	6.5–9.0	–	140	–	2.00	70	–	Less invasive	Control	Eom and Wood (2020)
232 ± 13	9 ± 1	2375	171	4.32	2.31	74	46	Mask	Control	Cameron and Davis (1970)
210 ± 2	8.6	1728	175	6.07	2.36	74	46	Mask	Control	Davis and Cameron (1971)
150–380	6–11	2289	194	5.09	2.77	70	41	Mask	Control	Smith and Jones (1982)
186–289	4–12	2289	251	6.58	–	–	–	Mask	Control	Jones and Schwarzfeld (1974)
100–300	10.5	2009	198	5.91	2.44	81	58	Mask	Control	Randall and Cameron (1973)
192–353	5–10	2200	264	7.21	3.11	85	39	Mask	Control	Iwana et al. (1987)
281 ± 6	15–16	2050	220	6.43	–	–	33	Mask	Control	Playle and Wood (1989)
200–350	15 ± 1	2730	350	7.69	–	–	43	Mask	Control	Playle et al. (1990)
100–300	14 ± 2	2220	270	7.30	2.84	92	43	Mask	Control	Wood and Jackson (1980)
900	10–12	2098	133	3.80	1.48	90	80	Van Dam	Control	Van Dam (1938)
		6951	705	6.09	6.13	115	50	Van Dam	Struggling	Van Dam (1938)
~350	15 ± 1	3000	750	15.00	9.86	76	27	Fick	Control	Wood and Munger (1994)
		8100	4300	31.85	38.39	112	17	Fick	Post-exercise	Wood and Munger (1994)
900–1500	9.5–10.0	1560	211	8.11	–	–	33	Fick	Control	Kiceniuk and Jones (1977)
		11640	1700	8.76	–	–	33	Fick	Severe exercise	Kiceniuk and Jones (1977)
200–600	10–12	1125	1427	76.11	18.53	77	9	Fick	Control	Stevens and Randall (1967a, b)
		5938	7138	72.13	69.98	102	9	Fick	Moder exercise	Stevens and Randall (1967a, b)
400–600	13.5	1562	200	7.68	3.57	56	70	Fick	Control	Hughes and Saunders (1970)

For Post-exercise and Post-feeding measurements in the present study, mean values in the 10-min period where $\dot{M}O_2$ was greatest have been reported. ($\dot{M}O_2$ O_2 consumption rate; *Vw* ventilatory flow rate; *CR* convection requirement for O_2; *Vsv* ventilatory stroke volume; *fr* ventilatory frequency; *%U* % O_2 utilization)

generally indicated by previous studies using more stressful or indirect techniques. (ii) based on available studies where fr was measured and other parameters estimated (Altimiras and Larsen 2000; Millidine et al. 2008), changes in Vsv would be more important in achieving increased $\dot{M}O_2$ after both exercise and voluntary feeding than would changes in fr; and (iii) exercise *versus* feeding would have different effects on post-treatment patterns of $\dot{M}O_2$, Vw, and %U. This latter idea was based on the fact that the time courses would differ. Excess post-exercise oxygen consumption (EPOC) peaks almost immediately after the end of chasing exercise (Scarabello et al. 1991; Zhang et al. 2020), whereas the specific dynamic action (SDA) following feeding builds slowly (Jobling 1981; Eliason et al. 2008; Eliason and Farrell 2014).

Materials and methods

Experimental animals

Rainbow trout (37.3 ± 1.7 g, $N = 32$) were transferred from a hatchery (Fraser Valley Trout Hatchery, Abbotsford, BC, Canada) and held in flow-though, charcoal-filtered dechlorinated Vancouver City tap water ($[Na^+]$, 0.17 mmol L^{-1}; $[Cl^-]$, 0.21 mmol L^{-1}; hardness, 30 mg L^{-1} as $CaCO_3$; pH 7.0; temperature, 6.5 – 9.0 °C) at the University of British Columbia. The fish were fed with commercial food pellets (EWOS, Surrey, BC, Canada) (crude protein 47%; crude fat 18%; crude fiber 1.5%; calcium 1.4%; phosphorous 1%; sodium 0.5%; vitamin A 5000 IU kg^{-1}; vitamin D3 3000 IU kg^{-1}; vitamin E 200 IU kg^{-1}) but fasted for one week prior to experiments. The experiments were approved by the University of British Columbia under animal care protocol #A17-0301 following the guidelines of the Canadian Council on Animal Care. The experiments were performed at 8ºC. After completion of the experiments, the fish were returned to their original tanks.

Experimental procedures and analytical techniques

A modified version of our "less invasive" ventilation measuring system (Eom and Wood 2020) was used to directly measure the ventilation flow (Vw) after exhaustive exercise (Series 1, $N = 10$), after voluntary feeding (Series 2, $N = 6$), and in the control treatments in each of the Series ($N = 10$ and $N = 6$ respectively). Detailed dimensions of the system are presented in Fig. 1. An ultrasonic blood flow probe (FP) (V-series, 10.0 mm, Transonic Systems Inc., Ithaca, NY, USA), recalibrated for water flow, is used to continuously record Vw. The system avoids the need for prior operative procedures or anesthesia, minimizes the need for handling, and involves no suturing or gluing of material to the fish. The original system described earlier was designed for 250—500 g trout (Eom and Wood 2020). The principal modifications were a reduction in size so as to accommodate 30—50 g trout, the replacement of the silicone funnel in the mouth chamber with rigid PVC pipe (Part 1) so as to reduce compliance and allow maximum buccal expansion at high ventilation stroke volumes, and the sealing of the rear of the chamber (Part 2) with a rubber stopper (RS) so that the outflow was on the upper surface. This ensures that there is no contamination of the expired P_EO_2 measurement by the outside water in which the whole apparatus is submerged, and also means that any additional O_2 consumption by the skin is captured. As in the original design, the trout's head was advanced through the elastic balloon piece layer (BPL), which was cut to fit each fish individually so as to form a tension seal, while allowing maximum expansion of the operculae, as may occur at high stroke volumes and during coughing. A video illustrating how the fish is mounted in the system can be found as Supplementary Media SM1 in Eom and Wood (2020).

A needle-type oxygen optode (PreSens, Microx TX 3, Precision Sensing GmbH, Regensburg, Germany) was inserted through the outflow into the rear of Part 2. Thus Part 2, which accommodated the operculae and body of the fish, served as a mixing chamber (water volume ~ 60 ml with fish present) to ensure that mean expired P_EO_2 was measured. It took about 6 min for system to equilibrate after the fish was placed in it. Inspired P_IO_2 was checked frequently during the experiments and was kept close to 100% air saturation.

The flow probe (V-series, Transonic Systems Inc., Ithaca, NY, USA) used for measuring the ventilation flow was connected to a dual-channel small animal blood flow-meter (T106 series, Transonic) (Fig. 1). The flow probe detected both the magnitude and direction of flow so the correct orientation was essential during experiments. The intrinsic calibration and

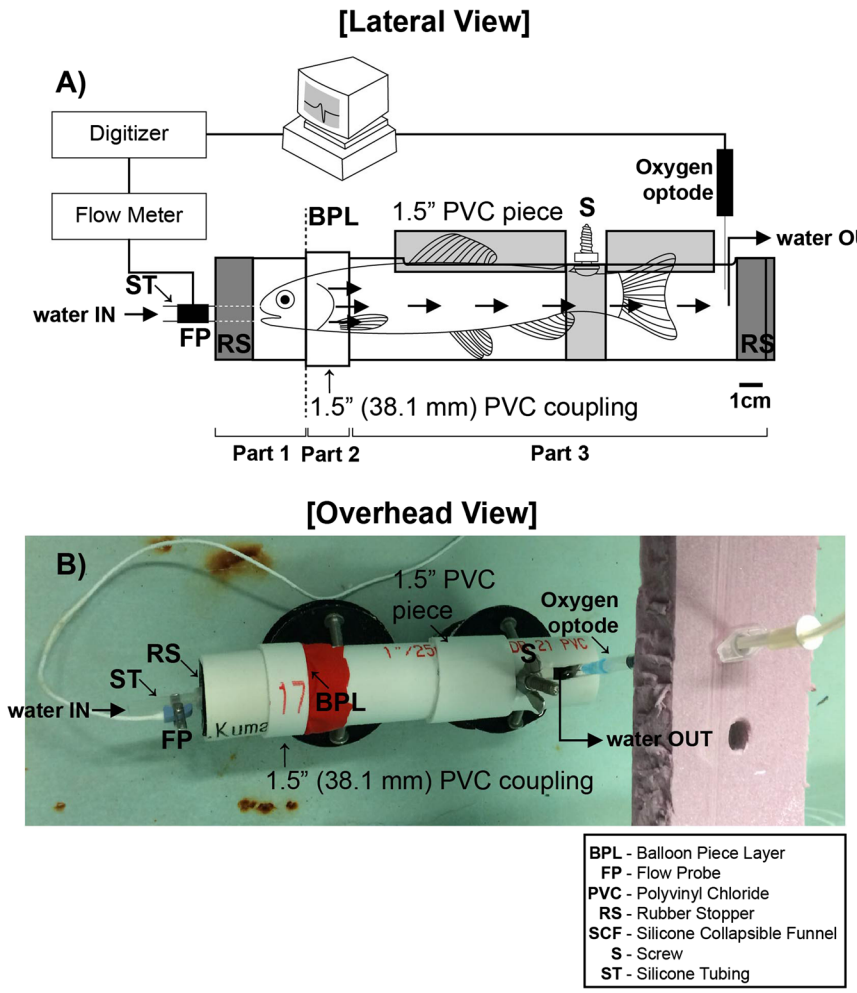

Fig. 1 **A** A schematic diagram (lateral view) and **B** a photograph (vertical overhead view) of the modified "less invasive" ventilation flow measuring system. With minimal handling and no anesthesia or surgery, the juvenile trout were placed into Part 3 (constructed from I.D. 38.1 mm PVC pipe). The head was advanced through the pre-fitted elastic balloon piece layer (BPL, Part 2) membrane, thereby separating the buccal opening for inhalation from the opercular openings for exhalation. Towards the rear of Part 3, an I.D. 25.4 mm PVC collar, which fitted snugly into the I.D. 38.1 mm PVC pipe, was slipped over the fish's tail and secured in place with a screw (S) to prevent backward movement. The BPL was fixed in place by the overlying I.D. 47.6 mm PVC coupling which snugly fitted over the I.D. 38.1 mm PVC pipe. The anterior end of the fish's head was sealed into Part 1 (again constructed from I.D. 38.1 mm PVC pipe) which forms the anterior chamber, sealed at the front end by a rubber stopper (RS) bearing the silicone tubing (ST) and flow probe (FP). Another rubber stopper sealed the rear end of Part 3. An oxygen optode was inserted into Part 3 through its superior exit for water flow to measure mean expired P_EO_2 continuously. The flow meter and optode signals were sent to the data processing system. The whole system was submerged in a water bath at 8ºC with PO_2 (representing inspired P_IO_2) maintained close to 100% air saturation

zero of the flow-meter were used for initial calibration of flow probe. Final calibration was performed by perfusing water at the experimental temperature through the flow probe at known rates, determined gravimetrically, using an aqua lifter vacuum pump (Cheng Gao Plastic and Hardware Electricity, Dogguan, Guandong, China). The measured ventilation flow and expired PO_2 were recorded continuously and visualized in a PowerLab data integrity system (ADInstruments, Colorado Springs, CO, USA), allowing calculation of mean ventilation flow rate (Vw), ventilation frequency (fr, from the pulsatility of the Vw recording), ventilatory stroke volume (Vsv), oxygen utilization (%U), oxygen consumption

rate ($\dot{M}O_2$), and ventilatory convection requirement for oxygen (CR) as outlined below. Every 60 min (Figs. 2, 5), 10 min (Figs. 3, 6), or 3 s (Figs. 4, 7), the respective measured and calculated ventilation parameters were averaged and plotted.

$$\dot{M}O_2(\mu mol\ kg^{-1}\ h^{-1}) = \dot{V}w(L\ kg^{-1}\ min^{-1}) x\ \alpha_{O2}\ x[P_IO_2(torr) - P_EO_2(torr)]x60(min\ h^{-1}) \quad (1)$$

$$\%U = [(P_IO_2(torr) - P_EO_2(torr))/P_IO_2(torr)]x100\% \quad (2)$$

$$Vsv = \dot{V}w(ml\ kg^{-1} min^{-1})/fr(min^{-1}) \quad (3)$$

$$CR = \dot{V}w\left(ml\ kg^{-1}\ min^{-1}\right)x\ 60min\ h^{-1}/\dot{M}O2(\mu mol\ kg^{-1}\ h^{-1}) \quad (4)$$

where α_{O2} is the appropriate O_2 solubility coefficient (2.3470 µmol $torr^{-1}$ L^{-1}) at 8 °C (Boutilier et al. 1984).

Series 1: Respiratory physiology after exhaustive exercise

The experimental fish were placed individually in a 200-L fiberglass tank and chased for 10 min using a plastic PVC pipe, by which time they were exhausted. Within one minute, each exercised fish was transferred to the modified less invasive system (Fig. 1) and the Vw, fr, Vsv, %U, and $\dot{M}O_2$ were continuously measured for 4 h. The control fish were handled identically but without exercise.

Series 2: Respiratory physiology after voluntary feeding

As trout feed better in groups, three juvenile trout were placed in a 200-L fiber glass tank, and were allowed to feed voluntarily on commercial food pellets delivered by hand 10 times in 10 min (1 feeding per min). This ensured that the fish were fed to satiation. One of the fed individuals was transferred to the system (Fig. 1) and Vw, fr, SVw, %U, and $\dot{M}O_2$ were continuously measured for 4 h. A separate experiment was performed in which the mass of food consumed was carefully measured so as to estimate the ration. This ration amounted to 2.72 ± 0.21% (N = 6) dry pellets relative to wet body weight. The control fish were handled identically, but without feeding.

Statistical analyses

All measured or calculated respiratory parameters have been reported as means ± S.E.M. (N) where N represents the number of fish. The data were analyzed in two different ways. In both, all data passed tests of normality and equal variance. The first analysis was designed so as to increase the statistical power by minimizing the number of comparisons. All respiratory parameters were averaged over each of the 4 h for each fish, in both the control and experimental treatments separately, within each of Series 1 and Series 2. Then a two -way ANOVA was performed (factors = time, treatment as control or experimental), and post-hoc comparisons among means were made by Tukey's test. This revealed the general trends over time, and whether the experimental means were significantly different from the control means measured at the same times. It also demonstrated that there were no significant variations over time in the control means for any of the measured parameters within either Series 1 or Series 2. The second analysis was designed to provide a finer time scale examination of patterns within only the experimental treatments, for each of Series 1 and Series 2. All respiratory parameters were averaged over 10-min periods for each fish, then a one-way repeated measures ANOVA was performed, followed by Tukey's post hoc test to identify significant variations over time within the experimental treatments. All the tests were performed with GraphPad Prism software version 6.0 (La Jolla, CA, USA), and a threshold for statistical significance of $p \leq 0.05$ was used throughout.

Results

Under control conditions, resting trout exhibited values of Vw ~ 300 ml kg^{-1} min^{-1}, fr ~ 90 min^{-1}, SVw ~ 3.3 ml kg^{-1}, % U ~ 67%, and routine $\dot{M}O_2$ ~ 4360 µmol kg^{-1} h^{-1} (Table 1). In general, the fish increased oxygen consumption rates after both exhaustive exercise and voluntary feeding but the patterns over time and the mechanisms by which these changes were achieved differed between the treatments.

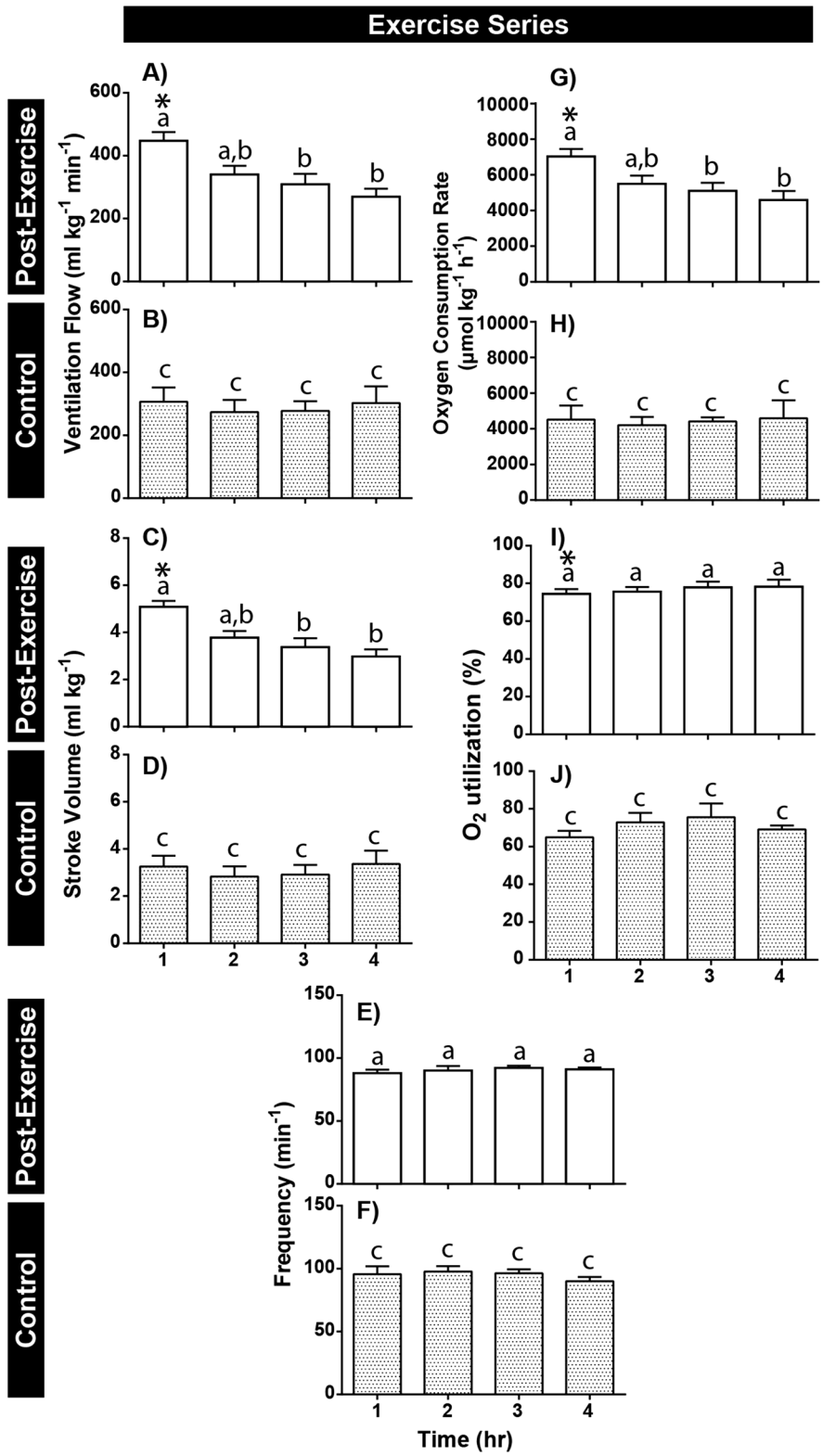

Fig. 2 Measured and calculated respiratory parameters (means ± 1 S.E.M.), averaged over 1-h intervals, in trout during 4 h of recovery from exhaustive exercise (experimental, $N = 10$), and in similarly handled but non-exercised trout (control, $N = 10$). (**A**, **B**) Ventilation flow rate, Vw (two-way ANOVA: treatment $p = 0.0794$, time $p = 0.0489$, interaction $p = 0.1349$); (**C**, **D**) Ventilatory stroke volume, Vsv (treatment $p = 0.0216$, time $p = 0.0225$, interaction $p = 0.0489$); (**E**, **F**) Ventilatory frequency, fr (treatment $p = 0.1499$, time $p = 0.8135$, interaction $p = 0.7098$); (**G**, **H**) Oxygen consumption rate, $\dot{M}O_2$ (treatment $p = 0.0201$, time $p = 0.1713$, interaction $p = 0.1986$); (**I**, **J**) Oxygen utilization, % U (treatment $p = 0.0322$, time $p = 0.2475$, interaction $p = 0.6482$). Within each treatment, means sharing the same letters are not significantly different ($p > 0.05$, Tukey's test). Asterisks indicate significant differences ($p \leq 0.05$) between experimental and control means simultaneously

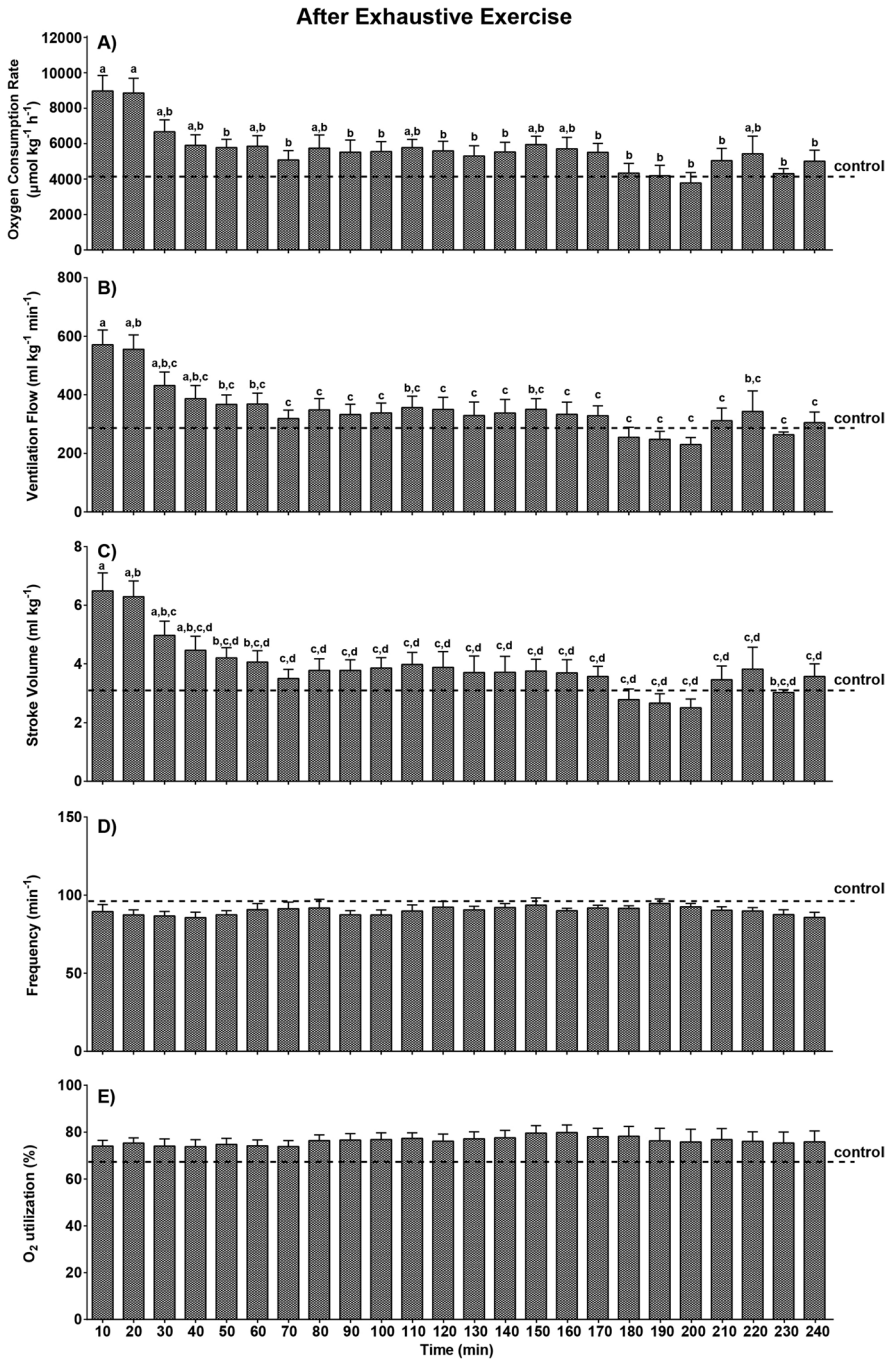

Fig. 3 Measured and calculated respiratory parameters (means ± 1 S.E.M.), averaged over 10-min intervals in trout during 4 h of recovery from exhaustive exercise ($N = 10$). (**A**) Oxygen consumption rate, $\dot{M}O_2$ (one-way repeated measures ANOVA $p < 0.0001$); (**B**) Ventilation flow rate, Vw (one-way repeated measures ANOVA $p < 0.0001$); (**C**) Ventilatory stroke volume, Vsv (one-way repeated measures ANOVA, $p < 0.0001$); (**D**) Ventilatory frequency, fr (one-way repeated measures ANOVA, $p = 0.7026$); (**E**) Oxygen utilization, % U (one-way repeated measures ANOVA, $p = 0.9997$). In each panel, means sharing the same letters are not significantly different ($p > 0.05$, Tukey's test). The dashed lines indicate the mean values for the similarly handled control fish (from Fig. 2) for reference

Series 1: Respiratory physiology after exhaustive exercise

There were no significant variations over the 4-h period in any of the respiratory parameters (Figs. 2B, D, F, H, J) in the control fish, showing that the minimal handling involved in catching the fish and placing them in the less invasive system had negligible effects on the measured parameters. However, the exhaustively exercised trout showed significantly greater Vw in hour 1 relative to hours 3 and 4, with a pattern of progressive decline (Fig. 2A; two-way

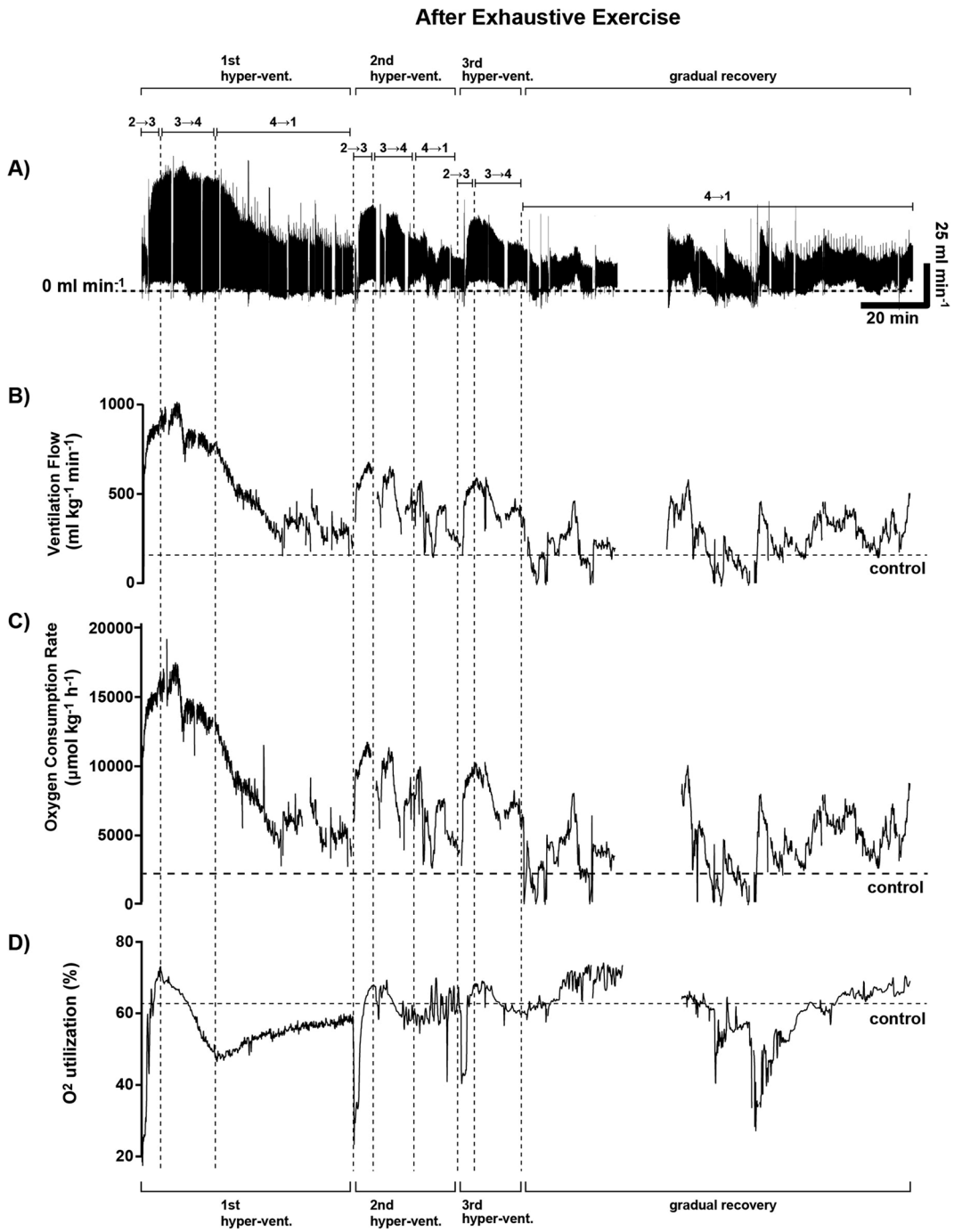

Fig. 4 Original recordings, averaged over 3-s intervals, of (**A**) pulsatile ventilation flow (Vw), (**B**) mean ventilatory flow rate (Vw), (**C**) oxygen consumption rate ($\dot{M}O_2$), and (**D**) oxygen utilization (%U) during a 4-h period of recovery from exhaustive exercise in a single trout. The horizontal dashed lines indicate the mean pre-exercise control values for the same fish. The dashed vertical lines and the numbers in panel (**A**) indicate the data areas circled in Fig. 8A

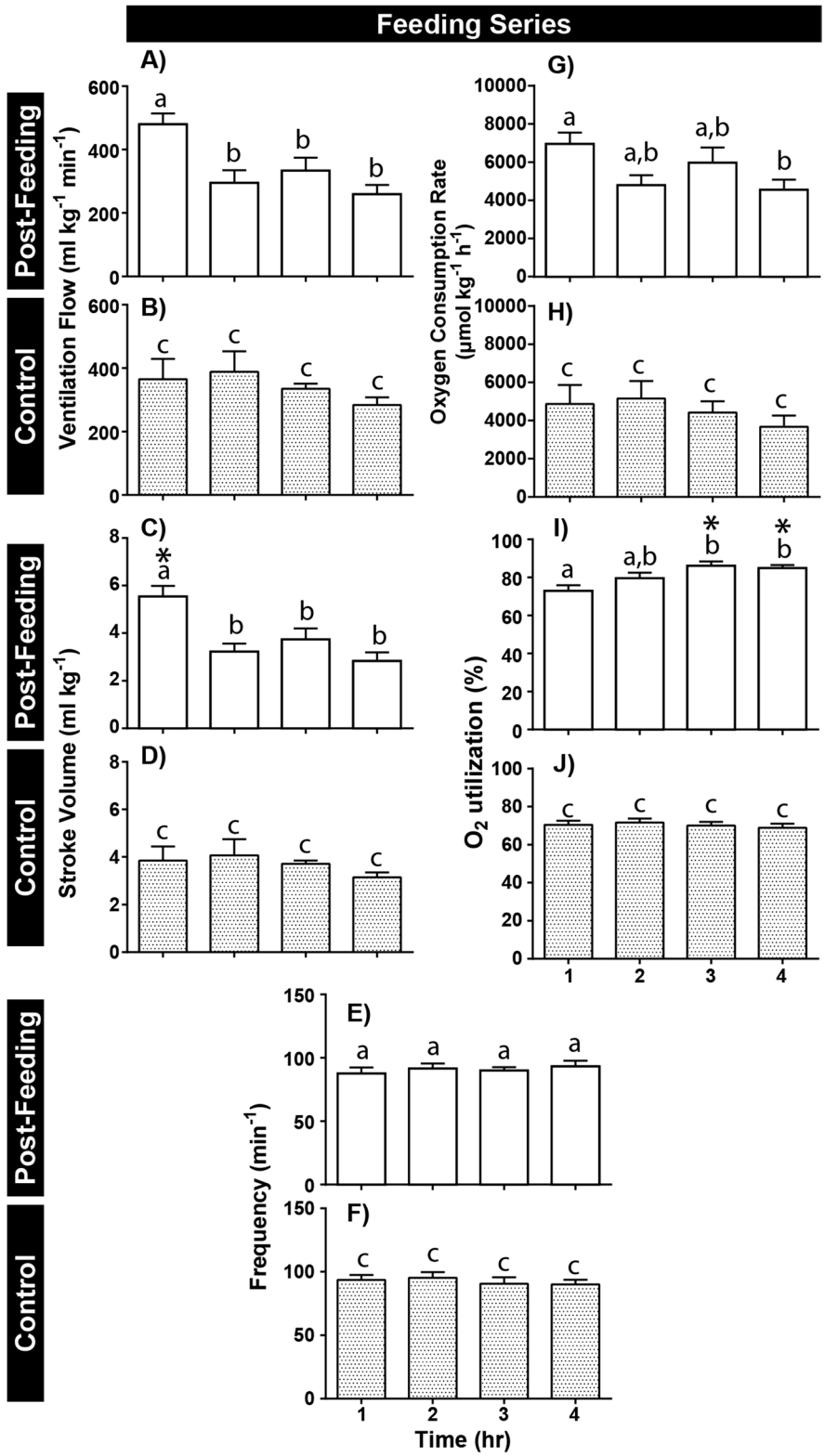

Fig. 5 Measured and calculated respiratory parameters (means ± 1 S.E.M.), averaged over 1-h intervals, in trout for 4-h after voluntary feeding (experimental, $N=6$), and in similarly handled but non-fed trout (control, $N=6$). (**A**, **B**) Ventilation flow rate, Vw (two-way ANOVA: treatment $p=0.9692$, time $p=0.0092$, interaction $p=0.1113$); (**C**, **D**) Ventilatory stroke volume, Vsv (treatment $p=0.6526$, time $p=0.0039$, interaction $p=0.0348$); (**E**, **F**) Ventilatory frequency, fr (treatment $p=0.5360$, time $p=0.8515$, interaction $p=0.6982$); (G, H) Oxygen consumption rate, $\dot{M}O_2$ (treatment $p=0.0394$, time $p=0.0933$, interaction $p=0.3375$); (**I**, **J**) oxygen utilization, %U (treatment $p<0.0001$, time $p=0.0375$, interaction $p=0.0083$). Within each treatment, means sharing the same letters are not significantly different ($p>0.05$, Tukey's test). Asterisks indicate significant differences ($p\leq0.05$) between experimental and control means simultaneously

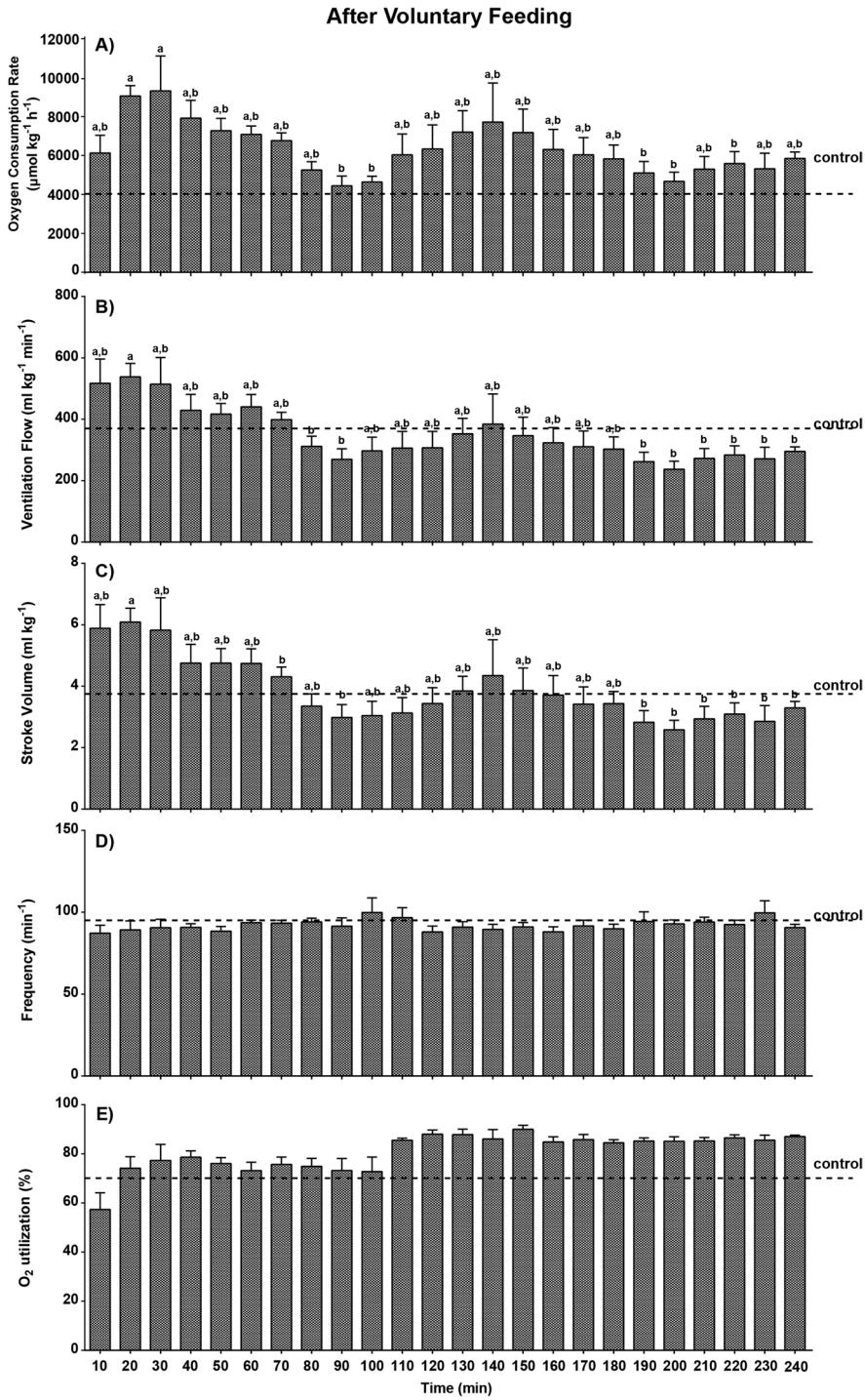

Fig. 6 Measured and calculated respiratory parameters (means ± 1 S.E.M.), averaged over 10-min intervals in trout for 4-h after voluntary feeding ($N = 6$). (**A**) Oxygen consumption rate, $\dot{M}O_2$ (one-way repeated measures ANOVA, $p = 0.0003$); (**B**) Ventilation flow rate, Vw (one-way repeated measures ANOVA, $p = 0.0407$); (**C**) Ventilatory stroke volume, Vsv (one-way ANOVA, $p = 0.0436$); (**D**) Ventilatory frequency, fr (one-way repeated measures ANOVA, $p = 0.4282$); (**E**) Oxygen utilization, % U (one-way repeated measures ANOVA, $p = 0.0083$. In each panel, means sharing the same letters are not significantly different ($p > 0.05$, Tukey's test). The dashed lines indicate the mean values for the similarly handled control fish (from Fig. 5) for reference

ANOVA: treatment $p = 0.0794$, time $p = 0.0489$, interaction $p = 0.1349$). This was due to significantly higher Vsv in the first hour relative to both the corresponding control value, and to hours 3 and 4, with a similar pattern of progressive decline over time (Fig. 2C; treatment $p = 0.0216$, time $p = 0.0225$, interaction $p = 0.0489$). There were no changes in fr (Fig. 2E; treatment $p = 0.1499$, time $p = 0.8135$,

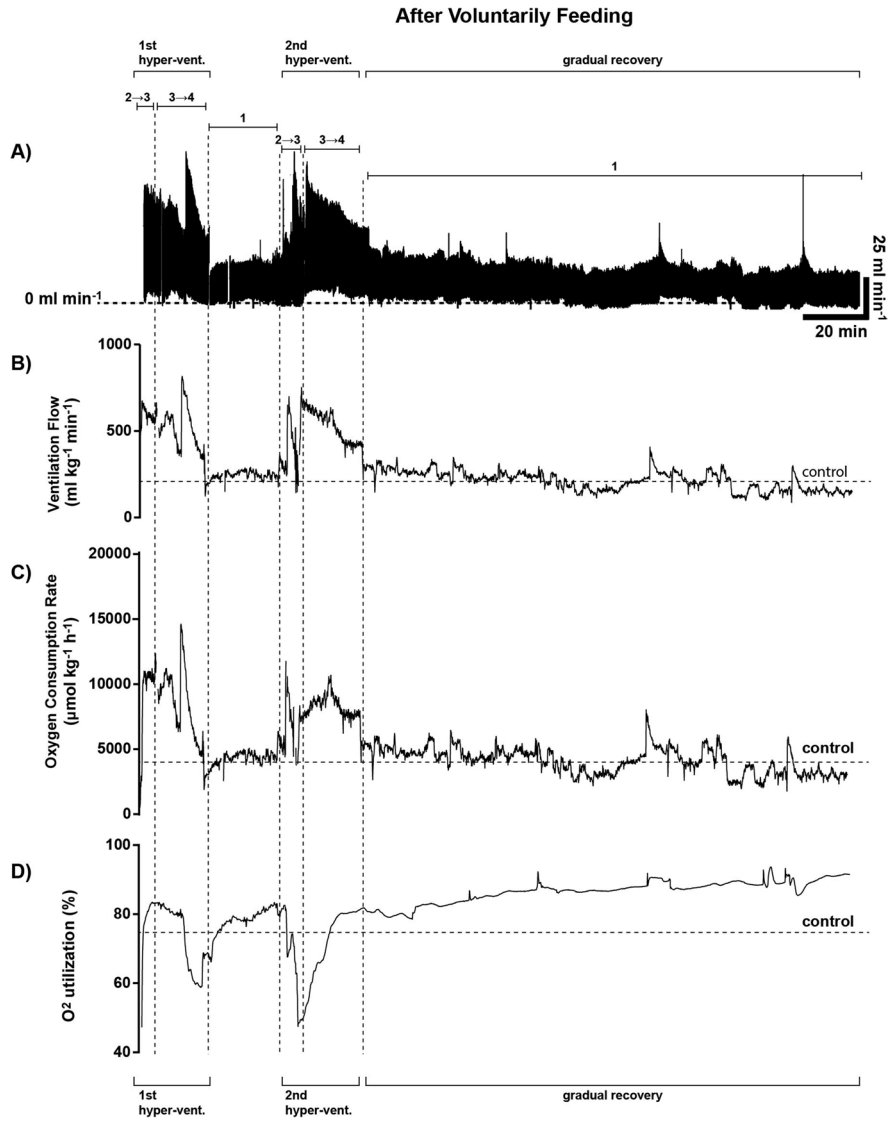

Fig. 7 Original recordings, averaged over 3-s intervals, of (**A**) pulsatile ventilation flow (Vw), (**B**) mean ventilatory flow rate (Vw), (**C**) oxygen consumption rate ($\dot{M}O_2$), and (**D**) oxygen utilization (%U) during a 4-h period following feeding in a single trout. The horizontal dashed lines indicate the mean pre-exercise control values for the same fish. The dashed vertical lines and the numbers in panel (**A**) indicate the data areas circled in Fig. 8B

interaction $p=0.7098$). $\dot{M}O_2$ exhibited a similar pattern to Vw, being significantly greater in the first hour relative to both the control and the fourth hour, falling gradually over the interim (Fig. 2G; treatment $p=0.0201$, time $p=0.1713$, interaction $p=0.1986$). % U was slightly but significantly elevated overall in the exercised treatment, though only the difference in hour 1 was significant relative to the control, and % U remained very constant throughout (Fig. 2I; treatment $p=0.0322$, time $p=0.2475$, interaction $p=0.6482$). Therefore, the $\dot{M}O_2$ changes were driven mainly by the variations in Vw (Fig. 2A) which in turn were due to variations in Vsv (Fig. 2C).

Analysis of post-exercise responses over a finer time scale (10-min intervals) revealed that the greatest increases in $\dot{M}O_2$ occurred in the first 20 min of recovery (approximate doubling relative to controls) (Fig. 3A). These were followed by a decline to a slightly elevated level that was more or less stable up to 150 min, with a slight further decrease at 180 – 200 min and some fluctuations thereafter (Fig. 3A; one-way repeated measures ANOVA $p<0.0001$). These were explained by very similar patterns in Vw (Fig. 3B; $p<0.0001$) and Vsv (Fig. 3C; $p<0.0001$). Both fr (Fig. 3D; $p=0.7026$) and %U (Fig. 3E, $p=0.9997$) remained remarkably constant

throughout, though note that %U was consistently higher than in the simultaneous controls.

Even greater insight was gained by looking at the data of individual fish over a very fine time scale (3-s intervals). No two fish were alike in their post-exercise patterns, though there were some common themes. Figure 4 shows one example, chosen because pre-exercise control data had been recorded for 4 h for this same individual, with the average values shown by the horizontal dashed lines. Invariably, Vw was greatest immediately after exercise or shortly thereafter, but there were almost always smaller secondary peaks (Figs. 4A, B). The number and timing of these secondary peaks varied greatly among individuals, so they were lost when data were averaged over either 1-h (Fig. 2) or 10-min periods (Fig. 3). Secondly, as in the aggregated data, changes in $\dot{M}O_2$ (Fig. 4C) were driven mainly by changes in Vw (Fig. 4B) even when Vw was exhibiting rapid fluctuations. Thirdly, %U (Fig. 4D) at times varied directly with Vw and other times inversely with Vw (Fig. 4B). Clearly, the two could be manipulated independently by the fish, a topic explored in greater detail in the Discussion section with reference to the numbered phases in Fig. 4A.

Series 2: Respiratory physiology after voluntary feeding

As in Series 1, the control fish exhibited no significant variations over the 4-h period in any of the respiratory parameters (Figs. 5 B, D, F, H, J). However, in the fish that had undergone voluntary satiation feeding (2.72 ± 0.21% dry pellets relative to wet body weight), Vw was significantly higher in hour 1 than in hours 2, 3 and 4 (Fig. 5A; two-way ANOVA: treatment $p = 0.9692$, time $p = 0.0092$, interaction $p = 0.1113$). This pattern was largely due to significantly higher Vsv in hour 1, relative to hours 2,3, and 4 (Fig. 5C; treatment $p = 0.6526$, time $p = 0.0039$, interaction $p = 0.0348$) because fr exhibited no significant variations (Fig. 5E; treatment $p = 0.5360$, time $p = 0.8515$, interaction $p = 0.6982$). $\dot{M}O_2$ was significantly elevated overall by feeding, though none of the individual differences were significant relative to the corresponding control values, and followed a similar pattern to Vw, being significantly greater in the first hour relative to the fourth hour (Fig. 5G; treatment $p = 0.0394$, time $p = 0.0933$, interaction $p = 0.3375$). % U was significantly higher overall in the post-feeding treatment, and increased significantly in hours 3 and 4 relative to hour 1, and also relative to the corresponding control values (Fig. 5I; treatment $p < 0.0001$, time $p = 0.0375$, interaction $p = 0.0083$). Therefore, $\dot{M}O_2$ changes were due to variations in both %U (Fig. 5I), as well as in Vw (Fig. 5A). In turn, the latter were due to variations in Vsv (Fig. 5C).

When the post-feeding responses were analyzed over a finer time scale (10-min intervals), clear biphasic patterns emerged (Fig. 6). The greatest increases in $\dot{M}O_2$ were seen at about 30 min after the meal, followed by a progressive decline back to control levels by 90 min. This was followed by a secondary rise to another peak at 140 min, and another progressive decline thereafter (Fig. 6A; one-way repeated measures ANOVA $p = 0.0003$). The post-feeding $\dot{M}O_2$ cycles were mirrored by very similar cycles in Vw (Fig. 6B; $p = 0.0407$) and Vsv (Fig. 6C; $p = 0.0436$), while fr remained constant (Fig. 6D; $p = 0.4282$). %U (Fig. 6E; $p = 0.0083$) tended to increase in synchrony with the rise to the first peak in $\dot{M}O_2$ at 30 min, then stabilized before increasing again at about 110 min as the second peak in $\dot{M}O_2$ was approached. At this later time, %U was considerably higher than control levels, surpassing 85%. Note that in contrast to the aggregated data of Fig. 5I, none of these stepwise changes in %U over 10-min intervals (Fig. 6E) were significant by Tukey's post hoc test, reflecting the conservative protection built into this multiple comparison test, though they were highly significant (p ranging from < 0.001 to 0.04) by Fisher's LSD test.

Examination of the data from individual fish over the 3-s time scale confirmed the biphasic pattern seen after feeding in Fig. 6. However, as with post-exercise recovery data, there were variations among fish. Figure 7 shows an example, a different trout from that in Fig. 4, but again chosen because pre-exercise control data had been recorded for this same fish, with average values indicated by the dashed horizontal lines. In this particular individual, the two peaks in Vw (Fig. 7A, B) and $\dot{M}O_2$ (Fig. 7C) occurred early in the post-feeding period. Again, changes in Vw (Fig. 7A, B) were a major determinant of changes in $\dot{M}O_2$ (Fig. 7C). However, alterations in %U (Fig. 7D) also played an important role, especially later in the post-feeding period when a rise in %U became prominent.

The roles of Vw and %U in determining $\dot{M}O_2$ after feeding are examined further in the Discussion, with reference to the numbered phases in Fig. 7A.

Discussion

Overview

Key findings of this study support our original hypotheses, and provide additional mechanistic detail on how changes in Vw and %U contribute to increased $\dot{M}O_2$ after both exhaustive exercise and voluntary feeding. When measured with the present less invasive system, breathing proved to be more efficient (i.e., higher %U, lower relative Vw) under both resting and active conditions than indicated by previous determinations using more invasive or indirect techniques, in support of our first hypothesis. Indeed, % U did not change or increased after both exercise and feeding. Secondly, as predicted, increases in Vsv were more important than increases in fr (which barely changed) in elevating Vw, and therefore $\dot{M}O_2$ after both exercise and feeding. Although peak levels of $\dot{M}O_2$ and Vw were similar after exhaustive exercise and feeding, there were marked differences in timing, pattern, and duration of the changes following the two different treatments, in support of our third hypothesis.

Respiratory and ventilatory parameters under control conditions

Table 1 compares the present measurements of $\dot{M}O_2$, Vw, Vsv, fr, and %U on rainbow trout, made using the less invasive system, with previous determinations of these same parameters on the same species, made using other techniques (attached mask, Fick equation, or original method of van Dam). Most data are for resting trout under control conditions, but a few are for the peak levels after exercise. There are no previous data for the peak levels after feeding. Focusing now on the control data, it is clear that comparisons are confounded in an expected manner by differences in body size and temperature (lower mass-specific rates in larger animals, higher mass-specific rates at higher temperatures).

Unfortunately, there are no previous measurements of $\dot{M}O_2$, Vw, and %U for trout of 37 g at 8ºC, as used here. However, the measurements of Cameron and Davis (1970), Davis and Cameron (1971), Randall and Cameron (1973), Jones and Schwartzfeld (1974), Smith and Jones (1982), and Iwama et al. (1987) made by the mask technique, were all done on trout averaging about 236 g at a similar temperature. The average control $\dot{M}O_2$ in the 236 g trout in these six studies was 2138 μmol kg^{-1} h^{-1}, in comparison to the 4363 μmol kg^{-1} h^{-1} in our 37 g fish (Table 1). This comparison yields an allometric scaling exponent of 0.615, well within the normal range for fish in general (Clarke and Johnston 1999). Exponents ranging from 0.30 to 1.51 have been reported in other studies on salmonids (Brett 1964; Hughes 1984; Staples and Nomura 1976; Norin and Malte 2011; Onukwufor and Wood 2020). Applying this scaling exponent of 0.615 (based on routine $\dot{M}O_2$) to the control Vw measured by the less invasive technique in the present 37 g fish yields a predicted Vw of 147 ml kg^{-1} min^{-1} for the 236 g fish, considerably lower than the actual mean Vw of 209 ml kg^{-1} min^{-1} measured by the mask technique in the six studies. In our earlier study (Eom and Wood 2020), the control Vw measured on much larger trout (260-501 g, mean = 380 g) at comparable temperature by the less invasive technique was 140 ml kg^{-1} min^{-1}. Application of the same allometric scaling coefficient again yields a lower predicted Vw of 168 ml kg^{-1} min^{-1} for the 236 g trout. Thus, trout in the less invasive system maintains the same relative $\dot{M}O_2$ with a lower Vw, after allometric scaling.

Even without allometric scaling, this more efficient respiration can be seen clearly by calculation of the ventilatory convection requirement for oxygen (CR). The CR under control conditions was 4.13 L $mmol^{-1}$ for the present 37 g fish in the less invasive system *versus* a mean of 5.86 L $mmol^{-1}$ (range 4.32–7.21) in the 236 g fish as measured by attached masks (Table 1). This greater efficiency is largely explained by the higher %U (about 67%) in the resting trout in the less invasive system. The lower %U (33–59%) in all the earlier mask studies seems to be consistent regardless of body size or temperature (Table 1). A notable exception is the original study by van Dam (1938) himself in which %U was about 80% in a single 900 g trout at higher temperature (10-12ºC). This was accompanied by a measured Vw value of only 133 ml kg^{-1} min^{-1} and a CR of 3.80, the latter very similar to the present study (Table 1). As in

the present study, van Dam (1938) did not attach the mask by stitching or gluing but rather by custom tailoring a form-fitting device, and reported that he was able to move his single trout in and out of the system a number of times, keeping it in good condition by daily feeding (Unfortunately, he did not present information on the effect of feeding!). Again, it seems that when trout are less stressed, %U can be appreciably higher, and both relative Vw and CR are appreciably lower.

Another consideration is that %U in our less invasive system will also capture any additional O_2 uptake by the skin after the water has been expired from the gills. Kirsch and Nonnotte (1977) reported that this could amount to 13% of total $\dot{M}O_2$ in trout in fully oxygenated water, though this would undoubtedly be lower in our system as the skin is in contact with post-gill water of lower PO_2. Likely, the devices used in previous mask studies would also have captured some of the O_2 uptake by the skin in their %U calculations, but with less reliability, whereas Fick studies with opercular catheters for sampling P_EO_2 would have missed it entirely. Control values in the ventilation studies based on the Fick equation (Wood and Munger 1994; Kiceniuk and Jones1977; Stevens and Randall 1967a, b; Hughes and Saunders 1970) are highly variable, with Vw ranging from 200 to 1427 ml kg^{-1} min^{-1} at $\dot{M}O_2$ values ranging from 1125 to 3000 μmol kg^{-1} h^{-1} (Table 1). This variability is not appreciably reduced by allometric scaling and is largely explained by massive variability in %U which ranges from 9 to 70% (Table 1). The principal explanation here appears to be that opercular cannulae used to sample expired water PO_2 in the Fick technique provide poor estimates of mean expired oxygen tension, such that apparent %U depends critically on the exact placement of the catheters (Davis and Watters 1970).

The relative contributions of changes in frequency (fr), ventilatory stroke volume (Vsv) and % utilization (%U) in altering ventilation volume (Vw) and oxygen consumption ($\dot{M}O_2$)

In the present study, increases in Vw after exercise were achieved mainly by increases in Vsv rather than by increases in fr (Figs. 2, 3, Table 1). This is consistent with the findings of van Dam (1938) when his trout struggled spontaneously, and those of Wood and Munger (1994) and Stevens and Randall (1967a, b) on exercised trout, both of which used the Fick technique to estimate Vw (Table 1). Similarly, Heath (1973) measured buccal and opercular pressure amplitudes as a proxy for Vsv and concluded that large elevations in Vsv were much more important than modest elevations in fr in increasing Vw during and after exercise in trout. This pattern is also consistent with previous measurements on trout with the mask technique that used temperature (Randall and Cameron 1973), hypoxia (van Dam 1938; Davis and Cameron 1971; Kinkead and Perry 1990; Eom and Wood 2020), hyperoxia (Wood and Jackson 1980; Eom and Wood 2020), and hypercapnia (Janssen and Randall 1975) to alter ventilation. With respect to water pump efficiency, there are obvious energetic advantages to varying stroke volume rather than frequency when pumping a medium of high density and viscosity such as water (Shelton et al. 1986; Perry and Wood 1989).

Similarly, in the present study, increases in Vw after feeding were mainly achieved by increases in Vsv rather than by increases in fr (Figs. 5, 6, Table 1). We are aware of no previous studies that made comparable measurements after feeding, but Millidine et al. (2008) reported that variations in fr explained only about 30% of the variation in $\dot{M}O_2$ in juvenile Atlantic salmon that were freely swimming and feeding; the other 70% was presumably due to changes in Vsv and/or %U. This raises the interesting question of the role of %U in altering $\dot{M}O_2$ after both exercise and feeding. Previous studies on trout suggest that %U either does not change (Stevens and Randall 1967a, b; Kiceniuk and Jones 1977) or falls (van Dam 1938; Wood and Munger 1994) as Vw increases after exertion (Table 1). In contrast, our results indicate that %U actually rises slightly and CR falls as Vw increases after exercise, and more so after feeding (Figs. 2I, J, 3E, 5I, J, 6E), as discussed below. The discrepancies with the previous literature may be explained by problems with opercular cannulae in the Fick studies (Davis and Watters 1970), and the possibility that "struggling" (van Dam 1938) may differ from true swimming exercise.

Respiratory and ventilatory responses to exercise

$\dot{M}O_2$ increased approximately twofold in the first 10-min period after exhaustive exercise relative to

control values (Fig. 3A, Table 1), whereas previous studies have generally reported greater increases of 3 to sixfold (Wieser 1985; Scarabello et al. 1991, 1992; Zhang et al. 2019, 2020; McArley et al. 2021). There are several reasons for this difference. Firstly, earlier $\dot{M}O_2$ studies used tail-grabbing, electric shocks, or intentional air exposure at the end of chasing to ensure maximal exhaustion, whereas we simply chased our fish. Secondly, there was a 1-min time lag in transferring our trout to the system at the end of the chasing exercise protocol, followed by about a 6-min equilibration period before reliable data could be recorded. Zhang et al. (2020) have shown that $\dot{M}O_2$ falls during this period, so it is likely that we are underestimating post-exercise maximum Vw as well as maximum $\dot{M}O_2$. Thirdly, there is accumulating evidence that longer measurement windows yield lower estimates of maximum $\dot{M}O_2$. We elected to average data in Fig. 3 over 10-min periods, whereas windows as low as 1.5 min have been recommended to capture the highest possible values (Zhang et al. 2019, 2020). Certainly, it is clear from our 3-s window recordings after both exercise and feeding (Figs. 4C, 7C, 8A, B) that much higher values than those captured in 10-min means can be seen. Lastly, we are comparing our post-exercise data with data on our control fish in the same apparatus representing "routine $\dot{M}O_2$", while some of the other studies have made their comparisons against lower "standard $\dot{M}O_2$" estimates which were obtained by statistical extrapolation or data selection.

Within the above limitations, the 10-min window analyses indicate that $\dot{M}O_2$ and Vw were greatest immediately in the first two periods after exercise, and thereafter declined in parallel such that routine rates were re-established statistically within 80–90 min, though small further reductions may have occurred thereafter (Fig. 3A, B). This contrasts with much longer periods before internal metabolite and acid–base status is re-established (Turner et al. 1983; Milligan 1996; Scarabello et al. 1991; Wood 1991; Wang et al. 1994; Zhang et al. 2018), and is consistent with the ability of rainbow trout and other salmonids to rapidly elevate $\dot{M}O_2$ again in repeat swim tests, long before complete metabolic recovery has occurred (reviewed by Zhang et al. 2019).

An important finding is that increased Vw after exercise occurred without any decrease in mean %U.

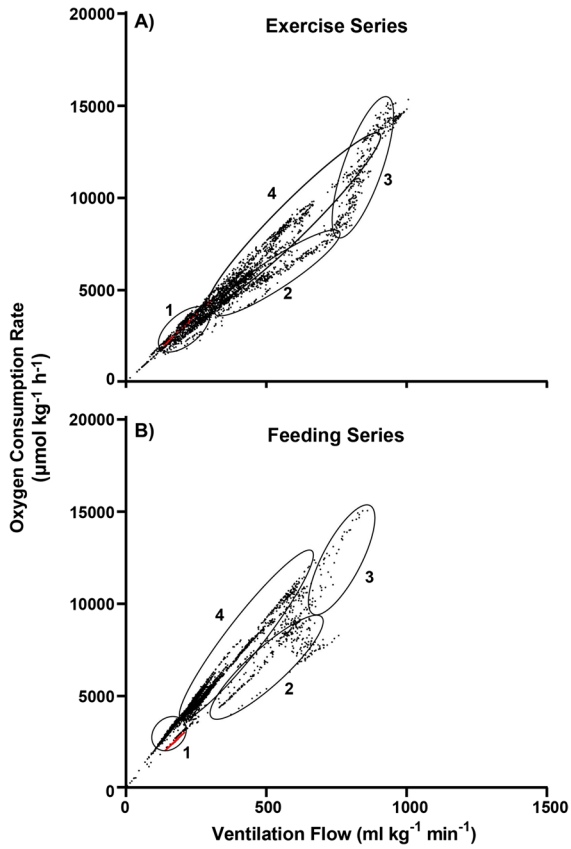

Fig. 8 Analyses of the relationships between oxygen consumption rate ($\dot{M}O_2$) and ventilatory flow rate (Vw) based on the data for (**A**) the individual fish of Fig. 4 during 4-h after exhaustive exercise, and (**B**) for the individual fish of Fig. 7 during 4-h after voluntary feeding. The numbered, circled data points relate to the numbered phases in Fig. 4A and 7A respectively. The red data points represent the resting control values measured in the same fish before the experimental treatments. See text for additional details

There is a general belief, based on the classic studies of van Dam (1938), Saunders (1962) and Davis and Cameron (1971), that as Vw increases, %U falls and CR increases because the water flows in a less effective manner across the gills, exhibiting turbulence, bypassing some of the secondary lamellae, or flowing too quickly to equilibrate. Certainly, there are instances where falling %U with increased Vw could be seen in the high-resolution recordings from individual fish (Fig. 4), but overall this did not occur, and indeed the already high mean %U tended to increase slightly after exercise (Figs. 2I, 3E) such that CR actually fell slightly (Table 1). Very likely, this can be attributed to an increase in effective gill

O_2 permeability (increased functional surface area and reduced diffusion distance) which is accomplished by blood flow redistribution in the gills (reviewed by Wood and Eom 2021) combined with a lower mixed venous blood PO_2 (i.e. increased diffusion gradient) in exhausted fish (Kiceniuk and Jones 1977; Neumann et al. 1983; McArley et al. 2021). The less stressful conditions and the impossibility of contamination of P_EO_2 by outside water in our system allowed the detection of this high %U.

Figure 8A presents an analysis of the relationship between Vw and $\dot{M}O_2$ for the post-exercise fish shown in Fig. 4; the numbered, circled data points relate to the numbered phases in Fig. 4A. Overall, it is clear that there was a tight correlation between these two parameters, with the variations reflecting changes in %U. Data in area 1 were achieved mainly at the end of the 4-h recovery period and were similar to control data. Data in area 2 were obtained at times when Vw and $\dot{M}O_2$ were increasing rapidly, either immediately after exercise or during later surges, and at these times, the points tended to fall below the general relationship, reflecting small decreases in %U. At these times gas exchange may have been less effective due to more turbulent water flow bypassing some lamellae and/or incomplete blood flow redistribution in the gills. Data in area 3 represented times when fairly stable peaks in Vw occurred, and here it can be seen that elevations in $\dot{M}O_2$ were relatively greater than those in Vw, reflecting increases in %U as water and blood flow patterns were optimized. Finally, those in area 4 were obtained at times when Vw and $\dot{M}O_2$ were both decreasing back towards control levels, and %U had also returned to control levels.

Respiratory and ventilatory responses to feeding

We examined the respiratory consequences of voluntary feeding, in contrast to many studies on the physiology of SDA that have used gavage or intubation to deliver the meal (e.g. Thorarensen and Farrell 2006; Eliason et al. 2007; Seth et al. 2009). As first shown by Brett and Zala (1975), there is a marked exercise and excitement component to voluntary feeding, so some of the same patterns in Vw and $\dot{M}O_2$ responses as seen after exhaustive exercise were expected. Indeed, this seemed to be the case, with initial increases in these parameters in the first 30 min post-feeding to levels comparable to those seen after exhaustive exercise (Table 1, Figs. 3, 6). Likely, this first peak had a substantial exercise-excitement component, but we interpret the second peak at 140 min as a response to food ingestion. Gavage and intubation studies suggest that the metabolic stimulation caused by food ingestion is not immediate but starts at 1–4 h after the meal enters the gastrointestinal tract (Eliason et al. 2007; Seth et al. 2009). By this time, blood flow to the gut has increased (Thorarensen and Farrell 2006; Seth et al. 2009; Seth and Axelsson 2010). Amino acid absorption into the systemic blood plasma was detected 3 h after food was placed in the stomach by gavage (Karlsson et al. 2006). At 1–4 h, the meal is almost exclusively confined to the stomach in rainbow trout that have undergone voluntary feeding (Bucking and Wood 2006a). The stomach plays a critical role in both the secretion of gastric acid and the absorption of major ions in this species (reviewed by Wood and Bucking 2010). The resulting alkaline tide (Bucking and Wood 2008), flux of Na^+ out of the stomach (Bucking and Wood 2006b), and increases in plasma $[Na^+]$ (Bucking and Wood 2006a) first become detectable at 2–3 h after voluntary feeding. We, therefore, interpret the rise in $\dot{M}O_2$ and Vw at this time as the first real SDA response, driven by the cost of HCl secretion, nutrient and ion absorption in the stomach, and associated systemic events (metabolic processing of absorbed nutrients, protein synthesis for growth, branchial base excretion). It is well established that SDA is a long-lasting event in salmonids (McCue 2006), often not peaking until 6–20 h after a meal (Medland and Beamish 1985; Eliason et al. 2007, 2008; Goodrich et al. 2022). Therefore, we predict that if measurements were to be extended to these times, further substantial increases in $\dot{M}O_2$ and Vw would be observed, especially once the food reaches the prime absorptive areas, the anterior intestine and pyloric caecae (Buddington and Diamond 1986; Bucking and Wood 2012).

Figure 8B presents an analysis of the relationship between Vw and $\dot{M}O_2$ for the fed trout shown in Fig. 7; the numbered, circled data points relate to the numbered phases on Fig. 7A. A clear difference from the post-exercise trout of Fig. 8A is the greater slope of the overall relationship, reflecting the greater %U in the fed trout. The rest of the analysis reveals superficially similar patterns between post-exercise recovery (Fig. 8A) and post-feeding treatments (Fig. 8B), though with subtle differences. In Fig. 8B, data in

area 1 were recorded at times when Vw had returned to control levels yet % U remained high and that can be seen as the elevation of these values above the resting control data points that are in red. Measurements in area 2 reflected times of high Vw when %U clearly fell. Data points in area 3, where elevated Vw and $\dot{M}O_2$ were both stable and %U was high, were relatively scarce. In area 4, where Vw and $\dot{M}O_2$ were both decreasing, % U remained elevated.

While $\dot{M}O_2$ and Vw were elevated approximately in parallel after both exhaustive exercise and voluntary feeding (Fig. 8), the principal difference appeared to be a greater reliance on increased %U after feeding. This was especially evident later in the 4-h post-prandial period when the SDA response became established (Figs. 5I, 6E). After exercise blood flow is preferentially diverted to red and white muscle (Neumann et al. 1983), whereas after feeding the gastrointestinal system receives preferential perfusion (Thorarensen and Farrell 2006; Seth et al. 2009; Seth and Axelsson 2010). One possible explanation for greater %U is that venous blood may have a lower PO_2 and/or a greater O_2 affinity after feeding than after strenuous exercise, thereby improving the diffusion gradient for $\dot{M}O_2$ at the gills. Another might be a more effective matching of water flow and blood flow patterns in the gills after feeding. However, we are aware of no evidence on these points, which are promising areas for future investigation.

Concluding remarks

In conclusion, this study provides the first direct measurements of Vw and %U after exhaustive exercise and feeding in fish. In the last two decades, we have learned a great deal about how external signals stimulate ventilation in teleosts (Porteus et al. 2012; Perry et al. 2023; Pan and Perry 2023), but we know very little about how internal signals related to exercise and feeding also stimulate ventilation. Recent findings on the role of plasma lactate (Thomsen et al. 2019; Leonard et al. 2022) and plasma ammonia (Zhang et al. 2011; Eom et al. 2020) in stimulating gill chemoreceptors, and thereby breathing, provide the first clues on the exercise and feeding signals, respectively. We now have the tools to quantify the extent of changes in Vw and %U after these treatments, which will facilitate future progress on these important topics.

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Data availability From the authors, on reasonable request.

Declarations

Ethical approval The methodology of this study was approved by the University of British Columbia Animal Care Committee.

Competing interests No competing interests were declared.

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