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RESEARCH ARTICLE

The physiological consequences of a very large natural meal in a voracious marine fish, the staghorn sculpin (*Leptocottus armatus*)

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ABSTRACT

Little information exists on physiological consequences when wild fish eat natural food. Staghorn sculpins at 10–13°C voluntarily consumed 15.8% of their body mass in anchovies. Gastric clearance was slow with >60% of the meal retained in the stomach at 48 h, and was not complete until 84 h. At 14–24 h post-feeding, pH was depressed by 3 units and Cl^- concentration was elevated 2-fold in gastric chyme, reflecting HCl secretion, while in all sections of the intestine, pH declined by 1 pH unit but Cl^- concentration remained unchanged. P_{CO_2} and total ammonia concentration were greatly elevated throughout the tract, whereas P_{NH_3} and HCO_3^- concentration were depressed. Intestinal HCO_3^- secretion rates, measured in gut sacs *in vitro*, were also lower in fed fish. Whole-animal O_2 consumption rate was elevated approximately 2-fold for 72 h post-feeding, reflecting 'specific dynamic action', whereas ammonia and urea-N excretion rates were elevated about 5-fold. Arterial blood exhibited a modest 'alkaline tide' for about 48 h, but there was negligible excretion of metabolic base to the external seawater. Pa_{CO_2} and Pa_{O_2} remained unchanged. Plasma total amino acid concentration and total lipid concentration were elevated about 1.5-fold for at least 48 h, whereas small increases in plasma total ammonia concentration, P_{NH_3} and urea-N concentration were quickly attenuated. Plasma glucose concentration remained unchanged. We conclude that despite the very large meal, slow processing with high efficiency minimizes internal physiological disturbances. This differs greatly from the picture provided by previous studies on aquacultured species using synthetic diets and/or force-feeding. Questions remain about the role of the gastro-intestinal microbiome in nitrogen and acid–base metabolism.

KEY WORDS: Voluntary feeding, Specific dynamic action, Alkaline tide, Gastrointestinal chemistry, Nitrogen metabolism

INTRODUCTION

Most physiological investigations on fish continue the long-held tradition of using fasting animals. However, in the last few decades, there has been growing interest in the physiological responses of fish to feeding (Grosell, 2011; Volkoff and Rønnestad, 2020; Hardy and Kaushik, 2022). Key findings relate to the re-organization of blood flow distribution to the gut after feeding (reviewed by Seth et al., 2011), elevations in metabolic rate ('specific dynamic action', SDA; reviewed by McCue, 2006; Secor, 2009; Chabot et al., 2016), nitrogenous waste excretion (reviewed by Wood, 2001; Bucking, 2017) and gastro-intestinal absorptive and secretory processes (reviewed by Bakke et al., 2011; Wood and Bucking, 2011). Additionally, the rise in pH in the systemic bloodstream ('alkaline tide') due to HCl secretion in the stomach and the accompanying changes in the trans-branchial fluxes of basic equivalents to the external water and secretion of HCO_3^- into the intestine have attracted attention. All of these processes are thought to involve exchange of basic equivalents for Cl^- (Grosell, 2011; Wood, 2019). However, most studies have been performed from an aquacultural perspective using domesticated species, often fed synthetic, nutrient-rich pellets, delivered artificially by intubation, gavage or other types of force-feeding. There are only a very few studies (e.g. Taylor and Grosell, 2006; Taylor et al., 2007; Wood et al., 2007a,b; Tirsgaard et al., 2015; Steell et al., 2019) where physiological responses have been followed in wild fish that have been allowed to feed voluntarily on natural diets. The reason for this is that wild fish are usually reluctant to feed in captivity, especially when confined in respirometry chambers or catheterized for repetitive blood sampling (Wood and Bucking, 2011).

These differences may be important because there is a 2- to 4-fold discrepancy in the caloric density, buffer capacity and water content of commercial pellet feeds versus natural diets (Wood and Bucking, 2011; Goodrich et al., 2022). Furthermore, there is some evidence that when fish feed voluntarily, the anticipation of feeding contributes to the post-prandial responses (e.g. Brett and Zala, 1975), and may result in smaller disturbances of internal homeostasis than when fish are force-fed a comparable ration (e.g. Cooper and Wilson, 2008).

In the present study, we have overcome these problems using the August Krogh principle (Krogh, 1929; Krebs, 1975), exploiting a happenchance observation to discover a species, the staghorn sculpin (*Leptocottus armatus*), that seems to feed whenever it is given the opportunity, regardless of most circumstances. The fish has a very large mouth, head and stomach, and apparently a very large appetite. We first noticed that freshly angled sculpins would attempt to eat smaller conspecifics when held on board in a covered container. We also found that the gut was empty in most of the sculpins that we caught, but a few contained a large meal of fish or shrimps. In preliminary experiments, we subsequently discovered that sculpins could consume a meal of dead fish amounting to 20% of their own body mass when housed overnight either outdoors in a large holding tank or indoors in a small respirometry chamber, as long as it was dark in the chamber. Furthermore, such eating could occur within a few hours of recovery from anaesthesia and surgery.

Therefore, we employed the staghorn sculpin to explore multiple aspects of the physiological responses to the voluntary consumption of a large meal of anchovies, a natural prey item. These include the

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Received 29 April 2023; Accepted 26 August 2023

Journal of Experimental Biology (2023) 226, jeb246034. doi:10.1242/jeb.246034

List of abbreviations

J_X	flux rate of substance X
$\dot{M}_{O_2}$	rate of oxygen consumption
NH_3	ammonia
NH_4^+	ammonium ion
NQ	nitrogen quotient
P_{CO_2}	partial pressure of carbon dioxide
Pa_{CO_2}	partial pressure of carbon dioxide in arterial blood
pHa	arterial blood pH
P_{NH_3}	partial pressure of ammonia
Pa_{NH_3}	partial pressure of ammonia in arterial blood
P_{O_2}	partial pressure of oxygen
Pa_{O_2}	partial pressure of oxygen in arterial blood
Rh	Rhesus
SDA	specific dynamic action

time course of stomach emptying, the profiles of pH, total ammonia, the partial pressure of NH_3 (P_{NH_3}), HCO_3^-, the partial pressure of CO_2 (P_{CO_2}) and Cl^- in the various sections of the gastrointestinal tract *in vivo* and the absorptive and secretory processes of these substances *in vitro* in gut sac preparations from fasted and fed animals. We also followed the temporal responses in the rate of oxygen consumption ($\dot{M}_{O_2}$), ammonia and urea-N excretion, and the net fluxes of basic equivalents with the external seawater after a large voluntary meal in intact sculpins. Finally, we used chronically cannulated sculpins to examine the time courses of acid–base status, P_{CO_2}, the partial pressure of O_2 (P_{O_2}), total ammonia, P_{NH_3}, urea-N and several nutrients (glucose, total amino acids, total lipids) in the arterial blood plasma *in vivo* following the large natural meal. Our overall working hypothesis was that despite the large meal size (a 20% body mass ration was offered), its voluntary nature and natural composition would result in much lower post-prandial disturbances in homeostasis than reported in previous studies on other species using force-feeding and/or concentrated synthetic diets.

MATERIALS AND METHODS

Experimental animals

Pacific staghorn sculpins (*Leptocottus armatus* Girard; 230±18 g, *N*=59) of both sexes were collected by angling at dusk near Brady's Beach, close to the Bamfield Marine Sciences Centre (BMSC, Bamfield, BC, Canada), in August–September 2019, and July 2022, under Fisheries and Oceans Canada collection permits XR 212-2019 and XR 119-2022. All procedures were approved by Animal Care Committees at both BMSC (AUP RS-19-15 in 2019 and RS-22-08 in 2022) and University of British Columbia (UBC; AUP A18-0271 in both years) and complied with the regulations of the Canada Council on Animal Care. At BMSC, sculpins were held with conspecifics of similar size in flow-through 400 l tanks covered with opaque netting that allowed a natural photoperiod. The bottom of the tank was covered with sand. Acclimation and experimental temperatures ranged from 10.5 to 13°C and salinity from 30 to 32 ppt. Animals were fed every 4 days with cut-up anchovies (*Engraulis mordax*; Alaska Bait Co., Anchorage, AL, USA), and were fasted for at least 6 days prior to experiments. We observed that in their holding tanks, sculpins remained buried in the sand during the day, and emerged to feed only during the hours of darkness, from dusk onwards.

In the laboratory (experimental series 2, 4, 5 and 6), the sculpins were held in individual covered chambers made from black Plexiglas (9×34×18 cm). The chambers were served with aeration and flowing seawater, and were partially submerged in a wet table that maintained the experimental temperature when flow was shut off for respirometry. For experimental feeding, a ration of cut-up anchovies (generally 4–8 pieces) equivalent to 20% of the fish's body mass was pre-weighed, and added to the chamber at 24:00 h, and then the lights in the lab were shut off. At 07:30 h the following morning, the lights were turned on, and at 08:00 h, uneaten food was removed and weighed, so as to calculate the consumed ration.

Series 1

This series examined conditions in the gastrointestinal tract of staghorn sculpins before (*N*=12, fasted fish) and approximately 14–24 h after feeding (*N*=9, fed fish). Sculpins were fed in groups in their outdoor holding tanks. An excess amount of food was added to the tanks at about 18:00 h and sampling was performed at 08:00–18:00 h the next day. The ration consumed was not measured. Fasted fish, in a separate tank, were not fed. For euthanasia and subsequent sampling, sculpins were netted individually into a bucket containing 2 g l^{-1} of MS-222 (Syndel, Nanaimo, BC, Canada) in seawater, adjusted to pH 7.8. The fish fork length and mass were recorded, then the peritoneal cavity was opened, and silk ligatures placed around the stomach, and anterior, mid and posterior intestines, established by dividing the intestinal length into three equal segments. The entire gastrointestinal tract from the oesophagus/stomach boundary to the rectum was then dissected out and, in some animals, the distance from the boundary to the pyloric sphincter (stomach length), and from the pyloric sphincter to the rectum (intestinal length), was measured. The chyme/intestinal fluid was collected from each section when present, though some samples were lost by spillage. The slurry was mixed, then a subsample was transferred to a 1.5 ml bullet tube and centrifuged at 5000 ***g*** for 5 min. The supernatant was decanted, measured immediately for pH and total CO_2 concentration, then frozen in liquid N_2, and stored at −70°C for later analysis of total ammonia and chloride concentrations.

P_{CO_2} and HCO_3^- concentrations were calculated from total CO_2 and pH measurements using rearrangements of the Henderson–Hasselbalch equation, and apparent dissociation constant pK′ and CO_2 solubility values for teleosts from Boutilier et al. (1984). P_{NH_3} and ammonium ion (NH_4^+) concentrations were calculated in a parallel fashion from total ammonia and pH measurements using the Henderson–Hasselbalch equation and dissociation constant pK and NH_3 solubility values for teleosts from Cameron and Heisler (1983).

It should be noted that these constants from Boutilier et al. (1984) and Cameron and Heisler (1983) are for teleost plasma, not for chyme/intestinal fluids where concentrations of ions, proteins and other substances may well differ. Two previous studies on three species (freshwater rainbow trout and goldfish – Wood and Eom, 2019; seawater English sole – Jung et al., 2020) have evaluated the concordance between calculated and directly measured values for P_{CO_2} and HCO_3^- concentrations. The general conclusions that can be drawn are that the degree of quantitative agreement is species dependent (better for trout than for the other two species), that the prediction of chyme HCO_3^- concentrations from direct measurements of total CO_2 and pH as in the present study is relatively good, but that the prediction of chyme P_{CO_2} values from these same direct measurements, again as in the present study, is less good, showing qualitative but only semi-quantitative agreement. We are aware of no similar comparisons for ammonia, but we anticipate that similar uncertainties may apply to calculated chyme P_{NH_3} values.

Series 2

This series tracked the rate at which ingested food left the stomach. The fish (*N*=12) were weighed, then allowed to settle for 24 h (i.e.

≥7 days fasting) in their individual chambers with a continuous flow of seawater and aeration. At 24:00 h, the fish were fed a 20% ration, and the amount consumed overnight was determined at 08:00 h by removing non-eaten food. Fish were then sequentially euthanized after a further 24 h (N=3), 48 h (N=3), 72 h (N=3), 84 h (N=1), 108 h (N=1) and 132 h (N=1). At each time, a concentrated anaesthetic stock of pH-adjusted MS-222 (Syndel) was poured into the fish's chamber so as to achieve a dissolved concentration about 2 g l^{-1} (pH 7.8). The peritoneal cavity was quickly opened, the stomach was ligated at both ends, the whole gastro-intestinal tract was removed, and the length of the tract as well as the fork length of the fish were measured as in series 1. The contents of the stomach were then weighed and compared with the ingested ration. Note that in contrast to synthetic pellets that are hygroscopic and quickly increase in weight after ingestion (e.g. Bucking and Wood, 2006), the hydration state of ingested fish flesh in the stomach stays relatively constant.

Series 3

This series employed isolated gut sac preparations incubated *in vitro* to examine absorption and secretion rates in the various sections of the gastrointestinal tract before (N=4 fasted fish) and approximately 14–24 h after feeding (N=5 fed fish). Fish were either fed overnight or fasted in outdoor tanks, then euthanized the next day exactly as in series 1. The four sections of the tract (stomach, and anterior, mid and posterior intestine) were dissected out on ice-chilled Petri dishes and thoroughly flushed with ice-cold Cortland saline (Wolf, 1963) supplemented with an additional 20 mmol l^{-1} NaCl to achieve appropriate tonicity for a seawater teleost. Each section was then made into a gut sac closely following the methodology described by Goodrich et al. (2020) and Jung et al. (2023), with the following exceptions. No organic buffers were used, the mucosal saline was supplemented with 1 mmol l^{-1} NH_4Cl and the serosal saline with 0.4 mmol l^{-1} NH_4Cl to approximate conditions measured in chyme and blood in sculpins *in vivo*, and the incubation time was 2 h. As in Jung et al. (2023), the mucosal and serosal salines had been pre-equilibrated with a 1% CO_2/99% O_2 gas mixture, and the small glass bottles used as flux chambers were partially submerged in a water bath to maintain the experimental temperature of 12°C. The serosal volume varied with the size of the gut sacs but was typically 50 ml for the stomach and 15 ml for the intestinal segments. Gut sacs were blotted and weighed in a standardized fashion at the start and end of the incubation. Samples were taken of the mucosal saline used to fill the sacs internally at the start of the incubation, and again after weighing at the end when the sac was drained. These samples were analysed for pH, Cl^-, total CO_2 and total ammonia as in series 1. The preparation was then thoroughly blotted so as to yield the empty mass, and the surface area (A) was measured by tracing onto graph paper (Grosell and Jensen, 1999). The initial (V_i) and final fluid volumes (V_f) were obtained by subtracting the empty mass from the initial mass, and the empty mass from the final mass, respectively. The flux rate of water (J_{H_2O}) was obtained from the mass change of the preparation (Eqn 1), and the flux rates (J_X) of other substances (Eqn 2) were calculated from their disappearance or appearance in the mucosal fluid over the experimental period (T), where X_i is initial concentration and X_f is final concentration:

$$J_{H_2O} = (V_i - V_f) \times A^{-1} \times T^{-1} \quad (1)$$

and

$$J_X = (V_i \times X_i) - (V_f \times X_f) \times A^{-1} \times T^{-1}. \quad (2)$$

Thus, absorptive fluxes have a positive value and secretory fluxes have a negative value.

Series 4

This series recorded the changes in O_2 consumption ($\dot{M}_{O_2}$) and the excretion of nitrogenous wastes (ammonia and urea-N – note: 1N in ammonia, but 2N in urea) after feeding. The sculpins (N=5) were weighed, then placed in their individual chambers and allowed to settle for 24 h with a continuous flow of seawater and aeration. The next day (i.e. ≥7 days fasting), several pre-feeding control measurements were made on each fish. For each measurement, aeration and seawater flow were stopped, the chamber was filled to the top, thereby providing a known volume (5.51 l), and a small submersible pump in the chamber was activated for 30 s to ensure thorough mixing. P_{O_2} was measured, and a water sample (10 ml) was taken for ammonia and urea-N measurements, then the chamber was sealed. At the end of the 1 h respirometry period, the procedure was repeated, then the chamber was flushed with fresh seawater without disturbance to the fish, and flow and aeration were re-established. At 24:00 h, each fish was fed a 20% ration. The next morning, the ration consumed overnight was measured by removing and weighing any non-eaten food, then respirometric measurements were made at 08:00–09:00 h, 09:30–10:30 h, 11:00–12:00 h, 12:30–13:30 h, 14:00–15:00 h, 15:30–16:30 h and 20:00–21:00 h (i.e. approximately 12 h after the end of the feeding period). Additional measurements were made at 08:00–09:00 h and 20:00−21:00 h over the next 3 days (i.e. 24 h, 36 h, 48 h, 60 h and 72 h post-feeding).

P_{O_2} was converted to O_2 concentration using the O_2 solubility coefficient at the appropriate temperature and salinity tabulated by Boutilier et al. (1984). $\dot{M}_{O_2}$ was calculated from the decline in O_2 concentration (ΔC), and the excretion rates of total ammonia and urea-N were calculated from the increases (ΔC) in their concentration. All ΔC values were expressed as positive values and factored by time (T), chamber volume (V, corrected for fish body mass, W) and body mass (W) itself to yield consumption or excretion rates:

$$\text{Consumption or excretion rate} = [\Delta C \times (V - W)] \times W^{-1} \times T^{-1}. \quad (3)$$

The nitrogen quotient (NQ) was calculated as the sum of ammonia and urea-N excretion rates divided by the simultaneously measured $\dot{M}_{O_2}$.

Series 5

This series examined the net flux of acidic and basic equivalents with the environment after feeding. As in series 4, the fish (N=6) were weighed, and allowed to settle for 24 h in their individual chambers with a continuous flow of seawater and aeration. The next day (i.e. ≥7 days fasting), the pre-feeding control flux measurement was made from 12:00 h to 18:00 h. At the start, seawater flow was stopped, while aeration continued. Water samples (3×10 ml) were taken at the start and end of the flux period; one was analysed for total ammonia, another for titratable alkalinity, while the third served as a back-up. At 18:00 h, seawater flow was restored, and at 24:00 h, the fish was fed a 20% ration. The ration consumed overnight was measured at 08:00 h by removing non-eaten food. Post-feeding fluxes were run using identical methodology but shorter time periods at 09:00–12:00 h and 18:00–21:00 h over the next 2 days, i.e. approximately 2.5 h, 11.5 h, 26.5 h and 35.5 h post-feeding. Titratable alkalinity fluxes were calculated using Eqn 2.

Journal of Experimental Biology (2023) 226, jeb246034. doi:10.1242/jeb.246034

The difference in titratable alkalinity (final minus initial titratable alkalinity concentrations) provided the net loss of titratable base from the fish where positive values represent titratable base loss (=titratable acid uptake), and negative values represent titratable base uptake (=titratable acid excretion). The difference between the titratable base excretion (positive or negative) and the simultaneously measured total ammonia excretion (always positive) yielded the net basic equivalent flux. Thus, the convention used here is that net base excretion to the water was positive, and net acid excretion to the water was negative.

Series 6

This series focused on the changes occurring in the arterial blood after feeding. Sculpins (N=6) were anaesthetized (MS-222, 0.67 g l^{-1}, adjusted to pH 7.8 with NaOH) on an operating table with continuous irrigation of the gills. A chronic indwelling catheter (PE50, with an external PE160 sleeve for security, Clay-Adams™, Becton and Dickinson Co., Franklin Lakes, NJ, USA) was implanted into the caudal artery. As the staghorn sculpin has a very similar morphology to the gulf toadfish, the methodology was identical to that described by Wood et al. (1997). The catheter was filled with the same NaCl-supplemented Cortland saline (Wolf, 1963) as in series 3, which was heparinized with 50 i.u. of lithium heparin (Sigma-Aldrich, St Louis, MO, USA). The wound was dusted with oxytetracycline (Sigma-Aldrich) and closed with silk suture. The gills were irrigated with anaesthetic-free seawater until ventilation resumed, and then the fish was weighed and placed in its individual chamber, which was continuously flushed with fresh seawater throughout the experiment.

The experiment started after 36–48 h recovery from surgery (i.e. ≥7 days fasting). A control blood sample was taken via the catheter in late afternoon, and then the fish was fed a 20% ration overnight at 24:00 h. The ration consumed overnight was measured at 08:00 h, non-eaten food was removed, and then blood samples were sequentially taken at 10:00 h and 20:00 h, and thereafter at 24, 36, 48, 60 and 72 h post-feeding. At each time, two 125 µl samples were drawn into ice-cold gas-tight glass syringes (Hamilton, Reno, NV, USA). One was immediately centrifuged at 5000 ***g*** for 1 min; the plasma was decanted, frozen in liquid N_2 and stored at −70°C for later analysis of the concentrations of total ammonia, urea-N, glucose, total amino acids and total lipids. The other sample was analysed immediately for arterial pH and partial pressure of O_2 (Pa_{O_2}), then centrifuged (5000 ***g***, 1 min) for measurement of arterial plasma total CO_2 concentration. The red cells from both samples were resuspended in non-heparinized saline and returned to the fish by the catheter. P_{CO_2}, HCO_3^- and P_{NH_3} in arterial plasma were calculated as in series 1 and 3, using appropriate constants for teleost plasma from Boutilier et al. (1984) and Cameron and Heisler (1983).

Analytical techniques

In series 1 and 3, total CO_2 in gastrointestinal fluid, chyme or mucosal salines was measured with a Corning 965 analyser (Corning Instruments, Corning, NY, USA), calibrated with $NaHCO_3$ standards, and Cl^- concentration was measured by coulometric titration using a CMT10 chloridometer (Radiometer, Copenhagen, Denmark) calibrated with a Radiometer standard. A thermo-jacketed (12°C) Orion ROSS glass combination micro-electrode (Fisher Scientific, Toronto, ON, Canada) coupled to an Accumet™ meter (Fisher Scientific) was used for pH measurements in chyme/gastrointestinal fluids and incubation salines. In series 6, the pH in arterial blood samples (pHa) was measured using a 238140 Biotrode™ (Hamilton), which is a glass combination micro-electrode, coupled to the same Accumet™ meter. In both cases, the micro-pH electrodes were thermostatically set to the experimental temperature and calibrated with precision buffers (Fisher Scientific and Radiometer).

In series 4, P_{O_2} in the seawater was measured using a DO 6+ galvanic oxygen electrode and meter (Oakton Instruments, Vernon Hills, IL, USA). In series 6, after the pHa measurement, Pa_{O_2} in the same blood sample was measured using an O_2 micro-optode (PreSens Precision Sensing GmbH, Regensburg, Germany). The sample was then centrifuged (5000 ***g***, 1 min) and the plasma was analysed for total CO_2 using the same Corning 965 analyser as in series 1 and 2.

In series 4 and 5, assays of total ammonia and urea-N concentrations in seawater were performed by the colorimetric methods of Verdouw et al. (1978) and Rahmatullah and Boyde (1980), respectively, with standards and blanks made up in seawater. In series 1 and 3, where ammonia was measured in chyme and gut sac incubation salines, mucus interfered with the colorimetric ammonia assay, so a commercial enzymatic assay kit designed specifically for plasma (Raichem ammonia kit based on glutamate dehydrogenase, Cliniqa Corporation, San Marcos, CA, USA) was employed. In series 6, we found that there was an unknown substance in the plasma of sculpins that interfered with both the enzymatic assay for ammonia and the colorimetric assay for urea-N. This problem was solved by adding 30 µl of 8% ice-cold $HClO_3$ to 30 µl of freshly thawed plasma (first thaw) in a 500 µl bullet centrifuge tube, mixing thoroughly, and allowing the acidified sample to sit on ice for 30 min. The tubes were then centrifuged at 10,000 ***g*** for 15 min at 4°C. For urea-N, a 20 µl aliquot of the acidified supernatant was diluted to 300 µl with 4% $HClO_3$, in a glass test-tube, then assayed at 525 nm after boiling for 10 min, as described by Rahmatullah and Boyde (1980). For ammonia, a 30 µl aliquot of the acidified supernatant was removed to a second bullet tube, then neutralized with 45 µl of saturated Tris buffer (pH 7.5). After thorough mixing, a 50 µl aliquot of the neutralized extract was analysed for total ammonia at 340 nm using the Raichem kit. For both assays, standards were put through exactly the same procedures, and the methods were cross-validated between seawater and plasma assays using common standards.

In series 5, titratable alkalinity concentrations were determined by titrating N_2-bubbled seawater samples to an endpoint of pH 4.00 with standardized 0.02 mol l^{-1} HCl (BDH™, VWR International Ltd, Poole, UK), as described by McDonald and Wood (1981). Samples were nominally 10 ml but exact volumes were determined gravimetrically. Micro-burettes (Gilmont Instruments, Great Neck, NY, USA) and Accumet AE 150 pH meters and electrodes (Fisher Scientific) were employed.

In series 6, for glucose, total amino acid and total lipid assays, addition–recovery tests revealed no interfering substances in sculpin plasma. Glucose concentrations in plasma (2×5 µl aliquots) were assayed directly at 340 nm using an Infinity™ glucose hexokinase liquid stable reagent kit (Thermo Fisher, Middletown, VA, USA). Total amino acid concentrations in plasma were determined using a modification of the ninhydrin assay of Moore and Stein (1954) with alanine as a standard. Plasma samples (15 µl) were deproteinized with 15 µl of 80% methanol, gently mixed, then centrifuged for 1 min at 5000 ***g***. A 20 µl aliquot of the supernatant was removed to a glass test-tube and 300 µl of 0.2% ninhydrin solution (Sigma-Aldrich) was added. After thorough vortexing, the tubes were boiled for 20 min. After cooling, 1.6 ml of 50% *n*-propanol was added, followed by vortexing again. Colour was allowed to develop for 10 min and then the samples and identically treated standards were read at 570 nm. Total lipid concentrations in plasma were assayed

using a modification of the method of Van Handel (1985), with canola oil dissolved in 100% chloroform as a standard. Vanillin reagent was made by dissolving 300 mg of vanillin in 50 ml of hot distilled water, followed by the addition of 200 ml of 85% phosphoric acid, and then stored in the dark. Plasma samples (15 µl) and standards were pipetted into glass test-tubes, which were then incubated at 90–100°C in a heater block for 30 min so as to evaporate to dryness, at which point 300 µl of concentrated H_2SO_4 (95–98%) was added. After a further 10 min of incubation at 90–100°C, the samples were cooled, then 3 ml of vanillin reagent was added, followed by thorough vortexing. Colour was allowed to develop for 10 min and then the samples and standards were read at 570 nm. A SpectraMax 340PC plate reader (Molecular Devices, San Jose, CA, USA) was employed for all colorimetric and enzymatic assays.

Statistics

Data are expressed as means±1 s.e.m. (N), where N is the number of sculpins or preparations from different fish. In series 1 and 3, data were analysed by two-way ANOVA with factors of treatment (fed and fasted), and gut section (stomach, and anterior, mid and posterior intestine), followed by Tukey's *post hoc* test to identify significant differences. In series 4, 5 and 6, time series data were analysed by repeated measures one-way ANOVA, followed by Dunnett's *post hoc* test to identify significant differences from pre-feeding control measurements. Prior to testing, all data were checked for normality of distribution and homogeneity of variance. If data failed, they were then subjected to standard transformations (log, square route, inverse). If data still failed, the non-parametric Kruskall–Wallis ANOVA on ranks was applied, followed by Dunn's *post hoc* test. Student's *t*-test, paired, unpaired or one sample as appropriate, was used for simple pair-wise comparisons. Significance was accepted at $P \leq 0.05$.

RESULTS

Feeding and gastric clearance

In series 1 and 2, the ratios of intestinal length and stomach length relative to body length were 1.057±0.048 (N=21) and 0.186±0.011 (N=21), respectively, and did not differ between fed and fasted fish. The average ration of anchovies consumed during the 8 h overnight feeding period was 15.76±0.92% (N=24) of the sculpin's body mass, and did not differ among experimental series 2, 4, 5 and 6. Gastric clearance of food, measured in series 2, was relatively slow, with about 70% left in the stomach at 24 h and about 63% at 48 h after the end of the feeding period (Fig. 1). Clearance surpassed 50% by 72 h, and was essentially complete by 84 h. Throughout 12–108 h, the intestine was full of a very loose chyme.

Gut chemistry in fed versus fasted sculpins

In series 2, sculpins were euthanized at 14–24 h post-feeding, a time when at least 70% of the meal was still in the stomach (see Fig. 1). Two-way ANOVA identified significant effects of feeding and segment (both P<0.0001), and their interaction (P<0.007), on pH in the luminal fluids of the gut (Fig. 2A). The interaction effect disappeared when the stomach was removed from the analysis. Fluid pH was consistently lower in all sections in fed versus fasted sculpins, and progressively increased from stomach to posterior intestine in both treatments. In fasted fish, mean pH was about 6.3 in the stomach, and increased to 8.1 in the anterior, 8.6 in the mid and 9.0 in the posterior intestinal segments. After feeding, pH was significantly depressed by about 3 units in the stomach relative to that in fasted fish, and by about 1 unit in each intestinal segment.

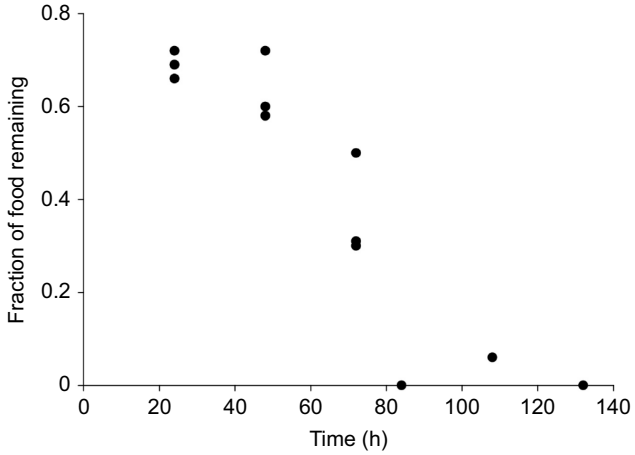

Fig. 1. The time course of food clearance from the stomach in staghorn sculpins of series 2. Each point represents the fraction of the voluntarily ingested satiation meal remaining in the stomach of an individual fish (N=12 in total) euthanized at the time shown, with 0 h representing the end of the 8 h overnight feeding period. Individual data points are shown.

There were significant overall effects of feeding (P<0.002) and segment (P<0.003) but no interaction effect on HCO_3^- concentration in luminal fluids (Fig. 2B). In fasted sculpins, mean HCO_3^- concentration progressively increased down the tract, from 16 mmol l^{-1} in the stomach to 70 mmol l^{-1} in the posterior intestine. In fed animals, the trend was similar, but the mean concentrations were reduced to 0.1 to 44 mmol l^{-1}, respectively. The differences were significant in the mid and posterior intestine. In contrast, opposite trends were seen in P_{CO_2}, where there were significant overall effects of segment (P=0.014), feeding (P<0.0001) and their interaction (P=0.038) (Fig. 2C). Mean P_{CO_2} was consistently higher in all sections in fed fish, falling from 103 Torr in the stomach to 11 Torr in the posterior intestine (where 1 Torr≈133 Pa), whereas the comparable gradient in fasted sculpins was 29 Torr (stomach) down to 2 Torr (posterior intestine). The differences between fed and fasted were significant in all intestinal sections but not in the stomach.

Cl^- concentration in gastrointestinal fluids was significantly affected by segment (P<0.0001), feeding (P<0.05) and their interaction (P<0.0001), with the both of last two effects being a function of the stomach (Fig. 2D). After feeding, gastric fluid Cl^- concentration was significantly elevated approximately 2-fold above the mean level (141 mmol l^{-1}) in fasted fish, but there were no feeding-related differences in the various intestinal sections. Mean Cl^- concentration fell progressively down the tract, to about 55 mmol l^{-1} in the posterior intestine.

Feeding also greatly influenced mean total ammonia levels (P<0.0001), with much higher concentrations in the chyme of fed animals (averaging about 1460 µmol l^{-1}) than in the fluids of fasted fish (310 µmol l^{-1}), with no significant effects of segment or interaction (Fig. 3A). The differences between fed and fasted fish were significant in each intestinal section but not in the stomach.

P_{NH_3} values in the digestive tract were highly variable (Fig. 3B). In fasted sculpins, moving down the tract, mean P_{NH_3} rose from 26 µTorr in the stomach to 805 µTorr in the posterior intestine. In fed sculpins, despite the much higher chyme total ammonia concentrations (Fig. 3A), mean P_{NH_3} levels tended to be lower, rising from 0.1 µTorr in the stomach to 356 µTorr in the posterior intestine. This reflected the much lower pH values in all sections of fed fish (Fig. 2A). After log transformation of the data, two-way ANOVA confirmed significant overall effects of segment (P<0.003) and feeding

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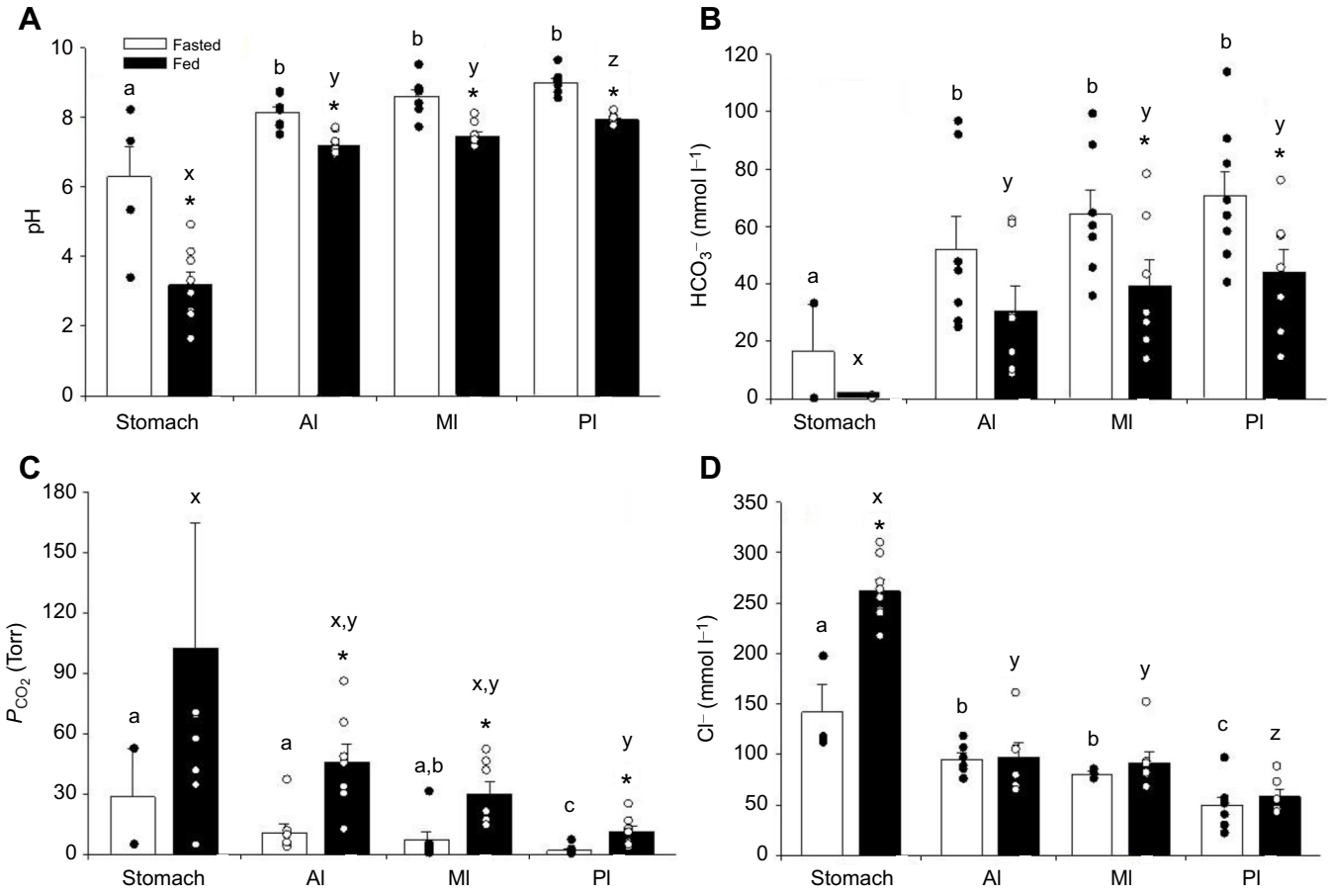

Fig. 2. Chemical conditions in the gastrointestinal fluids of staghorn sculpins of series 1. Data are for fish euthanized either 14–24 h after voluntary ingestion of a satiation meal (fed) or after not feeding for at least 6 days (fasted). Means±s.e.m. and individual data points are shown. Within a treatment, means not sharing the same letter are significantly different (P<0.05). Asterisks indicate significant differences (P<0.05) between treatments within the same segment. (A) pH. Two-way ANOVA: feeding P<0.0001, segment P<0.0001, interaction P<0.007. For fasted fish: stomach N=5, anterior intestine (AI) N=7, mid intestine (MI) N=8, posterior intestine (PI) N=8. For fed fish: stomach N=8, AI N=8, MI N=9, PI N=9. (B) HCO_3^- concentration. Two-way ANOVA: feeding P<0.002, segment P<0.003, interaction P=n.s. For fasted fish: stomach N=2, AI N=7, MI N=7, PI N=8. For fed fish: stomach N=7, AI N=7, MI N=7, PI N=7. (C) P_{CO_2}. Two-way ANOVA: feeding P<0.0001, segment P<0.014, interaction P=0.038. For fasted fish: stomach N=2, AI N=7, MI N=7, PI N=8. For fed fish: stomach N=7 (note that one data point at 470 Torr is off scale and not shown), AI N=7, MI N=7, PI N=7. (D) Cl^- concentration. Two-way ANOVA: feeding P<0.05, segment P<0.0001, interaction P<0.0001. For fasted fish: stomach N=3, AI N=6, MI N=3, PI N=8. For fed fish: stomach N=8, AI N=6, MI N=7, PI N=6.

(P=0.016) with no significant interaction effect. The feeding-related differences in P_{NH_3} were significant in the stomach and mid-intestine.

Transport rates measured *in vitro* in gut sacs from fed versus fasted sculpins

In series 3, all three intestinal sections, as well as the stomach in fasted fish, secreted HCO_3^- into the luminal fluid in preparations from both treatments, but rates were generally higher in fasted animals (averaging about −1.90 µmol cm^{-2} h^{-1}) than in fed ones (averaging about −0.20 µmol cm^{-2} h^{-1}) (Fig. 4A). The overall effect of feeding was significant (P<0.004) whereas the effects of segment and interaction were not. The feeding-related differences in HCO_3^- secretion rates were significant in the stomach, and anterior and posterior intestine.

Ammonia was absorbed on a net basis averaging about 0.055 µmol cm^{-2} h^{-1} in all three intestinal sections with no differences between fed and fasted sculpins, whereas ammonia flux in the stomach was negligible (Fig. 4B). There was a significant effect of segment (P=0.0017) but not of feeding or their interaction for net ammonia flux.

Net Cl^- flux was highly variable with no significant influence of feeding, section or their interaction (Table S1). Cl^- absorption rates averaged 1.99 µmol cm^{-2} h^{-1} in the intestinal sections. Fluid was absorbed on a net basis at a rate averaging 7.7 µl cm^{-1} h^{-1} by all intestinal sections from both fed and fasted sculpins, but was secreted by the stomach at 1.0 µl cm^{-1} h^{-1} in preparations from fed fish (Table S1). There was a significant overall effect of segment (P<0.0001) but not of feeding or their interaction.

Nitrogenous waste excretion and oxygen consumption rates after feeding

In series 4, the mean routine rates of ammonia and urea-N excretion in fasted sculpins were about 270 µmol kg^{-1} h^{-1} and 52 µmol kg^{-1} h^{-1}, respectively, while mean routine $\dot{M}_{O_2}$ was approximately 2050 µmol kg^{-1} h^{-1}. Thus, ammonia accounted for 84% of measured N-excretion. Throughout the first 12 h after feeding, mean ammonia excretion increased about 5.5-fold (Fig. 5A) and mean urea-N excretion by about 4-fold (Fig. 5B), with elevations remaining significant to 60 h and 36 h, respectively. The simultaneous increase in mean $\dot{M}_{O_2}$ was much lower, only about 2-fold, but the elevation

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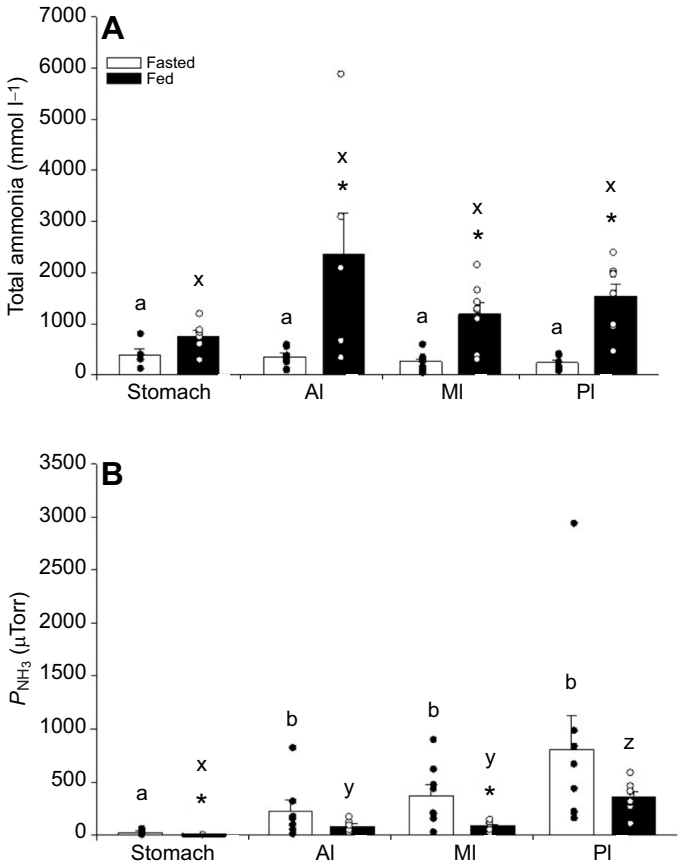

Fig. 3. Total ammonia concentration and partial pressure of ammonia (P_{NH_3}) in the gastrointestinal fluids of staghorn sculpins of series 1. Data are for fish euthanized either 14–24 h after voluntary ingestion of a satiation meal (fed) or after not feeding for at least 6 days (fasted). Means ±s.e.m. and individual data points are shown. Within a treatment, means not sharing the same letter are significantly different (P<0.05). Asterisks indicate significant differences (P<0.05) between treatments within the same segment. (A) Total ammonia. Two-way ANOVA: feeding P<0.0001, segment P=n.s., interaction P=n.s. (B) P_{NH_3}. Two-way ANOVA: feeding P=0.016, segment P<0.003, interaction P=n.s. In fasted fish: stomach N=5, AI N=7, MI N=8, PI N=8. In fed fish: stomach N=6, AI N=6, MI N=8, PI N=8.

remained significant and almost unchanged to 72 h (Fig. 6A). As a result, the mean NQ increased from about 0.16 in fasted animals to over 0.40 during the first 12 h post-feeding, declining slowly thereafter but remaining significantly elevated to 48 h (Fig. 6B).

Acid–base flux to the external seawater after feeding

In series 5, fasting sculpins exhibited a slight net excretion of acidic equivalents (averaging −70 µequiv kg^{-1} h^{-1}) to the external seawater Fig. 7A), though this was not significantly different from zero (one-sample t-test). At the first time point post-feeding (2.5 h), there was a changeover to net basic equivalent excretion (+202 µequiv kg^{-1} h^{-1}), but by 11.5 h this was declining and by 26.5 and 35.5 h, the low rate of net acid excretion was re-established. Overall, these changes were not significant (P=0.061) by one-way repeated measures ANOVA, though the initial shift from the negative control to the positive value at 2.5 h was highly significant (P=0.006) by a two-tailed paired t-test. These net acid–base fluxes were calculated as the difference between simultaneous measurements of titratable base excretion (Fig. 7B) and ammonia excretion (Fig. 7C), both of which increased 4- to 6-fold in the first 12 h with slight declines thereafter. The latter confirmed the responses seen in series 4 (Fig. 5A).

Responses in blood gases, acid–base, nitrogen and nutrient parameters after feeding

In series 6, arterial blood samples drawn from chronic catheters in fasted sculpins revealed a modest rise in mean pHa by about 0.08 units after feeding (Fig. 8A). This became significant at 12–24 h post-feeding, with fasting values gradually re-established thereafter. The rise in pHa was a classic metabolic alkalosis, reflecting a rise in mean plasma HCO_3^- concentration by about 1.8 mmol l^{-1} that remained significant from 2 to 48 h (Fig. 8B). There was no change in mean Pa_{CO_2}, which remained at the fasting level of 1.9 Torr throughout (Fig. 8C). Mean Pa_{O_2} was variable (60–85 Torr) over time but did not change significantly after feeding (Fig. 8D).

Arterial plasma total ammonia concentration rose by about 40% from the mean fasting level of 230 µmol l^{-1} by 2 h post-feeding, and then declined close to original levels thereafter (Fig. 9A); the changes were not significant (P=0.078). However, there was a similar but more pronounced pattern in mean Pa_{NH_3}, which was 77 µTorr in fasting sculpins, and then rose significantly by 56% at 2 h, declining thereafter (Fig. 9B). Mean plasma urea-N concentration (1645 µmol l^{-1} in fasting fish) was much higher than total ammonia, and exhibited significant (30%) increases at both 2 and 12 h post-feeding before declining thereafter (Fig. 9C).

Mean plasma glucose concentration was about 3.6 mmol l^{-1} in fasting fish, and became much more variable after feeding, but there were no significant changes (Fig. 10A). However, mean plasma total amino acid concentration, which was approximately 1.6 mmol l^{-1} in fasting sculpins, increased consistently by about 50% throughout the post-feeding period, with significant elevations at 2, 36 and 48 h (Fig. 10B). Mean plasma total lipid concentration was about 4.5 mmol l^{-1} in fasting animals and became highly variable after feeding. However, it rose in all fish at all times throughout the post-feeding period, with the overall increase being an approximate doubling (Fig. 10C). Non-parametric analysis demonstrated that the increases were significant at all times.

DISCUSSION

Overview

In our experiments, staghorn sculpins voluntarily ate a mean ration equivalent to 15.76% of their body mass within an 8 h period. This

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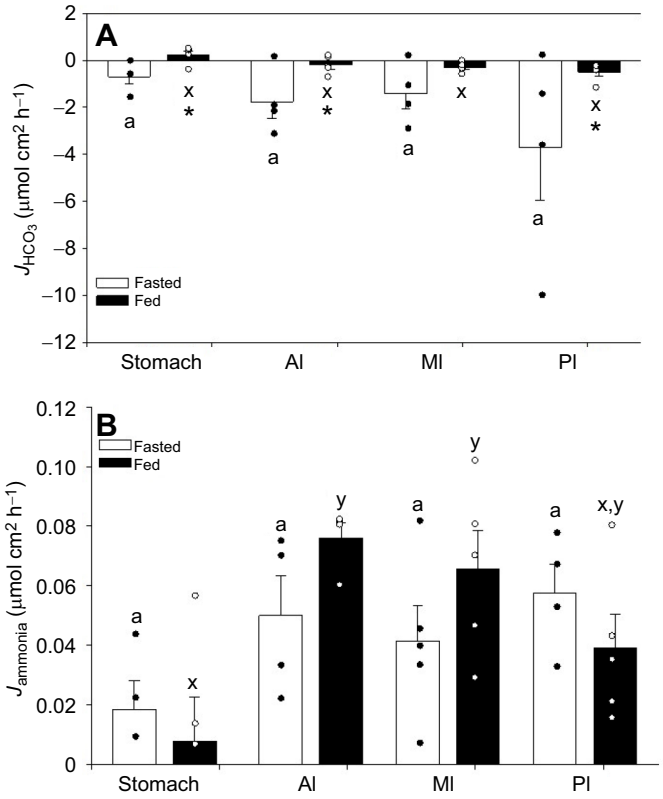

Fig. 4. Transport rates of HCO_3^- and total ammonia measured *in vitro* in gut sacs from staghorn sculpins in series 3. Data are for fish that had voluntarily ingested a satiation meal 14–24 h previously (fed) or were not fed for at least 6 days (fasted). Means±s.e.m. and individual data points are shown (N=4–5). Within a treatment, means not sharing the same letter are significantly different (P<0.05). Asterisks indicate significant differences (P<0.05) between treatments within the same segment. (A) HCO_3^- flux (J_{HCO_3}). Two-way ANOVA: feeding P<0.004, segment P=n.s., interaction P=n.s. (B) Total ammonia flux ($J_{ammonia}$). Two-way ANOVA: feeding P=n.s., segment P=0.0017, interaction P=n.s.

would correspond to 11 kg of food in a 70 kg human, an impressive feat. Of course, we do not know exactly when the ingestion occurred in the overnight period because the boxes remained covered, but based on the thrashing noises in the first few minutes after the anchovies were added, much of the eating probably occurred in the first hour. Thus, by 8 h in the experiments of series 2, 4, 5 and 6, a significant portion of the meal had probably been present in the stomach for some hours. As outlined in the Introduction and Materials and Methods, we believe this reflects the natural lifestyle of this animal, an opportunistic predator that emerges from the sand at night to eat infrequent, sometimes very large, meals. In support of our overall hypothesis, the physiological disturbances accompanying this huge ingestion of food were surprisingly modest relative to other studies where involuntary feeding and/or concentrated synthetic diets have been employed. We attribute this to the voluntary nature of the feeding, the natural diet, and the slow digestive and absorptive processes in the staghorn sculpin.

Oxygen consumption after feeding

Except during feeding, the sculpins were very quiet in their darkened chambers, so it is likely that the routine fasting $\dot{M}_{O_2}$ was close to standard metabolic rate. The post-feeding elevation in $\dot{M}_{O_2}$ (Fig. 6A) represents the well-known SDA response that has been attributed to numerous metabolic costs of processing the meal (reviewed by McCue, 2006; Secor, 2009; Chabot et al., 2016). The relative extent of the postprandial $\dot{M}_{O_2}$ elevation (about 2-fold) was very typical of most fishes, but the duration (>72 h) was much greater, reflecting the slow processing of the very large meal. Using the mean ingested meal size (157.6 g kg^{-1}), the energy content (9.92 kJ g^{-1}; Tirsgaard et al., 2015) for herring, which are very similar to anchovy in composition (Yeannes and Almandos, 2003), the mean SDA increment above pre-feeding control $\dot{M}_{O_2}$ integrated over 72 h (117,000 µmol O_2; Fig. 6A) and the standard oxycalorific coefficient (14.06 kJ g^{-1} O_2; Gnaiger, 1983), we calculate that the SDA coefficient is 3.4%. This represents the energy devoted to SDA as a percentage of the energy contained in the meal. This value

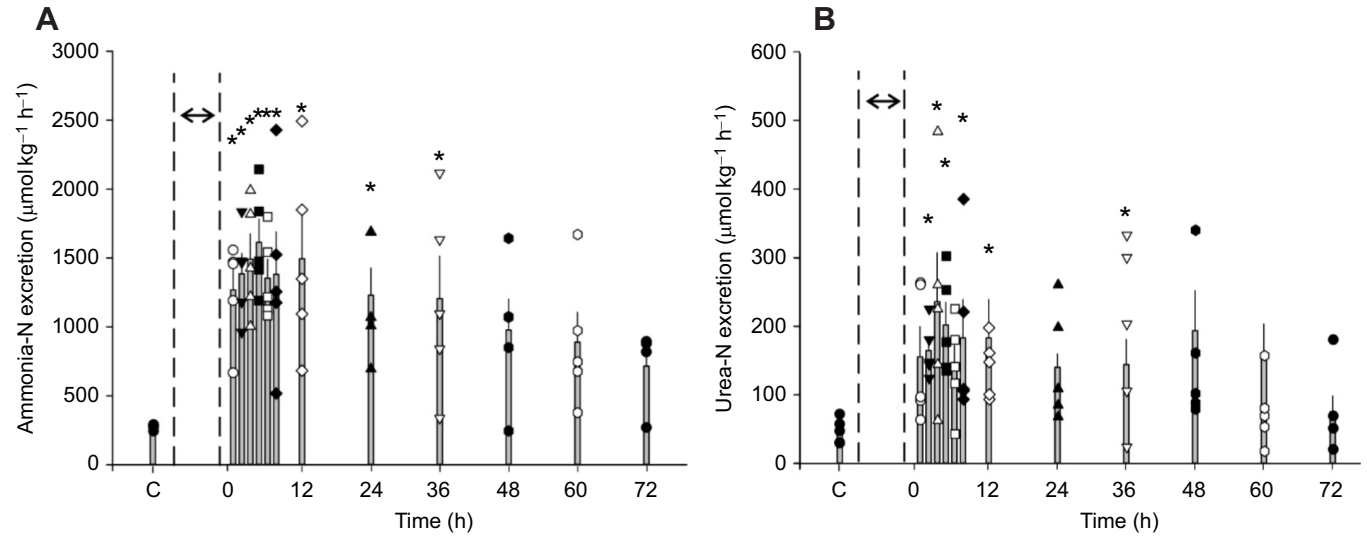

Fig. 5. The effect of voluntary ingestion of a satiation meal on total ammonia excretion rate and urea-N excretion rate in staghorn sculpins in series 4. Data are for fish that had been fasted for at least 7 days previously. C represents the control value measured in fasting fish prior to feeding, vertical dashed lines indicate the 8 h feeding period, and 0 h represents the end of the 8 h overnight feeding period. Means±s.e.m. and individual data points are shown (N=5). Asterisks indicate significant differences (P<0.05) from the fasted control value. (A) Ammonia-N excretion rate. (B) Urea-N excretion rate.

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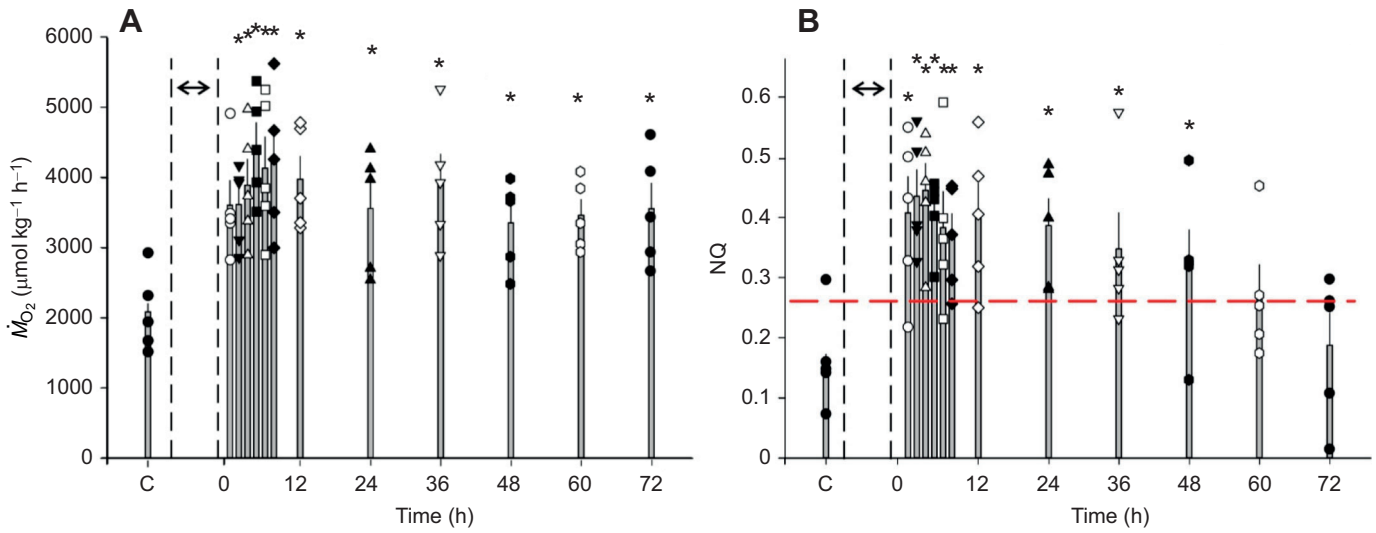

Fig. 6. The effect of voluntary ingestion of a satiation meal on oxygen consumption rate and nitrogen quotient in staghorn sculpins in series 4. Data are for fish that had been fasted for at least 7 days previously. C represents the control value measured in fasting fish prior to feeding, vertical dashed lines indicate the 8 h feeding period, and 0 h represents the end of the 8 h overnight feeding period. Means±s.e.m. and individual data points are shown (N=5). Asterisks indicate significant differences (P<0.05) from the fasted control value. (A) Oxygen consumption rate ($\dot{M}_{O_2}$). (B) Nitrogen quotient (NQ). The dashed line indicates NQ=0.27, the theoretical value at which 100% of aerobic metabolism is fuelled by the oxidation of protein (see Discussion).

(3.4%) is low relative to most reports in fish which are typically in the 7–40% range (McCue, 2006; Secor, 2009). As SDA obviously continued beyond 72 h (Fig. 6A), 3.4% is an underestimate. However, even if SDA continued at the same rate over the subsequent 3 days, then the SDA coefficient would only approach the lower end of the range reported in other species. Again, this reflects the slow processing of the large meal, which appears to be very efficient.

Nitrogen metabolism after feeding

The present data reveal several unusual features of N metabolism in staghorn sculpins. Firstly, in fish fasted for at least 7 days, the mean NQ was surprisingly high (0.164). This may be compared with a NQ of 0.27, indicated by the dashed line in Fig. 6B, which is the theoretical maximum value where all aerobic metabolism is fuelled by protein (van den Thillart and Kesbeke, 1978; van Waarde, 1983; Lauff and Wood, 1996; Wood, 2001). Therefore, 61% of routine metabolism is powered by protein oxidation in sculpins that have lived all their lives on a natural diet, a surprisingly high percentage. Most previous measurements have been made on fish raised on synthetic diets, and have uniformly indicated a much lower percentage (15–40%) in fasting or post-absorptive individuals (reviewed by van Waarde, 1983; Wood, 2001). The one exception is the tambaqui, an Amazonian fructivore raised on a commercial high-protein (and therefore likely inappropriate) diet, where 60–80% of routine metabolism was fuelled by protein oxidation in fasted fish (Wood et al., 2017).

Secondly, like other teleosts (Wood, 2001), sculpins exhibited a rise in N excretion after feeding (Fig. 5). This phenomenon was first shown in sockeye salmon by Brett and Zala (1975). Based on the classic work of Brown and Cameron (1991a,b), this reflects a surge in protein oxidation from part of the meal which is used to power the metabolically costly process of protein synthesis for growth from the rest of the meal, the latter accounting for much of the SDA. The unusual feature in sculpins is the extent of the rise in mean N excretion (about 5-fold) relative to that in mean $\dot{M}_{O_2}$ (about 2-fold; Fig. 6A). As a result, mean NQ was significantly greater than 0.27 (one sample t-tests) from 1 h to 48 h after the meal. In other words, even if there was no contribution of lipid or carbohydrate at this time of greatly elevated O_2 consumption (Fig. 6A), the oxidation of protein still could not explain all of the measured ammonia and urea-N excretion. We are aware of no previous investigations where NQ surpassed 0.27 in teleosts after feeding. Indeed, if one calculates the NQ based on the increment of N excretion over 72 h (70,000 μmol N) divided by the increment in $\dot{M}_{O_2}$ (i.e. SDA, 117,000 μmol O_2), the incremental NQ is 0.60! These observations suggest that other phenomena must be generating some of this exogenous N excretion in staghorn sculpin. Anaerobic metabolism in the fish is possible (Van Waarde, 1983), but unlikely in view of the fact that water O_2 levels, as well as blood Pa_{O_2}, remained high throughout (Fig. 8D). One possibility that should be explored in future is anaerobic production by the microbiome in the gut. Here, conditions are virtually anoxic (Jung et al., 2020, 2022), yet there are many reports of considerable generation of ammonia in the digestive tract of fed animals (Bucking and Wood, 2012; Bucking et al., 2013; Rubino et al., 2014; Pelster et al., 2014), as also shown in the present study (Fig. 3A), as well as several reports of urea-N generation (Bucking et al., 2013; Jung et al., 2021). Indeed, Rubino et al. (2014) estimated that gastrointestinal production could account for 18% of whole-body ammonia excretion in rainbow trout under fasting conditions, and 47% after feeding, though that study did not distinguish between aerobic and anaerobic production.

Thirdly, despite this ‘wastage’ of some nitrogen in the first 24 h, the overall process in sculpins appears to be highly efficient in terms of N retention, similar to the pattern in energy retention. In the anchovies used as food in the present study, the protein content was about 20% of the wet body mass (Yeannes and Almandos, 2003), and the N content of fish protein is about 0.16 g N g^{-1} protein (Wood, 2001). Therefore, the 157.6 g kg^{-1} meal of anchovies would represent the ingestion of about 360,000 μmol kg^{-1} of N. Based on integration of the total N excretion as ammonia plus urea-N and subtraction of the fasting rate (‘endogenous N’ excretion; Wood, 2001) (Fig. 5), about 30,000 μmol kg^{-1} of ‘exogenous N’ was excreted in the first 24 h, or only about 8.3% of

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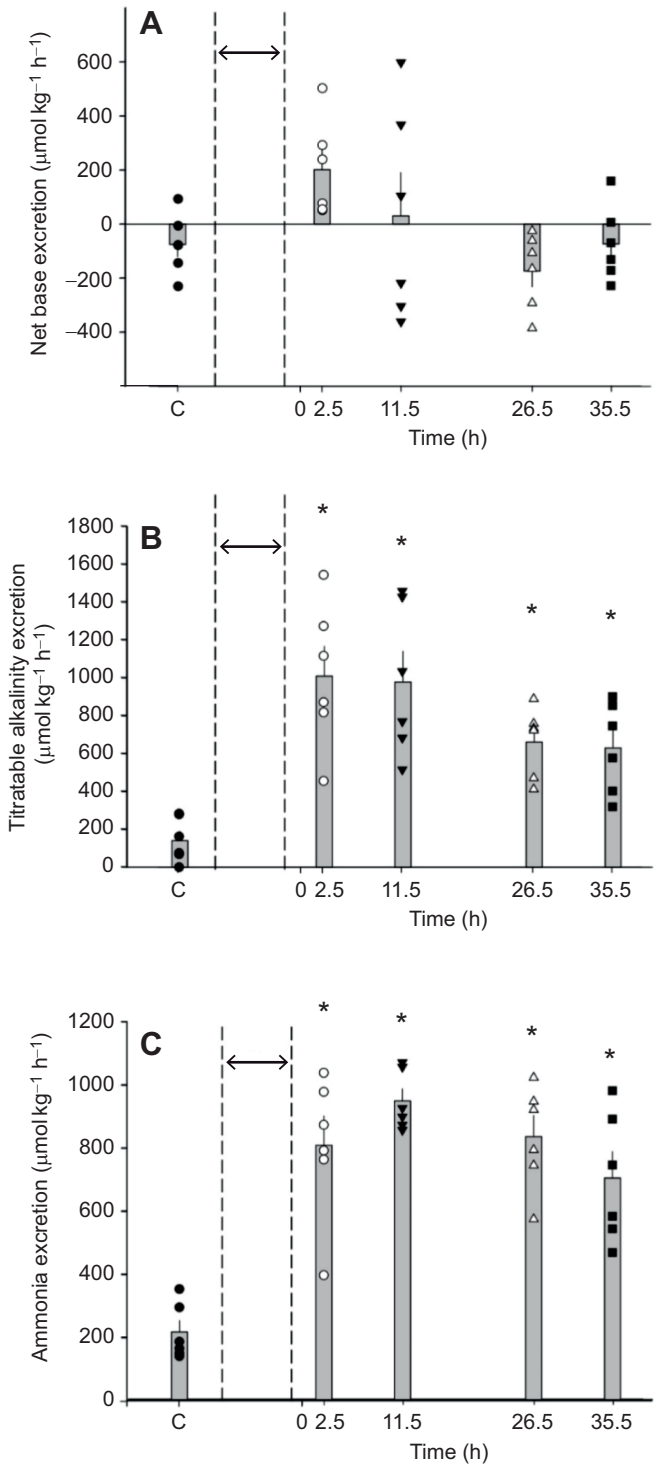

Fig. 7. The effect of voluntary ingestion of a satiation meal on the net excretion rate of basic equivalents, titratable base and total ammonia in staghorn sculpins in series 5. Data are for fish that had been fasted for at least 7 days previously. C represents the control value measured in fasting fish prior to feeding, vertical dashed lines indicate the 8 h feeding period, and 0 h represents the end of the 8 h overnight feeding period. Data are plotted at the midpoints of the 3 h flux periods. Means±s.e.m. and individual data points are shown (*N*=6). Asterisks indicate significant differences (P<0.05) from the fasted control value. (A) Net base excretion. Positive values represent excretion of basic equivalents, whereas negative values indicate excretion of acidic equivalents. (B) Titratable alkalinity excretion. (C) Ammonia excretion.

the N content of the meal, which increased to only 70,000 μmol kg^{-1} or 19.4% by 72 h. By way of contrast, on high rations of synthetic commercial diets, rainbow trout at 15°C excreted 16% in 24 h (Alsop and Wood, 1997), turbot at 12°C excreted 17% in 24 h (Dosdat et al., 1995), zebrafish at 21°C excreted 20% in only10 h (Ferreira et al., 2019), sockeye salmon at 15°C excreted >70% in 24 h (Brett and Zala, 1975) and tambaqui at 28°C excreted 82% in 24 h (Wood et al., 2017). This high N retention efficiency in staghorn sculpins may reflect the natural diet, the effects of lower temperature and/or a more efficient metabolism.

Acid–base regulation after feeding

The staghorn sculpin clearly belongs to that category of fishes (reviewed by Wood, 2019) that maintains a circumneutral mean pH (6.3) in the stomach during fasting, transitioning to a very acidic pH (3.2) after feeding (Fig. 2A), similar to sea bream (Yúfera et al., 2012). Activation of gastric HCl secretion by feeding was also signalled by the 2-fold elevation of Cl^- concentration in gastric fluid (Fig. 2D). Moving down the tract, alkaline conditions occurred, with mean pH (Fig. 2A) and HCO_3^- concentrations (Fig. 2B) progressively increasing from anterior to posterior intestine in both fed and fasted sculpins. This likely reflected secretion of HCO_3^- in exchange for Cl^- which has been described in many marine teleosts (reviewed by Grosell, 2011; Wood, 2019). Notably, however, mean pH and HCO_3^- concentration remained much lower in the chyme of fed versus fasted sculpins, which could be explained by the discharge of very acidic chyme from the stomach to the intestine, titrating the HCO_3^- to CO_2. Supporting this explanation is the much higher mean P_{CO_2} level in the gut fluids of fed versus fasted sculpins (Fig. 2C). However, the caveats noted in the Materials and Methods about quantitative uncertainties in absolute P_{CO_2} values calculated from measurements of total CO_2 concentration and pH in gut fluids require that these data should be interpreted cautiously. Nevertheless, similar exceptionally high P_{CO_2} values in chyme, both directly measured and calculated, have now been reported in a number of both freshwater and marine teleosts (Wood and Eom, 2019; Jung et al., 2020, 2022; Goodrich et al., 2020), and are thought to play a role in blood O_2 delivery to the enterocytes (Wood, 2019).

Reduced intestinal fluid HCO_3^- concentrations and lower pH values have also been seen after feeding in seawater-acclimated rainbow trout (Wilson et al., 1996), toadfish (Taylor and Grosell, 2006) and killifish (Wood et al., 2010), all of which were allowed to feed voluntarily. However, the killifish lacks both a stomach and HCl secretion, so the less alkaline intestine was explained by reduced HCO_3^- secretion rates measured after feeding in intestinal preparations *in vitro* from fed killifish (Wood et al., 2010). In contrast, elevated HCO_3^- secretion rates were seen in *in vitro* preparations from voluntarily fed toadfish (Taylor and Grosell, 2009) and force-fed rainbow trout (Bucking et al., 2009). Fortunately, in staghorn sculpins, the situation is more straightforward: HCO_3^- secretion rates *in vitro* were reduced after voluntary feeding (Fig. 4A), thereby also contributing to less alkaline conditions in the intestine.

An alkaline tide occurred in the systemic bloodstream after feeding, signalled by a rise in mean pHa (Fig. 8A) and plasma HCO_3^- concentration (Fig. 8B) with no change in Pa_{CO_2} (Fig. 8C), a phenomenon that has now been seen in a number of feeding studies in other fishes (Wood et al., 2005, 2007b; Bucking and Wood, 2008; Cooper and Wilson, 2008; Bucking et al., 2009; Li et al., 2010), though some exceptions exist (Taylor and Grosell, 2006; Taylor et al., 2007; Goodrich et al., 2022). The alkaline tide is attributed to the activation of HCl secretion via oxynticopeptic cells in the

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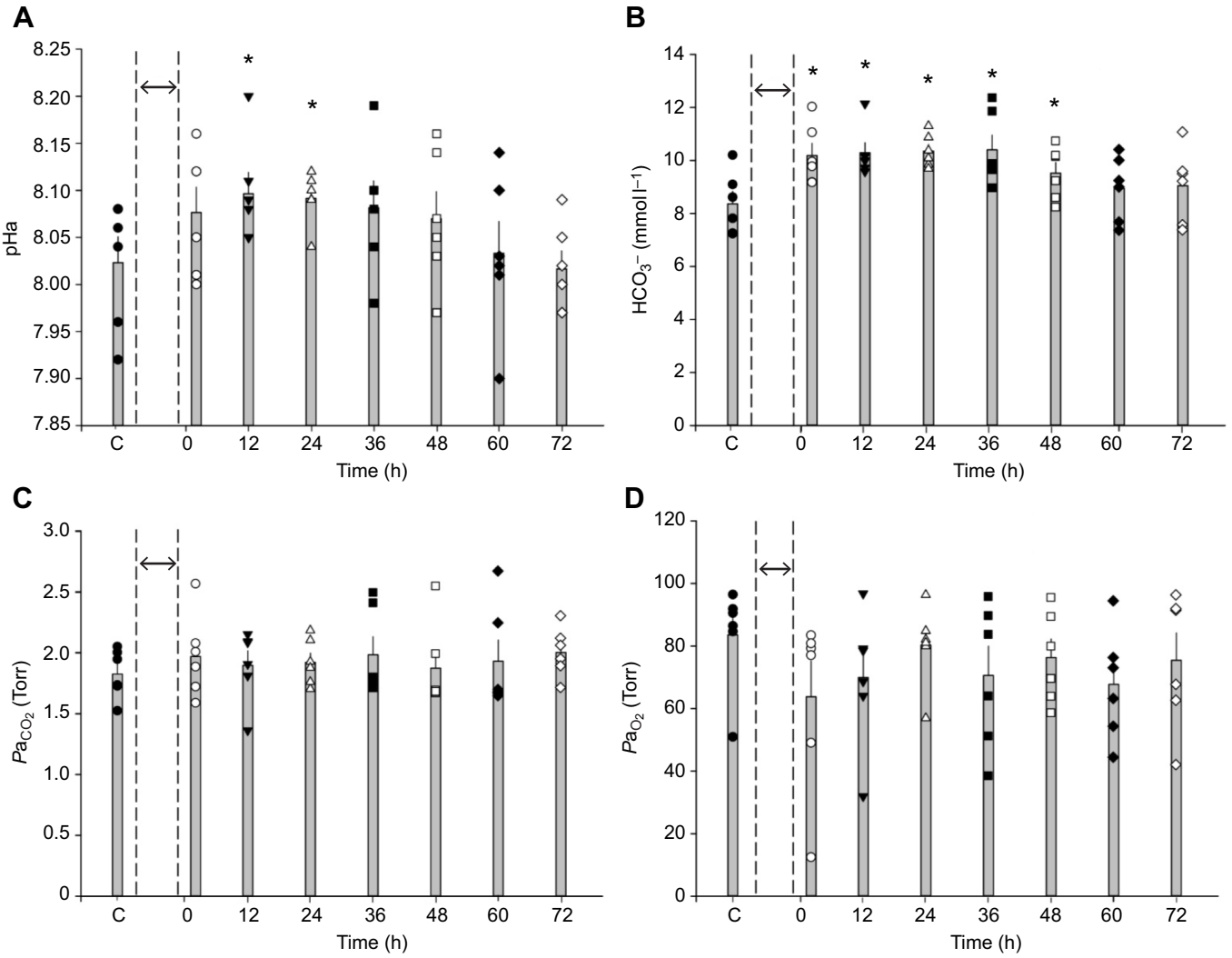

Fig. 8. The effect of ingestion of a satiation meal on arterial blood pH, plasma HCO_3^- concentration and arterial partial pressure of CO_2 and O_2 in staghorn sculpins in series 6. Data were obtained from arterial blood sampled by indwelling catheter from fish that had been fasted for at least 7 days previously. C represents the control value measured in fasting fish prior to feeding, vertical dashed lines indicate the 8 h feeding period, and 0 h represents the end of the 8 h overnight feeding period. Data are plotted at the midpoints of the 3 h flux period. Means±s.e.m. and individual data points are shown (N=6). Asterisks indicate significant differences (P<0.05) from the fasted control value. (A) Arterial pH (pHa). (B) HCO_3^- concentration. (C) Arterial partial pressure of CO_2 (Pa_{CO_2}). (D) Arterial partial pressure of O_2 (Pa_{O_2}).

stomach, which results in the equimolar efflux of HCO_3^- to the systemic bloodstream in exchange for Cl^- influx (Wood, 2019). In staghorn sculpins, despite the very large meal size, the magnitude of the alkaline tide was small (mean increases of 0.08 pH units, 1.8 mmol l^{-1} HCO_3^-), the latter significant up to 48 h. By way of contrast, in the studies cited above, the acid–base disturbance was larger (typically 0.2–0.4 pH units, 3–4 mmol l^{-1} HCO_3^-), but shorter lasting (<24 h). In dogfish sharks (Wood et al., 2007a, 2009) and freshwater rainbow trout (Bucking and Wood, 2008; Bucking et al., 2010; Goodrich et al., 2022), the alkaline tide was partly attenuated by the vigorous excretion of basic equivalents, mainly across the gills, to the external environment. However, this was not the explanation for the limited disturbance in the sculpin. A small shift to net excretion of basic equivalents was seen at one time point only (2.5 h; Fig. 7A), and its extent was very minimal relative to the cited studies.

At least in part, the explanation may lie in the finding of Cooper and Wilson (2008) that the alkaline tide was much smaller when trout ate the same ration voluntarily, rather than being force-fed. Another possibility is the finding of Bucking et al. (2009) that a large uptake of basic equivalents from the external seawater occurred after force-feeding in seawater-acclimated trout, presumably to augment HCO_3^- secretion in the intestine. This might have swamped basic equivalent excretion at the gills, but intestinal HCO_3^- secretion by the intestine was actually reduced in the sculpin (Fig. 4A). This contrasts with the situation in both the trout (Bucking et al., 2009) and the toadfish (Taylor and Grosell, 2009), where intestinal HCO_3^- secretion rates, measured *in vitro*, significantly increased after feeding. Indeed, Taylor and Grosell, (2009) calculated that the increase was of sufficient magnitude to prevent the alkaline tide in the toadfish. Thus, in the sculpin, it is not clear what happened to the excess HCO_3^- generated by HCl secretion in the stomach; again, we suggest that the potential role of the gut microbiome in this regard should be explored in future studies. Regardless, it is clear that the staghorn sculpin can process a very large meal with minimal acid–base disturbance.

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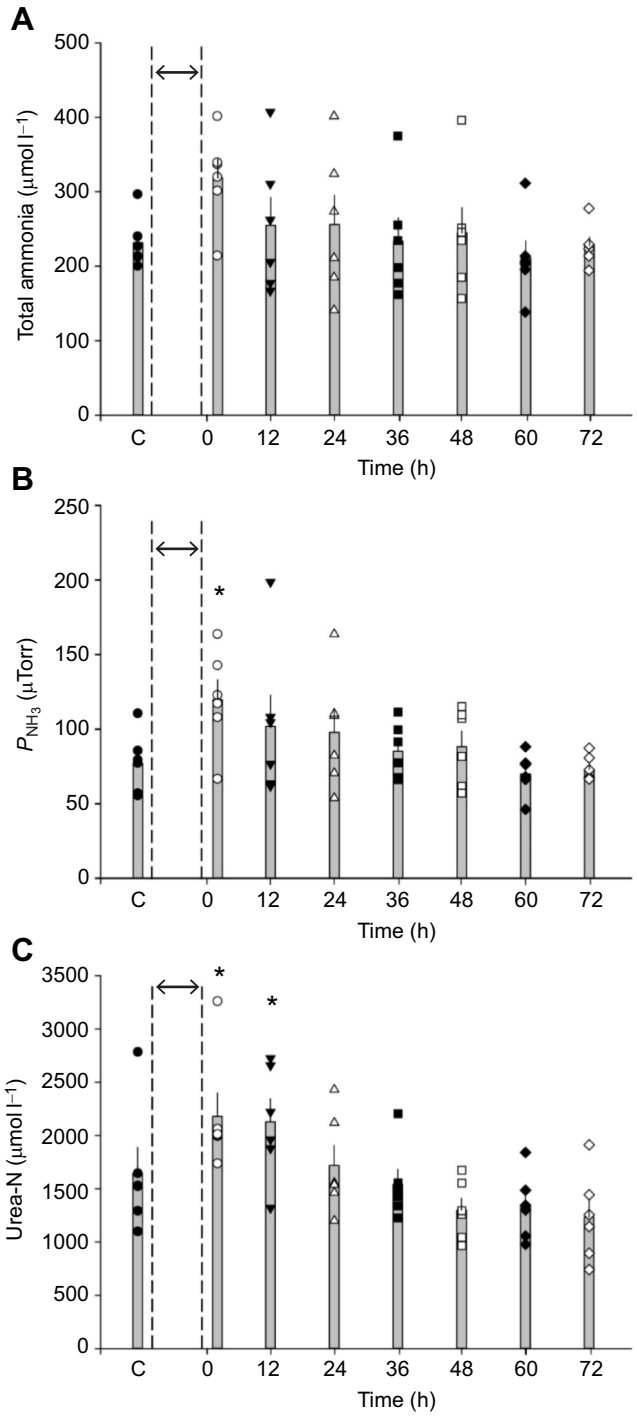

Fig. 9. The effect of ingestion of a satiation meal on plasma total ammonia concentration, P_{NH_3} and plasma urea-N concentration in staghorn sculpins in series 6. Data were obtained from arterial blood sampled by indwelling catheter from fish that had been fasted for at least 7 days previously. C represents the control value measured in fasting fish prior to feeding, vertical dashed lines indicate the 8 h feeding period, and 0 h represents the end of the 8 h overnight feeding period. Means±s.e.m. and individual data points are shown (N=6). Asterisks indicate significant differences (P<0.05) from the fasted control value. (A) Total ammonia concentration. (B) P_{NH_3}. (C) Urea-N concentration.

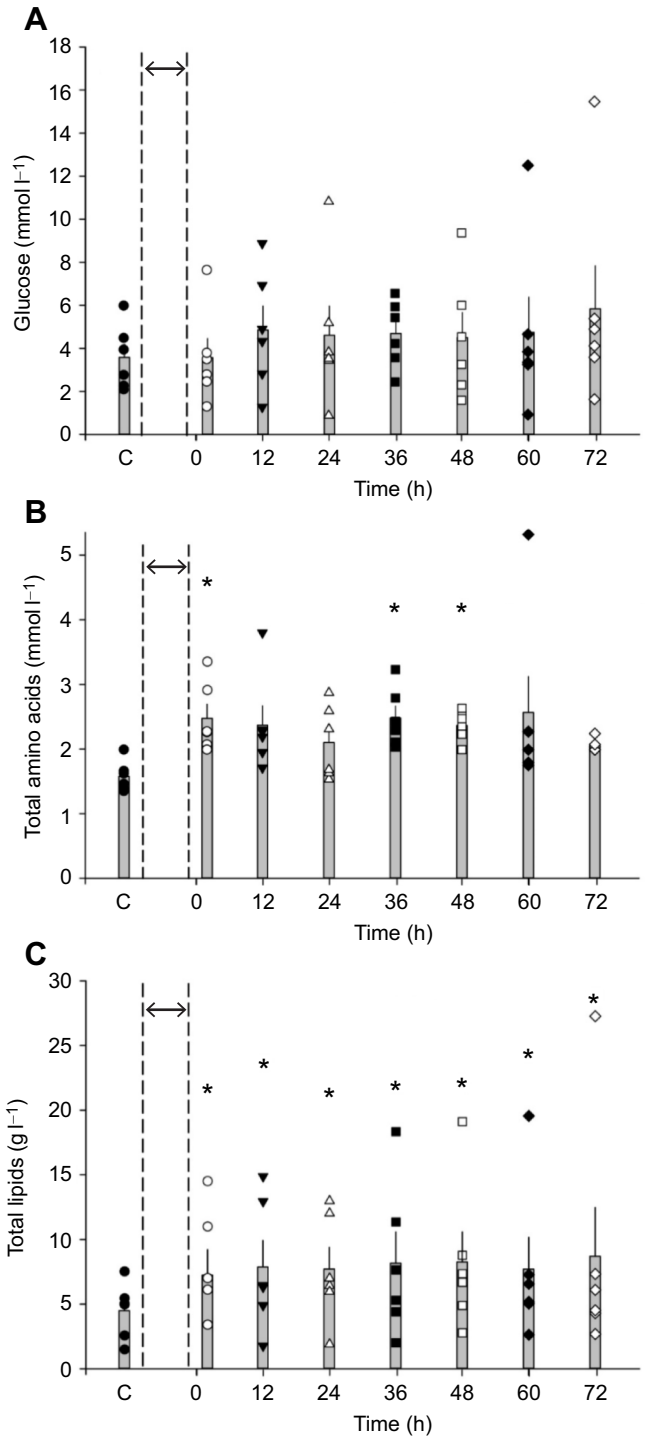

Fig. 10. The effect of ingestion of a satiation meal on plasma glucose, total amino acid and total lipid concentration in staghorn sculpins in series 6. Data were obtained from arterial blood sampled by indwelling catheter from fish that had been fasted for at least 7 days previously. C represents the control value measured in fasting fish prior to feeding, vertical dashed lines indicate the 8 h feeding period, and 0 h represents the end of the 8 h overnight feeding period. Means±s.e.m. and individual data points are shown (N=6). Asterisks indicate significant differences (P<0.05) from the fasted control value. (A) Glucose concentration. (B) Total amino acid concentration. (C) Total lipid concentration.

Gastrointestinal transport after feeding

The staghorn sculpin holds the large meal in the stomach for a long time (Fig. 1), slowly releasing a very loose chyme to the intestine. Absorption of nutrients was seen by the first post-prandial blood-sampling time (2 h), signalled by increases in mean plasma urea-N concentration (Fig. 9C), total amino acids (Fig. 10B) and total lipids

(Fig. 10C), with the last two remaining elevated for a prolonged period. Interestingly, the elevation in plasma total ammonia concentration was very small (non-significant) and ephemeral (Fig. 9A) in contrast to the large, long-lasting surges reported in many other feeding studies using synthetic commercial diets (e.g. Wicks and Randall, 2002a,b; Bucking and Wood, 2008; Bucking et al., 2009; Zimmer et al., 2010). The increases in mean plasma P_{NH_3} (Fig. 9B) and urea-N concentration (Fig. 9C) were also quickly corrected. In contrast, ammonia and urea-N excretion rates were greatly elevated for at least 36–60 h (Fig. 5A,B). This temporal disconnect argues for a rapid and sustained up-regulation of ammonia transporters [Rhesus (Rh) proteins] and urea transporters (UT proteins) in the gills after feeding, so as to strictly control the plasma levels of these potentially toxic N wastes. Indeed, there is evidence that this occurs for Rh proteins and associated ammonia transport pathways in rainbow trout (Zimmer et al., 2010; Zhang et al., 2015), but we are aware of no information on whether branchial urea transporters are similarly upregulated by feeding.

The *in vitro* experiments with gut sacs indicated no upregulation of intestinal ammonia absorptive pathways by feeding (Fig. 4B), and negligible ammonia absorption in the stomach. Note that these measurements were performed under identical conditions for fed versus fasted preparations so as to detect possible changes in transporter activity. *In vivo*, the total ammonia concentrations (and, by default, NH_4^+ concentrations) were much higher but the P_{NH_3} levels were lower in the luminal fluids of fed animals (Fig. 3A,B). As for calculated P_{CO_2} values, the caveats noted in the Materials and Methods for calculated P_{NH_3} values require that these data should be interpreted cautiously. The situation in sculpins appears to be very different from that of rainbow trout fed synthetic pellets, where similar gut sac experiments showed that the greatest ammonia uptake was from the stomach (Jung et al., 2023), and ammonia transport rates were upregulated after feeding (Rubino et al., 2014, 2019; Jung et al., 2021). In accord, the expression levels of intestinal ammonia transporters (Rh proteins for NH_3, NKCC for NH_4^+) (Bucking and Wood, 2012; Rubino et al., 2019) were similarly upregulated after feeding in trout. In the staghorn sculpin, the unchanged ammonia transport capacity of the gut after feeding, together with reduced P_{NH_3} due to lower pH in the luminal fluids and lack of absorptive capacity in the stomach, may all be adaptations to ensure that blood ammonia levels (Fig. 9A,B) do not surge during digestion of a large protein-rich meals when chyme total ammonia concentrations become very high (Fig. 3A). Furthermore, the absolute ammonia absorption rates were very low, about 10–40% of those measured in gut sac preparations from rainbow trout in the above-mentioned studies.

This was not the case for other intestinal transport rates. As noted earlier, intestinal HCO_3^- secretion was reduced after feeding (Fig. 4A) and the mean rates in gut sacs from fasted fish were 3- to 5-fold higher than in similar preparations from other marine teleosts at comparable temperatures (Grosell and Jensen, 1999; Grosell et al., 1999, 2001, 2005; Bucking et al., 2009). The mean fluid and Cl^- absorption rates (which did not change with feeding; Table S1) also tended to be higher.

Concluding remarks

The picture that emerges is that of a wild fish, opportunistically eating a very large natural meal, then processing it very slowly and efficiently while at the same time minimizing internal homeostatic disturbances. This is very different from the picture provided by previous studies on aquacultured species using synthetic diets and/or force-feeding. An unanswered question remains the extent to which the natural microbiome in the digestive tract contributes to the remarkable post-prandial homeostasis seen in the staghorn sculpin.

Acknowledgements

We thank BMSC staff, especially Research Coordinator Tao Eastham, for their support, Carolyn Morris for help with ammonia assays, and Dr Sunita Nadella for help with graphing.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.M.W., B.P.; Methodology: C.M.W., J.W., E.J., B.P.; Formal analysis: C.M.W., B.P.; Investigation: C.M.W., J.W., E.J., B.P.; Resources: C.M.W., B.P.; Data curation: C.M.W., B.P.; Writing - original draft: C.M.W.; Writing - review & editing: C.M.W., J.W., E.J., B.P.; Visualization: B.P.; Supervision: C.M.W.; Project administration: C.M.W., B.P.; Funding acquisition: C.M.W.

Funding

This work was funded by Natural Sciences and Engineering Research Council of Canada Discovery Grants to C.M.W. (RG-PIN 2017-03843, RGPIN-2023-03714).

Data availability

Data are available from the authors, on reasonable request.

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