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Post-prandial respiratory gas and acid-base profiles in the gastrointestinal tract and its venous drainage in freshwater rainbow trout (*Oncorhynchus mykiss*) and seawater English sole (*Parophrys vetulus*)

Ellen H. Jung [a,b,*], Colin J. Brauner [a], Chris M. Wood [a,b]

[a] Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada
[b] Bamfield Marine Sciences Centre, Bamfield, BC V0R 1B0, Canada

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ABSTRACT

The basic respiratory gas and acid-base conditions inside the lumen of the gastrointestinal tract (GIT) and blood draining the tract are largely unestablished in teleost fishes after feeding, though there have been some recent novel discoveries on freshwater rainbow trout (*Oncorhynchus mykiss*) and seawater English sole (*Parophrys vetulus*). The present study examined in greater detail the gas (PO_2, PCO_2, PNH_3) and acid-base profiles (pH, $[HCO_3^-]$, total [ammonia]) in the lumen of the stomach, the anterior, mid, and posterior intestine, as well as the venous drainage (subintestinal and/or hepatic portal vein) of the GIT in these two species 20 h post-feeding. Both species had high PCO_2, PNH_3, and total [ammonia], and low PO_2 (virtual anoxia) in the lumens throughout all sections of the GIT, and high $[HCO_3^-]$ in the intestine. Total [ammonia], PNH_3, and $[HCO_3^-]$ increased from anterior to posterior intestine in both species. *P. vetulus* had higher intestinal total [ammonia] and lower $[HCO_3^-]$ than *O. mykiss* post feeding, but total [ammonia] was much higher in the stomach of *O. mykiss*. Despite the extreme conditions in the lumen, both arterial and venous blood showed relatively lower PCO_2, total [ammonia] and higher PO_2, implying limited equilibration between the two compartments. The higher $[HCO_3^-]$ and lower total [ammonia] in the intestinal lumen of the freshwater *O. mykiss* than the seawater *P. vetulus* suggest the need for future comparative studies using conspecifics fed identical diets but acclimated to the two different salinities in order to understand the potential role of environmental salinity and associated osmoregulatory processes underlying these differences.

1. Introduction

The fish gastrointestinal tract (GIT) is a metabolically dynamic tissue, being a site for endogenous tissue metabolism, microbiome metabolism, acid-base and osmoregulatory ion and water transport, and nutrient degradation and transport following feeding. In combination, these processes generate high levels of CO_2 (Wood, 2019) and ammonia (Bucking et al., 2013b; Pelster et al., 2015; Rubino et al., 2014) and a virtually anoxic luminal environment (Jung et al., 2020). Indeed, recent direct measurements showed high PCO_2 (2–10 fold greater than blood levels) in the lumen of freshwater (FW) rainbow trout (*Oncorhynchus mykiss*) and goldfish (*Carassius auratus*) (Wood and Eom, 2019), and in the seawater (SW) English sole (*Parophrys vetulus*; Jung et al., 2020). Despite these extreme luminal conditions, it remains unclear to what degree the blood passing through the GIT is affected by these conditions.

Digestion can generate PCO_2 in the digestive tract as a result of a combination of increased endogenous metabolism of the GIT tissues, reaction of gastric HCl with endogenous carbonates in the food and HCO_3^- secretion in the lower tract, and bacterial fermentation (Grosell, 2011; Guffey et al., 2011; Jung et al., 2020; Kurbel et al., 2006; Suarez et al., 1997; Tomlin et al., 1991; Wood, 2019; Wood and Eom, 2019). Thus, feeding could further elevate the luminal PCO_2, creating greater PCO_2 diffusion gradients between the lumen and the blood, and thus potentially increase blood PCO_2 (Jung et al., 2020; Wood and Eom, 2019). In these earlier studies on *O. mykiss* (Wood and Eom, 2019) and *P. vetulus* (Jung et al., 2020), the blood in the subintestinal vein (SIV) draining the intestine exhibited higher (but variable) PCO_2 values than in the arterial blood. Notably, these PCO_2 levels were higher than the typical values reported for mixed venous blood in fish (reviewed by Tufts and Perry, 1998), which suggests some degree of equilibration

* Corresponding author at: Dept. of Zoology, University of British Columbia, Vancouver, B.C. V6T 1Z4, Canada.
E-mail addresses: jung@zoology.ubc.ca (E.H. Jung), brauner@zoology.ubc.ca (C.J. Brauner), woodcm@zoology.ubc.ca (C.M. Wood).

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between the high luminal PCO_2 and the blood passing through the GIT.

If the high luminal PCO_2 diffuses into the blood, it could benefit in unloading O_2 to the tissues *via* Bohr and Root effects (Cooper et al., 2014; Nikinmaa, 2006; Rummer and Brauner, 2015). This could be especially important in delivering O_2 to the metabolically active transport epithelia of the intestine where it may be virtually anoxic on the apical side, as first reported in *P. vetulus* (Jung et al., 2020). The PO_2 in the subintestinal venous blood in fed *P. vetulus* was low (8.8 mmHg) but still significantly higher than the lumen ($\leq$ 0.3 mmHg) suggesting incomplete equilibration between the lumen and the blood. The luminal PO_2, however, did not seem to be affected by feeding in *P. vetulus*. PO_2 in the GIT lumen of *O. mykiss* is completely unknown, and was measured for the first time in the present study. To understand the potential effect of feeding on the lumen and blood gas profiles, post-prandial conditions of both species were investigated. Due to an insufficient blood flow in the SIV of *P. vetulus* in fasting conditions, we were unable to insert micro-optodes into the vessel for comparison with fasting *O. mykiss*.

Furthermore, the catabolism of dietary proteins generates high concentrations of ammonia in the lumen reaching up to 1–2 mM during digestion in *O. mykiss* (Bucking and Wood, 2012; Rubino et al., 2014), creating a concentration gradient for ammonia into the plasma (Kaushik and de Oliva Teles, 1985; Wicks and Randall, 2002). A portion of this ammonia could be detoxified by GIT bacteria (Turner and Bucking, 2019) or absorbed into the enterocytes, which exhibit some capacity to metabolically detoxify ammonia loads (Jung et al., 2021; Rubino et al., 2014). The plasma ammonia level of mixed systemic blood (Bucking and Wood, 2008) and hepatic portal vein (HPV) blood (Karlsson et al., 2006) both increased significantly post feeding, suggesting transport of dietary-sourced ammonia into the blood. The majority of the plasma ammonia surplus is then excreted to the environment through the gills (Beamish and Thomas, 1984; Karlsson et al., 2006), and to a much lesser extent by the kidneys (Bucking et al., 2010). The possible transport of ammonia across the GIT membrane could be *via* K^+ channels, Rhesus (Rh) glycoproteins, and/or linked to Na^+ uptake partly through NKCC and aquaporins (Bucking et al., 2013a; Bucking and Wood, 2012; Rubino et al., 2019, 2015; Wright and Wood, 2009). All of the above information was generated on FW *O. mykiss*, and nothing is known about ammonia handling in the GIT of SW *P. vetulus*. Given the necessity of Na^+ uptake for osmoregulation, SW teleosts may experience greater uptake of ammonia into the blood than FW teleosts.

These previous novel findings indicated that there is much more of interest to be learned about respiratory gas exchange in the GIT of FW and SW teleosts. The present study surveyed the profiles of the three respiratory gases - O_2, CO_2, and ammonia - inside the lumen and in the blood in FW *O. mykiss* and SW *P. vetulus*. Questions of particular interest were whether the virtually anoxic conditions seen in *P. vetulus* GIT lumen would also occur in *O. mykiss*, whether there would be differences between the two species possibly linked to environmental salinity, and whether luminal gases would equilibrate with the bloodstream. Specifically, we hypothesized that the extreme conditions of PO_2, PCO_2 and ammonia in the lumen would be reflected in the blood draining the tract, and would become more marked after feeding.

2. Material and methods

2.1. Experimental animals

Rainbow trout *O. mykiss* ($N = 12$; 112 - 470 g) were obtained from Little Cedar Falls Hatchery (Nanaimo, BC) and transferred to University of British Columbia (UBC) where they were held for several months prior to experiments. Fish were held at 9 °C in flowing dechlorinated Vancouver tap water ($Na^+ = 0.09$, $Cl^- = 0.10$, $Ca^{2+} = 0.10$, $Mg^{2+} = 0.011$, $K^+ = 0.004$ mmol L^{-1}, hardness as $CaCO_3 = 3.3$ mg L^{-1}, pH = 7.0). During this time, *O. mykiss* were fed to satiation daily with commercial pellet food (BioTrout 4.0 mm, Bio-Oregon™, Long-view, WA, USA). English sole *P. vetulus* ($N = 21$; 195-565 g) experiments were performed at Bamfield Marine Sciences Centre (BMSC; Bamfield, BC) in August–September 2019 and 2020. Fish were collected from the wild (Barkely Sound, BC) by angling under Department of Fisheries and Oceans Canada collection permits XR-204.18 and XR-212.19, and transferred to outdoor seawater flow-through tanks at 10–12 °C and 32 ppt salinity at BMSC. Tanks were filled with 4 cm of sand to allow the animals to burrow; during this time *P. vetulus* were fed previously frozen anchovies (*Engraulis mordax*).

We investigated *O. mykiss* in starved and fed conditions, while only the fed state of *P. vetulus* was measured. For both species, in the fed groups, fish were fed 20 h prior to experimentation while in the fasted group feeding was withheld for 7 days prior to experimentation. All experiments were approved by the UBC (AUP 14–0251 and 18–0271) and BMSC Animal Care Committees (RS-18-20, RS-19-15, and RS-20-17).

2.2. Cannulation

Fish were anaesthetized with 0.1–0.2 g L^{-1} NaOH-neutralized MS-222 (Syndel Laboratories, Parksville, BC) in either freshwater (*O. mykiss*) or seawater (*P. vetulus*). Once anaesthetized, the fish were transferred to an operating table and their gills were continuously irrigated with temperature-controlled (acclimation temperature and salinity), anesthetic (~0.03 g L^{-1}; Stage 5; McFarland, 1959) water. The dorsal aorta of *O. mykiss* was cannulated according to the procedure described in Soivio et al. (1972). The caudal artery of *P. vetulus* was cannulated as previously described by Watters and Smith (1973) and Jung et al. (2020). Anaesthesia and gill irrigation were then maintained at the same level throughout the ensuing *in situ* measurements, following which fish were euthanized.

2.3. PCO_2, PO_2, total O_2 content and hemoglobin ([Hb]) measurements

The PCO_2 micro-optodes (PreSens, Regensburg, Germany), mounted in #23 hypodermic needles, were prepared and calibrated as described in Jung et al. (2020). These PCO_2 micro-optodes were prototype devices (PreSens 200,001,368) connected to an electronic transmitter (PreSens 300,000,114), with the output displayed on a personal computer running prototype software (PreSens 200,001,488). All PO_2 was measurements were made using micro-optodes (PreSens, Regensburg, Germany) mounted in #23 hypodermic needles and calibrated with air-equilibrated and sodium sulfite saturated saline kept in gas tight bottles. Arterial blood samples (approximately 1 mL) were collected from the catheter by blood pressure-driven flow into 2-mL microcentrifuge tubes, and then PCO_2 and PO_2 micro-optodes were inserted to the bottom of the tube sitting in the temperature-controlled bath. Whole blood O_2 content was measured using the Tucker method (Tucker, 1967). 10 μL of blood was collected using a gas-tight syringe (Hamilton, Reno, Nevada, USA) and injected into a custom-made 2344 μL Tucker chamber with the PO_2 micro-optode inserted through a gas-tight septum. The chamber was filled with potassium ferricyanide and saponin to release O_2 bound by hemoglobin. The [Hb] was measured as described by Kampen and Zijlstra (1961).

Then, the peritoneal cavity was surgically opened and the PO_2 micro-optode was directly inserted into the lumen of the gastrointestinal tract (GIT) at four sites (stomach, anterior, mid, and posterior intestine) for luminal measurements. The PCO_2 and PO_2 micro-optodes were inserted into the subintestinal vein (SIV) near the posterior intestine of both species, and into the hepatic portal vein (HPV) of *O. mykiss* in random order. In fasted *P. vetulus*, the SIV was not engorged enough for insertion of PO_2 and PCO_2 micro-optodes.

2.4. pH, total CO_2 content (TCO_2), and total [ammonia] measurements

The pH of the collected arterial blood was measured using a micro-combination probe (MI-414; 6 cm beveled tip; Microelectrodes Inc.,

Bedford, NH, USA). For both species, not enough blood could be taken from the SIV or HPV for pH, TCO_2 and [ammonia] measurements. After completion of all blood and luminal gas measurements, the fish was euthanized by an over-dose of anesthetic, and weighed. The four regions of the GIT (stomach, anterior intestine, mid intestine, posterior intestine) were ligated with 2–0 silk threads and excised. The GIT fluid/chyme was collected into 2-mL centrifuge tubes, centrifuged (2 min, 5000 *g*), and the pH of the supernatants was measured, using the same micro-electrode as for blood. The supernatants were then flash-frozen in liquid N_2. Samples were later thawed on ice and immediately assayed for TCO_2 using a Corning 965 CO_2 analyser (Ciba-Corning Diagnotstics, Halstead, Essex, UK) and total [ammonia] using a commercial ammonia kit (Raichem Cliniqa™; glutamate dehydrogenase method). Prior tests have demonstrated that chyme TCO_2 values do not change due to freezing.

2.5. Calculations and statistical analyses

Graphs were made and statistical analyses were performed using Graphpad Prism software (version 7.0a). All data are expressed as means ± SEM (N = number of fish). Calculated PCO_2 and [HCO_3^-] values were derived from rearrangements of the Henderson-Hasselbach equation (see Wood et al., 1983) using values for pK' and CO_2 solubility for teleost plasma (Boutilier et al., 1984). Partial pressures of ammonia (PNH_3) of both plasma and chyme were calculated from measured total [ammonia] and pH using the solubility coefficient of *O. mykiss* plasma reported in Cameron and Heisler (1983). Species comparisons of data within the same blood or GIT region, as well as differences between fasted and fed treatment groups within a species employed Student's unpaired *t*-test. Comparisons between blood and GIT regions within a species used one-way ANOVA and Tukey's *post hoc* test. All other data were analyzed using two-way ANOVA with blood and feeding state as factors, or species and blood or GIT regions as factors, followed by Tukey's *post hoc* tests. A significance level of $p < 0.05$ was used in all tests.

3. Results

3.1. Direct-measurement of blood and luminal O_2

The PaO_2 values of the two species in the fed state were not significantly different from each other, and the oxygenation status of arterial blood did not change with feeding in either species (Table 1). However, *P. vetulus* had significantly lower levels of [O_2] and [Hb] than *O. mykiss*, differences of about 75% and 50% respectively (Table 1). Feeding had no significant effect on the PO_2 of blood sampled at different locations of *O. mykiss*, but the PO_2 values of SIV and/or HPV were significantly lower than PaO_2 in both species, by 60% or more (Table 1 and Figure 1). Luminal PO_2 was almost anoxic (< 1 mmHg) in both species (Figure 1A, B), and was not affected by feeding in *O. mykiss*; note the differences in scale between the left and right y-axis in Figure 1. Luminal PO_2 was significantly lower than SIV or HPV PO_2 in both species (Figure 1).

Table 1
Effect of feeding on arterial blood characteristics of *O. mykiss* (*N* = 5–6) and *P. vetulus* (*N* = 7–13). Means ± SEM. * represent significant differences between fasted and fed states within a species. † represents significant difference between species in the same feeding state.

	O. mykiss		*P. vetulus*	
	Fasted	Fed	Fasted	Fed
PO_2 (mm Hg)	99.5 ± 6.5	90.3 ± 3.5	–	80.3 ± 7.9
PCO_2 (mm Hg)	3.5 ± 0.7	4.7 ± 0.8	–	7.5 ± 1.1
Total [ammonia] (μM)	37.9 ± 11.4	45.9 ± 14.0	–	238.3 ± 49.9†
PNH_3 (μmm Hg)	9.0 ± 2.4	15.8 ± 3.4	–	49.3 ± 14.3†
[HCO_3^-] (mM)	10.5 ± 0.3	14.3 ± 1.2*	–	3.9 ± 0.7†
pH	7.80 ± 0.09	7.94 ± 0.14	–	7.66 ± 0.10
[O_2] (mM)	3.9 ± 0.2	4.4 ± 0.6	0.8 ± 0.2†	1.0 ± 0.2†
[Hb] (mg mL^{-1})	106.0 ± 19.2	126.5 ± 14.7	62.1 ± 3.5†	63.8 ± 5.0†
[O_2]/[Hb] (mmoles g^{-1})	0.042 ± 0.006	0.038 ± 0.007	0.012 ± 0.002†	0.014 ± 0.004†

3.2. Direct-measurement of blood PCO_2

O. mykiss had 4-fold higher SIV and HPV PCO_2 than that in the arterial blood, but for *P. vetulus*, the 1.6-fold higher SIV PCO_2 was not significantly different than the arterial PCO_2 (Figure 2). There were no significant differences between species in either arterial or SIV PCO_2. However, as illustrated by the individual data symbols in Figure 2, direct measurements of PCO_2 in the veins draining the gastrointestinal tracts of both species (SIV and/or HPV) had greater variability (lowest – highest

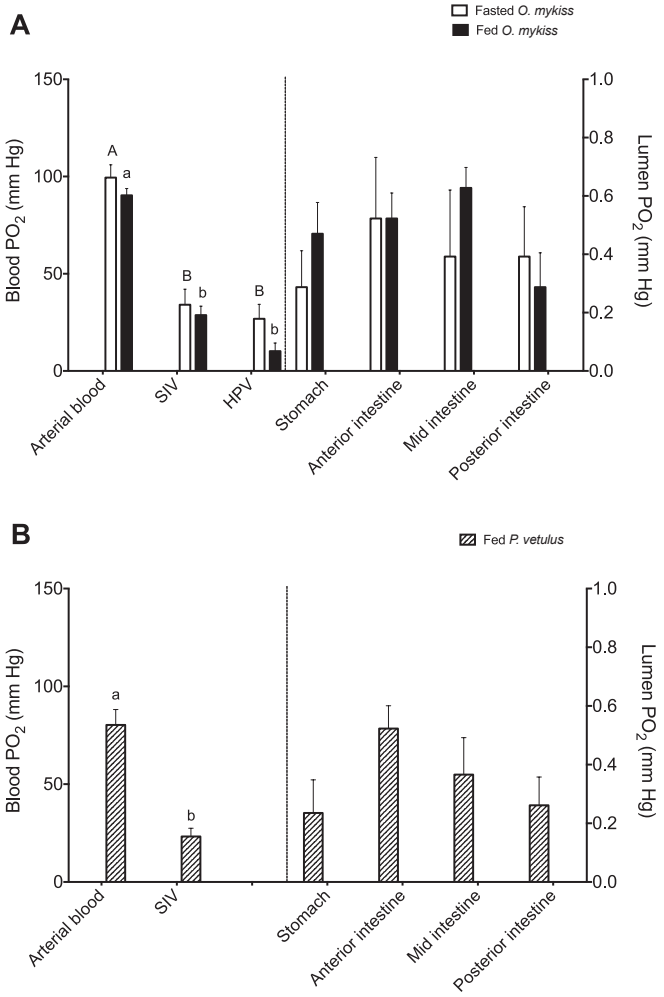

Fig. 1. Direct measurements of PO_2 in the blood and four regions of the gastrointestinal tract (GIT) lumen of (A) fasted and fed *O. mykiss* (N = 5–6) and (B) fed *P. vetulus* (*N* = 6–7). Means ± SEM. Blood data are reproduced from Table 1 to permit direct comparison with the luminal PO_2 values. Note that the left y-axis is for blood values, and right y-axis is for GIT lumen values. Upper case letters represent significant difference between blood samples from different sites in (A) fasted fish, and lower case in (A & B) fed fish. There were no significant differences between fasted and fed states in (A) *O. mykiss*, and there were no significant difference between fed species in either blood samples or luminal measurements. All luminal PO_2 values were significantly lower than blood PO_2 in both species.

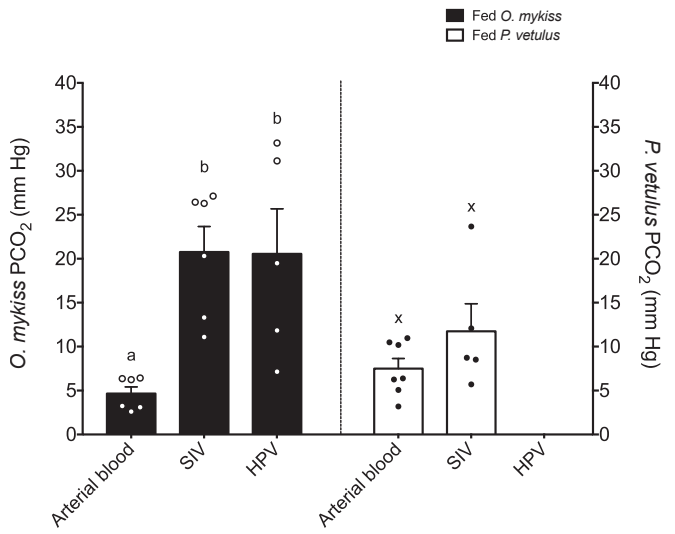

Fig. 2. Direct measurements of PCO_2 in arterial, subintestinal vein (SIV) and hepatic portal vein (HPV) blood after feeding in *O. mykiss* (left y-axis; N = 5–6) and *P. vetulus* (right y-axis; N = 5–7). Means ± SEM. Letters represent significant difference between blood measurements within a species. Arterial blood PCO_2 data are reproduced from Table 1 to permit direct comparison with SIV and HPV values. There were no significant differences between species. Individual data are overlaid on the bars as round symbols to illustrate the data distribution.

values: in *O. mykiss* SIV = 11.1–27.1 mmHg; HPV = 7.2–33.2 mmHg; in *P. vetulus* SIV = 5.7–23.7 mmHg) than in arterial blood samples (in *O. mykiss* 2.6–6.4 mmHg; in *P. vetulus* 3.2–11.0 mmHg).

3.3. $[HCO_3^-]$, pH and calculated PCO_2 of blood and Chyme

There was a significant 35% increase in $[HCO_3^-]$ of the arterial blood after feeding in *O. mykiss*, but the increase in pH was not significant (Table 1). Stomach chyme contained almost no $[HCO_3^-]$ (< 0.3 mM), but $[HCO_3^-]$ gradually increased as chyme proceeded down the intestinal tract in both species, resulting in the highest concentration in the posterior intestine (*O. mykiss* mean = 50.7 mM; *P. vetulus* mean = 20.5 mM) (Figure 3A). In general, *O. mykiss* had 2- to 3-fold higher $[HCO_3^-]$ in the arterial blood and intestinal chyme than *P. vetulus* (Table 1 and Figure 3A). In both *O. mykiss* and *P. vetulus*, the arterial blood pH values were typical of FW and SW teleosts respectively at these temperatures (Table 1). The stomach pH was the lowest in the GIT for both species (3.6 and 4.7 respectively), but pH increased close to blood levels in the intestine (7.7–8.1 and 7.1–7.6 respectively) (Figure 3B). The direct measurements of total CO_2 and pH allowed calculation of PCO_2 in the arterial blood and chyme, for which there were no significant differences between the species (Figure 3C). In both species, the calculated PCO_2 was highest in the stomach at 112–115 mmHg, but there were no differences among the intestinal segments (20–38 mmHg). The calculated PCO_2 in the arterial blood was low in both species at 3.4 mmHg for *O. mykiss* and 2.8 mmHg for *P. vetulus*. These were not significantly different from the directly measured values reported in Figure 2 for *O. mykiss* ($p = 0.21$), but measured values were significantly higher than calculated values in *P. vetulus* ($p = 0.0093$).

3.4. Blood and chyme ammonia

P. vetulus had approximately 5-fold higher total [ammonia] in the arterial blood plasma than *O. mykiss* (Table 1 and Figure 4A). In both species, chyme total [ammonia] was significantly higher (*O. mykiss* = 415–3709 μM; *P. vetulus* = 2180–3062 μM) than that of the arterial blood (Figure 4A; *O. mykiss* = 46 μM; *P. vetulus* = 238 μM). It was

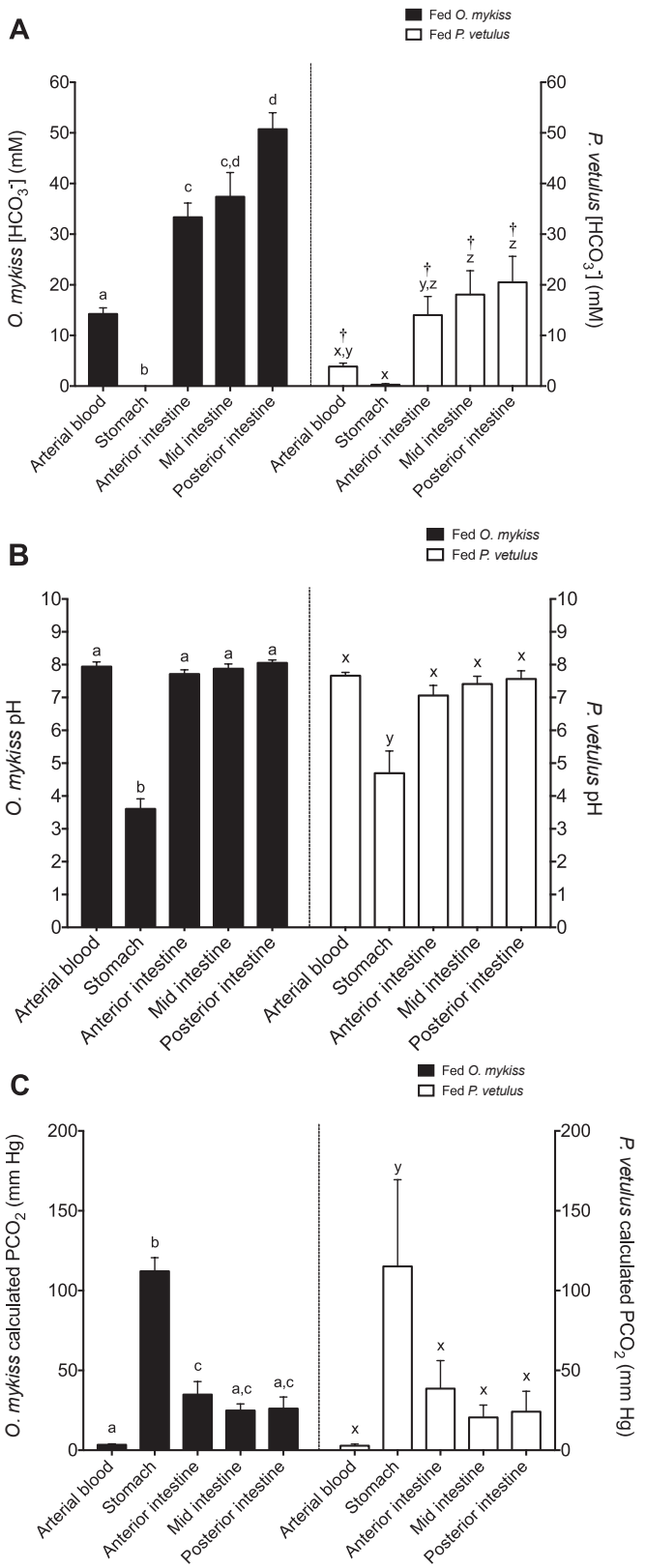

Fig. 3. (A) Measured $[HCO_3^-]$ and (B) pH of arterial blood and chyme and (C) Calculated PCO_2 of blood and chyme in fed *O. mykiss* (left y-axis; N = 6), and *P. vetulus* (right y-axis; N = 7). Means ± SEM. Arterial blood pH values are reproduced from Table 1 to permit direct comparison with the luminal values. Letters that differ represent significant differences between blood and regions of the GIT within a species. Dagger represents significant difference between species.

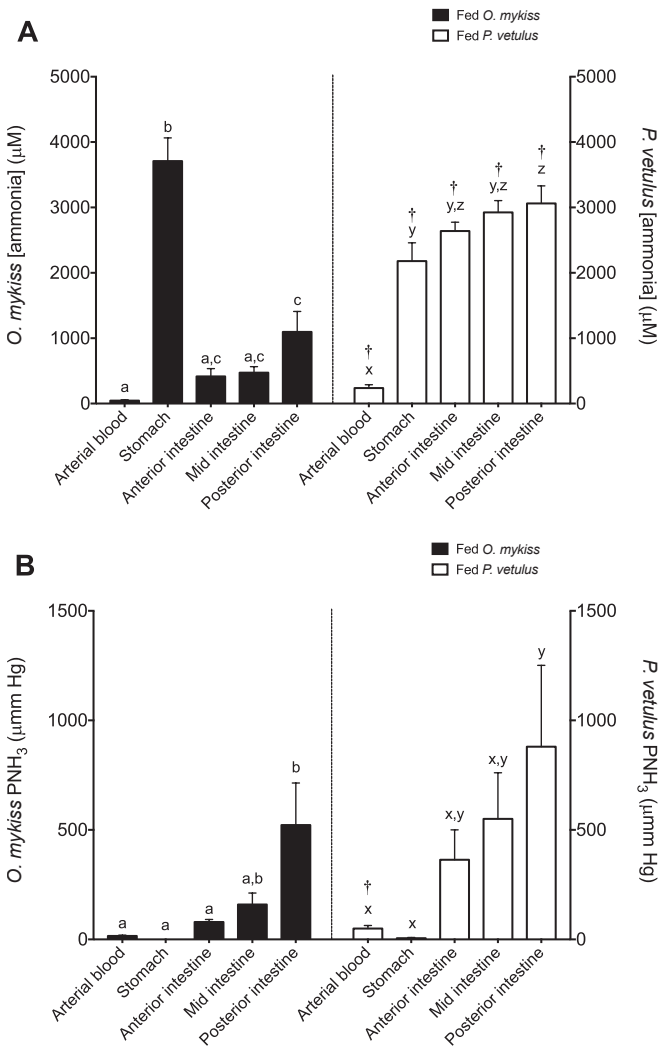

Fig. 4. Measured (A) total [ammonia] of arterial blood and chyme and (B) Calculated PNH_3 from direct measurements of pH (Figure 3B) and total [ammonia] (panel A) in fed *O. mykiss* (left y-axis; N = 5–6), and *P. vetulus* (right y-axis; N = 6–7). Means ± SEM. Letters that differ represent significant differences between blood and regions of the GIT within a species. Dagger represents significant difference between species. Arterial blood pH and PNH_3 values are repeated from Table 1 for comparison with the luminal values.

particularly high in the stomach of *O. mykiss*, followed by the posterior intestine then the anterior and mid intestines. In contrast, *P. vetulus* stomach chyme had the lowest total [ammonia], but it increased moving down the intestinal tract, as in *O. mykiss*. Therefore, *O. mykiss* had 1.7-fold higher stomach total [ammonia] than *P. vetulus*, but much lower concentrations (by 60–80%) in the intestine. There were no significant differences between species in chyme PNH_3 values (Figure 4B) calculated using measured pH (Figure 3B) and total [ammonia] (Figure 4A). Both species had the greatest PNH_3 value in the posterior intestine, which was up to 30-fold higher than in the arterial blood.

4. Discussion

The present study found extreme conditions in the lumen of the GIT, with high PCO_2, PNH_3, and total [ammonia], concurrent with extremely low PO_2 (almost anoxic) in both FW *O. mykiss* and SW *P. vetulus*. Overall, our findings did not support our original hypotheses. The venous blood draining from the tract did not seem to reflect the extreme luminal conditions. Furthermore, we did not find any remarkable differences in patterns between the species, though there were some interesting quantitative differences. Feeding had no effect on any of the blood measurements in *O. mykiss* with the exception of arterial blood [HCO_3^-], which was elevated (Table 1) likely as a consequence of HCl secretion into the stomach, inducing an alkaline tide (Hersey and Sachs, 1995). However, there were no significant differences in arterial blood pH or PCO_2 post feeding (Table 1). The lack of change in PCO_2 and consistent blood oxygenation status are indicative of an absence of respiratory compensation for the alkaline tide, similar to previous findings in teleosts (Bucking and Wood, 2008; Cooper and Wilson, 2008). The [O_2]/[Hb] did not change following feeding in *P. vetulus* as well, indicating that both species may be compensating acid-base status using base excretion or other means, rather than by respiratory mechanisms observed in other vertebrates (Andrade et al., 2004; Busk et al., 2000; Niv and Fraser, 2002).

4.1. Direct-measurement of blood and luminal O_2

The present study is the first to report a nearly anoxic lumen in a FW teleost, similar to the previous observation in SW *P. vetulus* (Jung et al., 2020). We found there to be little or no equilibration of PO_2 between the lumen and the blood in the SIV in both species (Figure 1). The anoxic lumen is similar to that in mammals which is a requirement to maintain favorable conditions for the GIT microbiome (Espey, 2013; Tiso and Schechter, 2015). In fact, the average luminal PO_2 of a mouse is reported to be <1 mmHg, similar to our findings, but the mucosa near the intestinal tissue (< 200 μm from the epithelial surface) is relatively well-oxygenated and colonized by oxygen-tolerant bacteria (Albenberg et al., 2014; Espey, 2013). A more recent study on germ-free mice revealed that mechanisms other than microbial respiration such as respiration of the intestinal epithelium tissue itself, and/or oxidative chemical reactions may also contribute to O_2 depletion (Friedman et al., 2018). The present study and Jung et al. (2020) found that the lumen was virtually anoxic regardless of feeding conditions in both species, implying oxygen depletion may not be dependent on the presence of food material. Whether similar heterogeneous PO_2 conditions and oxygen consumption pathways as those found in mouse also exist in fish requires further investigation.

4.2. Direct-measurement of blood PCO_2

The PCO_2 values in both the SIV and the HPV were higher than that of the arterial blood in *O. mykiss*, but not in *P. vetulus*, where only SIV PCO_2 could be measured (Figure 2). High venous PCO_2 could be due to an increase in luminal PCO_2, as observed after feeding in *O. mykiss* (Wood and Eom, 2019), where the measured PCO_2 in the lumen of the posterior intestine was about 25–30 mmHg. This is similar to the mean measured PCO_2 in the SIV and the HPV of approximately 20 mmHg in the present study (Figure 2), values that were below the calculated PCO_2 in the intestinal chyme, and far below the calculated PCO_2 in the stomach (Figure 3C). If luminal PCO_2 does diffuse into the blood, it could potentially affect acid-base regulation and blood O_2 transport (Cooper et al., 2014; Nikinmaa, 2006). However, it is important to point out the variability of the venous blood PCO_2 measurements for both species, as illustrated in Figure 2. Such variability has also been seen previously by Wood and Eom (2019) in *O. mykiss* (2–34 mmHg) and Jung et al. (2020) in *P. vetulus* (4–48 mmHg) and thus makes interpretation challenging. We speculate that this could be caused, at least in part, by the interruption of the blood flow during direct insertion of the PCO_2 micro-optodes. Further study with less invasive sampling will be required, perhaps by chronic cannulation of the SIV in much larger fish (Karlsson et al., 2006), before concluding that there is substantial PCO_2 diffusion from the lumen to the blood.

4.3. [HCO_3^-], pH and calculated PCO_2 of blood and Chyme

We found there to be almost no [HCO_3^-] in the stomach and that

[HCO_3^-] increases as chyme moves down the intestinal tract in both species (Figure 3A). The secretion of [HCO_3^-] into the intestinal lumen may be part of general digestive function in FW as well as in SW teleosts, but may be additionally utilized for osmoregulation in SW teleosts (Grosell, 2011). Calculated PCO_2 in the lumen was high in both species as predicted, but calculated stomach PCO_2 was higher (almost double) than directly measured values in previous studies (Figure 3C; Wood and Eom, 2019; Jung et al., 2020). Goodrich et al. (2020) also calculated particularly high stomach PCO_2 in FW climbing perch (*Anabas testudineus*) relative to the intestinal values. There may be a discrepancy between calculated and measured values as shown in Jung et al. (2020), but the same study also reported significant correlation between calculated and measured PCO_2 values. Thus, while some discretion should be used for interpreting absolute values, species comparisons using calculated PCO_2 could be relatively reliable. Nonetheless, arterial blood pH and PCO_2 showed no disturbance with feeding despite dynamic levels in the GIT.

P. vetulus had lower [HCO_3^-] in the chyme and similar chyme and SIV PCO_2 levels to *O. mykiss* (Figures 2 and 3A, C). Comparison between salinities is confounded by differences in diet (commercial pellets *versus* anchovies), temperature (9 °C *versus* 10–12 °C), and of course species. Nevertheless, there is no indication that the additional role of the intestine in osmoregulation in SW fish (Grosell, 2011) and the accompanying greater blood flow to the GIT (Brijs et al., 2015) is associated with more extreme levels of PCO_2 and [HCO_3^-] in the lumen. In SW teleosts, intestinal Cl^-/HCO_3^- exchange results in elevated luminal HCO_3^- concentrations, which help to precipitate Ca^{2+} and Mg^{2+} from the ingested seawater, thereby increasing the osmotic gradient for water absorption across the GIT (Grosell, 2011; Grosell et al., 2009; Guffey et al., 2011; Wood et al., 2010), with associated acid-base relevant ion movements between the lumen and the blood (Cooper et al., 2014). Our data, together with other recent data (Goodrich et al., 2020; Wood, 2019) question the general belief that high [HCO_3^-] in the intestine is exclusive to SW teleosts. As summarized in Wood (2019), the majority of the high intestinal [HCO_3^-] values measured in SW teleosts are for fasted animals. The few data on fed animals in SW summarized in Wood (2019) and our data for fed *P. vetulus* in the present study and Jung et al. (2020) are more comparable to those measured in fed FW teleosts, including other species investigated by Goodrich et al. (2020). A comparative study of FW and SW teleosts in the fasting state to eliminate feeding effects would be informative in teasing out these differences. Nevertheless, our observations indicate the need for further studies on conspecifics acclimated to FW and SW (*e.g.* the euryhaline *O. mykiss*) on similar diets to understand the effect of environmental salinity on luminal and blood gas conditions.

4.4. Blood and chyme ammonia

Luminal total [ammonia] was high in both species, but particularly high in the stomach of *O. mykiss* (Figure 4A). To our knowledge, this is the first study to report total [ammonia] in the chyme of the stomach, a region that has often been overlooked in ammonia handling abilities. This high [ammonia] disappears once the chyme moves into the intestine, suggesting that a large portion of the ammonia load may be absorbed in the stomach, similar to dietary Na^+ and K^+ ions (Bucking and Wood, 2006). If true, ammonia may be moving against the PNH_3 gradient in the stomach (Figure 4B), because the low gastric pH (Figure 3B) results in very low luminal PNH_3, despite high total [ammonia] (Figure 4A). In the mouse, Rh isoforms are expressed in the stomach (Handlogten et al., 2005), which may be involved in secreting and absorbing ammonia (Gips, 1973; Summerskill et al., 1966). At present, there is no information on possible ammonia absorptive pathways in the teleost stomach. An alternative explanation would be a very rapid absorption of large amounts of ammonia in the very first part of the anterior intestine, which could be driven by an increase in chyme PNH_3 (Figure 4B) in response to the almost 4 unit increase in pH encountered as chyme leaves the stomach (Figure 3B). Future experiments with isolated gut sac preparations (*e.g.* Jung et al., 2021; Rubino et al., 2014) may be able to address this uncertainty.

To date, there has been a focus on the intestine as a site of ammonia detoxification, excretion and absorption. Rubino et al. (2014) estimated that almost half of the ammonia produced from feeding in *O. mykiss* originates from ammonia being generated in the chyme or synthesized within the intestinal tissue, both of which may enter the blood. In various ammoniotelic teleosts, ammonia detoxifying enzymes such as glutamine synthetase (GS) and glutamate dehydrogenase (GDH) have been discovered in the enterocytes (Bucking et al., 2013b; Bucking and Wood, 2012; Mommsen et al., 2003; Pelster et al., 2015; Tng et al., 2008; Turner and Bucking, 2019). The same enzymes are also found in intestinal bacteria (Andersson and Roger, 2003; Müller et al., 2006; Turner and Bucking, 2019), indicating the importance of the microbiome in handling ammonia as well.

Concurrent with the assumed ammonia load in the lumen after feeding in their study, Karlsson et al. (2006) found a significant increase in plasma total [ammonia] levels in the HPV. While nothing is known about possible gastric ammonia absorption in fish, the mechanism of absorption of ammonia across the intestinal epithelium has now been investigated in some detail (Bucking et al., 2013b, 2013a; Jung et al., 2021; Rubino et al., 2019, 2015, 2014). Pathways include substitution for K^+ at K^+ channels and NKCC, and possibly passage through aquaporins (Rubino et al., 2019, 2015). Another possible route is *via* Rh glycoproteins that are present in the mammalian intestine (Handlogten et al., 2005; Weiner, 2006; Worrell et al., 2008), and are also expressed and responsive to feeding in the intestine of teleosts (Bucking et al., 2013a; Bucking and Wood, 2012; Rubino et al., 2015). Present evidence indicates that *O. mykiss* Rh glycoproteins transport NH_3 rather than NH_4^+ (Nawata et al., 2010), but this remains unproven. The HPV receives venous blood draining from the stomach as well as from the intestine (SIV), so the large ammonia load in the stomach found in the present study (Figure 4A) could be an additional or major source of elevated HPV ammonia levels reported in Karlsson et al. (2006). If the PNH_3 gradient is a driver of ammonia uptake, the posterior intestine could be a major site of ammonia absorption (Figure 4B). Overall, our data argue for the importance of measuring ammonia in the SIV to compare to the HPV levels, on a paired basis, in concert with simultaneous measurements of ammonia levels in stomach and intestinal chyme.

In the species comparison, *P. vetulus* had higher plasma and intestinal total [ammonia] levels than *O. mykiss* (Figure 4). Initially, given the necessity of Na^+ uptake in the gut for osmoregulation, we had speculated that SW teleosts may experience greater uptake of ammonia into the blood than FW teleosts, due to ammonia transport through Na^+ linked NKCC and aquaporins (Rubino et al., 2019, 2015). Although we did find higher plasma ammonia levels in *P. vetulus*, this may simply be due to the greater intestinal ammonia level in this species. Once ammonia is absorbed *via* the GIT, the fish is able to excrete some, but not all, of this ammonia across the gills, before the arterial blood sampling site (Bucking et al., 2013b; Bucking and Wood, 2008; Karlsson et al., 2006).

4.5. Conclusion

We performed a survey study on FW *O. mykiss* and SW *P. vetulus* of their postprandial profiles in the lumen and the circulatory systems supplying and draining the GIT, with respect to O_2, CO_2, ammonia, and pH. Despite the extreme conditions inside of the lumen, blood experienced much less disturbance. Therefore, there was no or limited equilibration of PCO_2, PO_2, ammonia between the lumen and blood in both species. There was a significant increase in post-intestinal venous PCO_2 in *O. mykiss*, but the variability in the data need to be addressed with a less invasive and/or *in vivo* sampling approach. We found minimal differences between the species, except for luminal total [ammonia] and

[HCO_3^-] levels that are potentially due to feed differences. Thus, our study suggests the need for further comparative investigation between FW and SW conspecifics under standardized conditions of diet and temperature, in order to understand any effects of the osmoregulatory role of the intestine in SW on the O_2, CO_2, ammonia, and acid-base conditions in the GIT lumen and the vascular system.

Authors' contributions

E.H.J. and C.M.W and devised the study. E.H.J. conducted the experiments, analyzed data and wrote the first draft. C.M.W. and C.J.B. provided supervision and edited the manuscript.

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Declaration of Competing Interest

All authors (Ellen H. Jung, Junho Eom, Colin J. Brauner, and Chris M. Wood) of the manuscript "Post-prandial respiratory gas and acid-base profiles in the gastrointestinal tract and its venous drainage in freshwater rainbow trout (*O. mykiss*) and seawater English sole (*P. vetulus*)," declare we have no competing interests.

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