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H^+ EXCRETION IN THE MARINE TELEOST *PAROPHRYS VETULUS*

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SUMMARY

This study examined the branchial, renal and blood acid-base responses of a marine teleost to intravascular infusion of mineral acid (HCl at 1000 μ-equiv/kg). In all animals the renal response was insignificant for at least 3 days following infusion. Two types of branchial and blood acid-base response were seen. Either the infused load disappeared rapidly from the extracellular into the intracellular compartment and was slowly cleared from the body (+16 h following infusion) or, more commonly, remained in the extracellular compartment until rapidly cleared by a pronounced increase in branchial excretion. In the latter the amount excreted exceeded that infused by about twofold on average. These responses are discussed in relation to those of freshwater teleosts.

INTRODUCTION

It is now well established that freshwater and marine fish rapidly defend their blood acid-base status against a variety of short-term challenges. Acute blood acidoses produced by either acid infusion (Wood & Caldwell, 1978; Kobayashi & Wood, 1980; Cameron, 1980) or severe exhaustive activity (Piiper, Meyer & Drees, 1972; Wood, McMahon & McDonald, 1977; Heisler, 1980; Turner, Wood & Hõbe, 1981) are usually corrected within 1–6 h of the disturbance. Recent studies have revealed that correction of these extracellular disturbances involves proton transfers to both the intracellular compartment and to the environment, the former being the first line of defence against acidosis and the latter being responsible for longer term regulation (Cameron, 1980; Heisler, 1980).

Proton transfers to the environment may occur either at the gills and/or at the kidney. Studies on marine elasmobranchs (Heisler, 1980) and freshwater teleosts (Cameron, 1980; Heisler, 1980) indicated that the gills were the dominant site for H^+ excretion while the kidney made only a minor contribution (1–10% of total acid output). In contrast, two recent studies on the freshwater rainbow trout showed a major role for the kidney. Wood & Caldwell (1978) demonstrated a pronounced

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elevation in renal H^+ excretion following acid infusion that was eventually sufficient to account for the load. More recently, McDonald & Wood (1981) have shown that the kidney of trout exposed to low environmental pH was solely responsible for H^+ excretion, eventually reaching a rate that was about 60% of the net uptake of H^+ at the gills. Both of these studies emphasize the slow nature of the renal response to acid loading, a factor perhaps not fully accounted for in other work.

In the present study on the lemon sole, *Parophrys vetulus* in sea water, we have examined branchial, renal and blood acid-base responses to intravascular mineral acid infusion. Our aim here was to determine the extent of intracellular buffering of the acid load and, in the light of apparently contradictory current findings, the relative participation of the gills and the kidney in the eventual clearance of the acid load. By employing water and urine titration techniques (McDonald & Wood, 1981; Wood & Caldwell, 1978) we have followed both branchial and renal H^+ excretion for 3 days following acid infusion, a period designed to ensure detection of any responses that may occur. We have chosen a flatfish for this study as the bladder (Hickman, 1968) and caudal vessels (Watters & Smith, 1973; Wood *et al.* 1977) can be chronically cannulated without difficulty. Choosing the lemon sole also allowed us to explore more fully the mechanisms of acid-base regulation in a marine teleost; studies to date have been largely confined to elasmobranchs and freshwater teleosts.

MATERIALS AND METHODS

Experimental animals

Lemon sole (*Parophrys vetulus*; 267–930 g) were collected by otter trawl in the waters off Bamfield Marine Station, Vancouver Island, British Columbia. Prior to experimentation the animals were held at least 1 week in large fibreglass tanks continuously supplied with fresh sea water at 11 ± 1 °C. While in the holding facilities and during subsequent experimentation the animals were starved to remove any influence of diet on renal acid output (Wood & Caldwell, 1978).

Experimental protocol

Before experiments, animals were surgically fitted under MS222 anaesthesia (1:10000 dilution in sea water) with chronic cannulae in a caudal vessel (artery or vein; Watters & Smith, 1973; Wood *et al.* 1977) and in the urinary bladder (Hickman, 1968) and then transferred to individual low volume (2–9 l) plastic chambers. The animals were allowed to recover in flowing sea water in these chambers for at least 36 h. The experimental regime which followed comprised a 24 h period prior to, and a 72 h period following, an intravascular infusion of mineral acid (1000 μequiv/kg infused as 1·0 N-HCl at 10 μl/min). During this time renal and branchial H^+ excretion, and blood acid-base status were monitored on all animals. For measurement of renal H^+ output, urine was collected over 12 h intervals, into covered vials via a siphon of 7 cm H_2O. Each 12 h collection was analysed for volume, pH, NH_4^+ and TA-HCO_3^- (see below).

To allow measurement of branchial net H^+ uptake/excretion, the sea-water flow into the fish chambers was stopped and the chamber volume adjusted to a know

value (usually 1 l per 100 g body weight). Chamber aeration was provided at a flow rate of 2–3 l/min by a multi-perforated plastic tube which lined the bottom edges of the fish chamber. This created a fine curtain of air bubbles which surrounded the fish and served to maintain constant high O_2 and low CO_2 levels ($P_{O_2} > 140$ torr; $P_{CO_2} < 1$ torr) and provide adequate mixing of chamber contents. Temperature control (11 ± 1 °C) was achieved by bathing the chambers in flowing sea water. The fish were held under these conditions for 11 h intervals interrupted by 1 h flushes with fresh sea water. Water samples, collected at the beginning and end of each 11 h period, were analysed for net branchial H^+ excretion/uptake (see below). During that 11 h period in which acid was infused, additional water samples were collected for analysis at 1, 2, 4 and 8 h. Sea water pH averaged 8·1 ± 0·1 over the course of the experiment. During the 11 h intervals the pH change from initial values did not exceed 0·2 pH units, so no pH adjustments were made to the water.

Blood samples (0·3 ml) for acid-base analysis (see below) were withdrawn anaerobically from the caudal vessel at 12 h prior to acid infusion and at +30 min, 1, 2, 8, 24, 48 and 72 h post-infusion.

Analytical techniques

Blood pH, haematocrit and total CO_2 content (whole blood and plasma, C_{CO_2}) were determined immediately on collected samples by methods described in McDonald, Hōbe & Wood (1980). These measurements were used to calculate P_{CO_2} and HCO_3^- (whole blood and plasma) from the Henderson–Hasselbalch equation. The quantity of non-respiratory acid buffered in blood (in m-equiv H^+/l) was calculated according to the following equation (equation 2 of McDonald *et al.* 1980):

$$\Delta H^+_b = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2), \quad (1)$$

where β is the slope of the whole blood non-bicarbonate buffer line ($\Delta HCO_3^-/\Delta pH$ expressed as m-equiv/l.pH unit) and the subscripts 1 and 2 refer to whole blood $[HCO_3^-]$ and plasma pH measurements made prior to and following acid infusion. The β value was calculated from the following relationship determined by *in vitro* CO_2 titration of blood from the closely related flat-headed sole *Hippoglossoides elassodon* (J. Turner, C. M. Wood & H. Hōbe, unpublished):

$$\beta = 32{\cdot}55 \times h_t - 2{\cdot}06, \quad (2)$$

where β is in m-equiv/l.pH and haematocrit (h_t) is expressed as a decimal. The methods employed to determine this relationship are described in detail in Wood *et al.* (1977) and Wood, McDonald & McMahon (1981).

The H^+ load to extracellular fluids (ΔH^+_{ECFV} in μequiv/kg body weight) was estimated from measurements of plasma pH and HCO_3^- according to the equation:

$$\Delta H^+_{ECFV} = BV.\Delta H^+_b + [IS - BV(1 - h_t)].\Delta H^+_{IF}, \quad (3)$$

where BV and IS are blood volume and inulin space respectively, and where ΔH^+_b and ΔH^+_{IF} (interstitial fluid) were calculated from equation (1). For the latter, a β value of −2·06 m-equiv/l.pH unit (the buffer capacity of separated plasma;

J. Turner, C. M. Wood & H. Hõbe, unpublished) was employed. The values used for inulin space (i.e. interstitial fluid volume plus plasma volume) and blood volume were respectively, 190 ml/kg (Eddy & Bath, 1979; Cameron, 1980) and 50 ml/kg (Stevens, 1968). This calculation assumes that the protein concentration and, therefore, buffer capacity of interstitial fluid is similar to that of separated plasma. While this has not been demonstrated in lemon sole, Hargens, Millard & Johansen (1974) have reported nearly identical protein concentrations in plasma and extra-vascular fluids of the flounder *Pleuronectes platessa* and the cod *Gadus morhua*.

Net branchial H^+ excretion/uptake (in μequiv/kg.h) was determined as the difference between the apparent H^+ uptake (apparent base loss) and the ammonia excretion by procedures similar to those described by Cameron (1980) and McDonald & Wood (1981). The former was determined from changes in titration alkalinity and the latter from changes in ammonia concentration in the water bathing the fish. For determinations of titration alkalinity, 10 ml water samples, thermostatted to the experimental temperature and continuously aerated, were titrated to pH 3·5 with stepwise addition of 0·2 N-HCl. The aeration provided for sample mixing and, together with 20–30 min of equilibration once a pH of $\leqslant 4{\cdot}5$ was reached, ensured the removal of CO_2 liberated by titration of water HCO_3^-. It should be pointed out that the excretion by the fish of any buffer bases (i.e. proton acceptors) with pK values in the range of 3–8 will cause an overestimate of apparent base loss. The most likely candidate here is the protein component of mucus secretion. However, at a typical mucus secretion rate of 1 mg/kg of body weight.h (calculated from Lock & Van Overbeeke (1981) assuming a protein content of dried mucus of 62%; Harris & Hunt, 1973) with a total H^+ binding capacity of 80 μequiv/g dry weight (Marshall, 1978), the additional 'base loss' due to mucus would amount to only 0·08 μequiv/kg.h. Since in the present study the apparent base loss (as determined by titration) varied from 30 to 300 μequiv/kg.h, the effect of mucus secretion is therefore likely to be negligible.

Urine pH and titratable acidity (TA-HCO_3^-) were determined immediately after collection as described in Wood & Caldwell (1978) and McDonald & Wood (1981). TA-HCO_3^- was determined as a single value in the double titration procedure recommended by Hills (1973). Titrants were 0·02 N-HCl and 0·02 N-NaOH, and the final end point of the titration was the blood pH value determined closest to the time of urine collection. Total renal acid output was calculated as the sum of the titratable acid efflux (TA-HCO_3^- × urine flow rate) and the ammonia efflux (NH_4^+ × urine flow rate).

Ammonia levels in water and urine were determined by the phenol hypochlorite method of Solorzano (1969). For increased accuracy, all samples were assayed in duplicate. Replicates differing by more than 5% were repeated.

RESULTS AND DISCUSSION

Blood acid-base disturbances

Prior to acid infusion, all animals had recovered from handling and anaesthesia as indicated by an absence of locomotor activity and by blood acid-base parameter

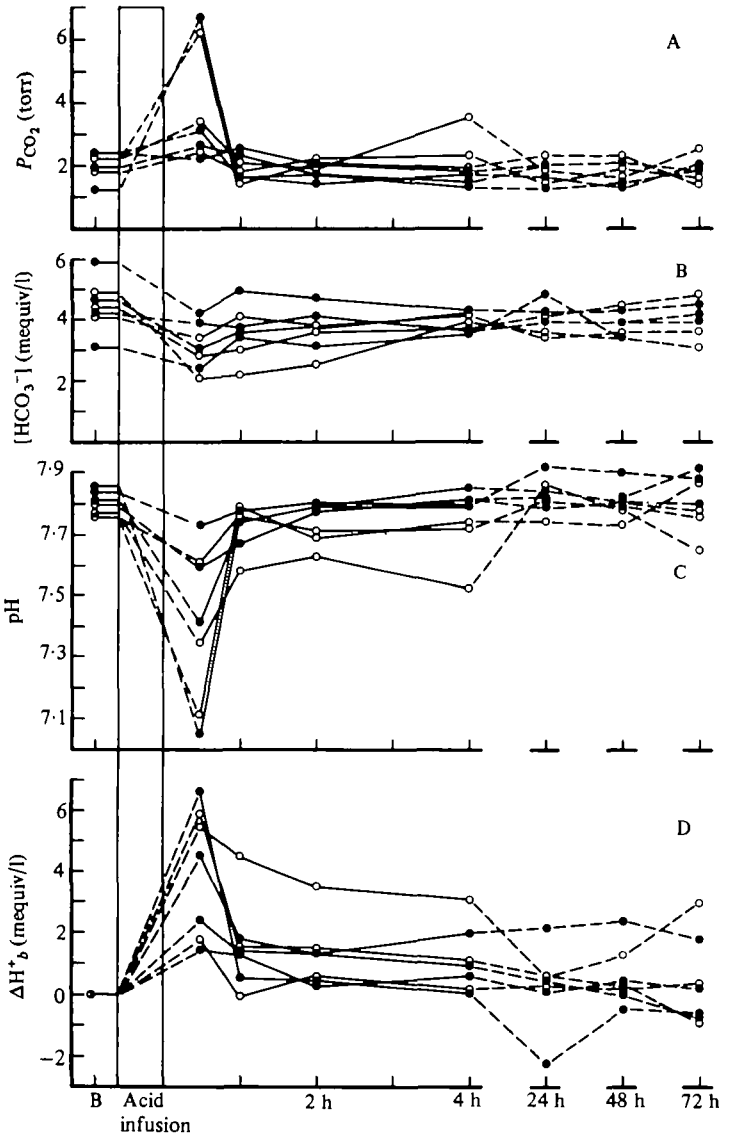

Fig. 1. (A) Plasma P_{CO_2}, (B) whole-blood bicarbonate concentration, (C) plasma pH and (D) Non-respiratory acid added to blood buffers (ΔH^+_b) in lemon sole ($N = 7$) prior to (point B) and following intravascular infusion of 1000 μequiv/kg of HCl. Individual values are plotted, closed symbols represent venous samples; open symbols, arterial samples. Bar = infusion period, duration 0·5–1·5 h (depending on body weight of fish).

nearly identical to those defined for the well-acclimated state of a related species at 10 °C, the starry flounder *Platichthys stellatus* (Wood, McMahon & McDonald, 1979). Intravascular infusion of 1000 μequiv/kg of HCl over 0·5–1·5 h provoked a blood acidosis in all animals but in no instance was any muscular activity observed. The blood pH depression which occurred was due not only to addition of non-volatile acid to the blood (i.e. ΔH_b^+, Fig. 1D) but also to an increase in P_{CO_2}, an increase which was evident in both arterial and venous blood (Fig. 1A). This respiratory

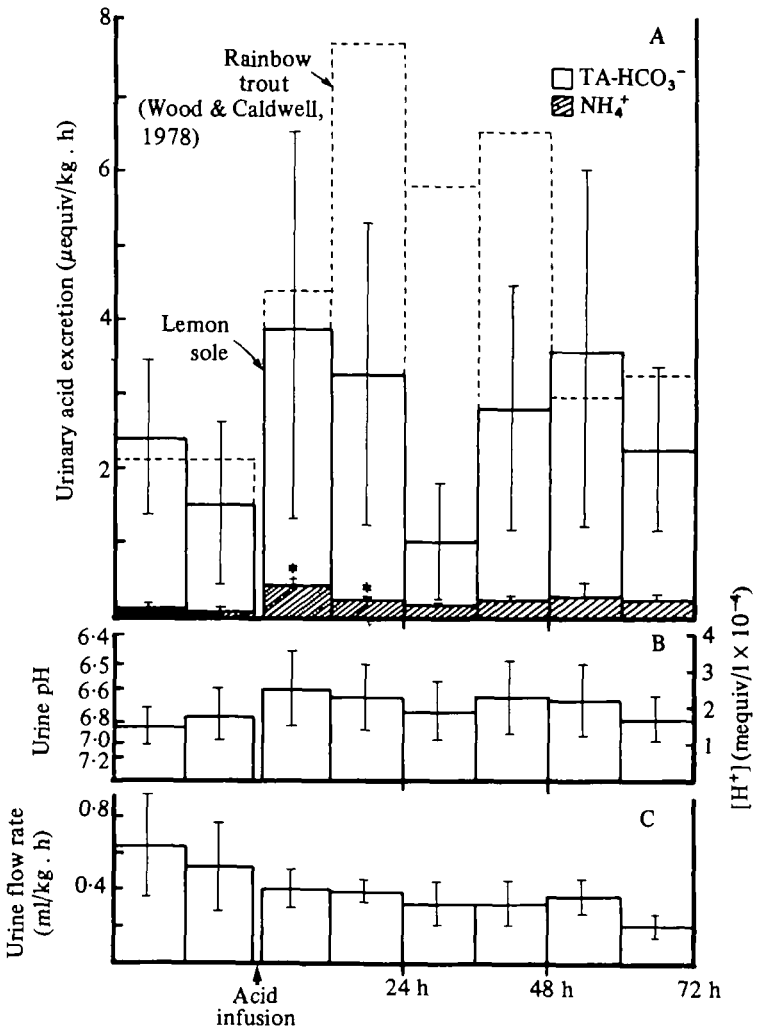

Fig. 2. (A) Total urinary acid excretion, $NH_4^+ + TA\text{-}HCO_3^-$, (B) Urine pH, and (C) Urine flow rate of lemon sole (means ± 1 S.E.M., $N = 7$) prior to and following intravascular infusion of 1000 μequiv/kg of HCl. Asterisks indicate significant difference ($P < 0.05$) from pre-infusion values (by paired t test). Dotted lines in A indicate total urinary acid excretion in fresh water rainbow trout (means, $N = 10$) prior to and following intra-arterial infusion of 100 μequiv/kg of HCl. Trout data from Wood & Caldwell (1978).

component of the acidosis may reflect either a stimulation of aerobic metabolism related to the stress of acid infusion or simply acid titration of blood HCO_3^- followed by slow branchial washout of CO_2. Similarly slow kinetics for CO_2 exchange have been reported in the starry flounder *Platichthys stellatus* (Wood *et al.* 1977) following strenuous exercise. In this animal a respiratory acidosis persisted for about 4 h.

Also evident in the lemon sole was a considerable variability in the magnitude of the acid-base disturbance provoked by acid infusion. This was particularly evident in ΔH_b^+ (Fig. 1 D), a parameter which estimates the non-respiratory component of the acidosis. Here, two types of response were seen; in one group of four animals

the concentration of non-volatile acids in the blood exceeded 4·5 m-equiv/l at 30 min post-infusion, while in the remainder ΔH_b^+ was less than 2·5 m-equiv/l. These differences are discussed below.

Urinary acid excretion

Under resting conditions the fasting sole produced an acid urine (Fig. 2B) with a pH that was at least one unit below that of blood. Such urines are typical of marine teleosts (Hickman & Trump, 1969) and are thought to be necessary in order to minimize the precipitation of Ca^{2+} and Mg^{2+} salts in the urinary bladder. The urine acidity consisted mainly (> 95%) of the titratable component, TA-HCO_3^-, with ammonia as NH_4^+ (the non-titratable component) contributing only a small fraction of the total (Fig. 2A). The urinary acid output prior to acid infusion averaged 2·05 μ-equiv/kg.h, a value nearly identical to that found in the acclimated freshwater rainbow trout (dotted lines, Fig. 2A; data of Wood & Caldwell, 1978). A major difference between the two species was that in the trout the urine acid content was about tenfold lower and the urine flow rate tenfold higher (Wood & Caldwell, 1978; McDonald & Wood, 1981).

Over the first 12 h following acid infusion in the sole there was a significant increase in urine ammonia excretion (Fig. 2A). This was a much more rapid and proportionately larger response than that seen in rainbow trout following either HCl infusion (Wood & Caldwell, 1978) or external acid exposure (McDonald & Wood, 1981). However, in absolute terms, the ammonia excretion remained a small component of urine acidity with the result that total renal acid output post-infusion was insignificantly different ($P > 0{\cdot}05$, by paired t test) from control values. In contrast, the acid excretion by the rainbow trout kidney (Wood & Caldwell, 1978) progressively increased following acid infusion to approximately 4 times the control values. The difference here is particularly striking in view of the fact that the acid load to the trout was one-tenth of that to the sole: 100 μequiv/kg *v.* 1000 μequiv/kg. This comparison emphasizes the inability of the marine teleost kidney to respond to acid challenge within at least 3 days of a blood acid-base disturbance, a result confirming and extending earlier observations on the marine sculpin *Myoxocephalus scorpius* (Hodler *et al.* 1955). A similar lack of renal responses has been reported for marine elasmobranchs (e.g. Cross *et al.* 1969; Heisler, 1980). The low urine flows of marine fish (Hickman & Trump, 1969) and the possible mandatory basal production of an acid urine to prevent bladder precipitates are probably important factors limiting the kidney's performance in acid-base regulation in sea water.

Branchial H^+ and ammonia excretion

Prior to acid infusion there was, on average, a slight net excretion of H^+ across the gills (dotted line, Fig. 3A) which, because of the variability between the two pre-infusion flux periods, was not significantly different from zero. With infusion of the acid load there was an immediate and substantial increase (*ca.* 9-fold) in H^+ excretion (Fig. 3A). This was highest during the actual period of acid infusion (0·5–1·5 h), indicating that a portion of the infused load was excreted almost im-

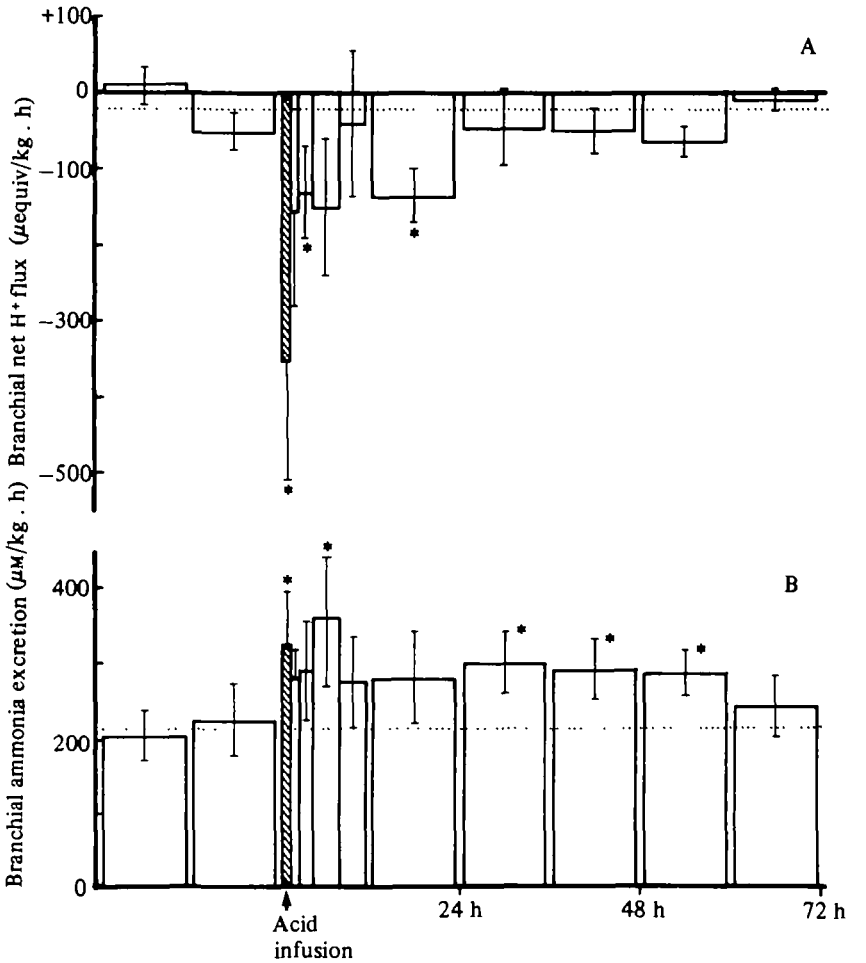

Fig. 3. (A) Branchial net acid flux and (B) ammonia excretion in lemon sole (means ± 1 S.E.M., $N = 7$) prior to and following intravascular infusion of 1000 μequiv/kg of HCl. Shaded bar = period of acid infusion. Dotted lines = average pre-infusion H^+ flux and ammonia excretion. Asterisks indicate significant difference ($P < 0.05$) from pre-infusion mean (by paired t test).

mediately upon entering the vascular compartment. Branchial H^+ excretion remained elevated for the subsequent 24 h; beyond this time it returned to near resting values.

Accompanying the increase in H^+ excretion was a significant increase in branchial ammonia excretion (Fig. 3B). Ammonia excretion as NH_4^+ is, of course, equivalent to H^+ excretion. Its role in acid-base regulation, however, cannot be directly assessed since the proportions of the total ammonia excretion occurring as NH_3 and NH_4^+ cannot be determined from the current data. Nevertheless it is now apparent that substantially increased branchial ammonia excretion is a response to disturbances which provoke blood acidoses in fish, namely severe exercise-induced lactacidosis (Heisler, 1980), environmental hypercapnea (Lloyd & Swift, 1976) and exposure to low environmental pH (Maetz, 1973; Heisler, 1980; D. G. McDonald, unpublished

results). In relation to the latter, Maetz (1973) and Heisler (1980) have argued that increased ammonia excretion was simply due to a marked improvement in the NH_3 diffusion gradient and thus had no role to play in acid-base regulation. However, in the freshwater rainbow trout (D. G. McDonald unpublished; Wood & McDonald, 1981) we have recently observed that branchial ammonia excretion rose gradually and progressively to 2 × resting levels over 4 days of acid exposure (pH = 4·0–4·5) and that coincident with this was a reduction of net H^+ uptake to near zero. Thus, it appears that fish may exploit and, indeed, stimulate endogenous nitrogen metabolism under a variety of circumstances to provide for the regulation of acid-base state. As Evans (1980) has pointed out, it remains to be demonstrated under what conditions H^+ or NH_4^+ excretion may predominate and whether one has relative advantages over the other.

Clearance of the acid load

As the amount of acid infused is known, the distribution of the load between extracellular and intracellular compartments and its rate of clearance from the body can be determined in individual fish. The former involves a calculation of the load to extracellular fluids (see Methods); that portion not accounted for must either have entered the ICF or have been excreted. In order to calculate the clearance of the load from the body, endogenous acid or base production must be taken into consideration. The approach adopted here was to assume that the average excretion for each fish prior to infusion (i.e. the mean of the two 11 h measurements, Fig. 3) represented its basal production; these values were then subtracted from all post-infusion measurements. It has also been assumed that acid infusion provoked no significant increase in endogenous acid production via anaerobic glycolysis. While blood and tissue lactate measurements have not been made in the present study, significant increases would only be expected in cases of substantially increased metabolic demand and/or pronounced impairment of oxygen delivery. Neither phenomenon is likely to have been important in these experiments as no overt locomotor activity was observed and as the Bohr shift in Hb O_2 affinity would have been relatively mild and short lived (see Fig. 1). In fact, given the high haemoglobin affinity in benthic flatfish under resting conditions (P_{50} = 8·6 torr at pH_a = 7·87; Wood *et al.* 1979) the infusion-induced acidosis may have actually improved O_2 delivery to tissues.

This analysis confirms that the animals in this study can be divided into two groups on the basis of their response to acid infusion. In one group of four animals (group 2, Table 1), the acid load was apparently transferred very rapidly to the intracellular compartment. There was a minimal blood acidosis by 30 min post-infusion (Table 1) and only about 1/3 of the acid load (287 μequiv/kg) remained in the ECF. Subsequently the load was cleared very gradually from the body. Net H^+ excretion by 2 h in this group amounted to only 191 μequiv/kg (Table 1) or 19% of the total, and it was not until about +16 h, on average, that there was sufficient branchial H^+ excretion to account for the infused load.

In the remaining animals (group 1, Table 1) on the other hand, the acid load remained largely in the extracellular compartment until excreted at the gills. By

Table 1. *Two types of response seen in lemon sole,* Parophrys vetulus, *to intravascular infusion of* 1000 *μequiv/kg of HCl: values are means* ± 1 S.E.M. (*see text for details*)

	Group 1	Group 2
N	4	3
Mean weight (range) (g)	712 (479–930)	459 (267, 290, 820)
Blood pH 30 min post-infusion	7·23 ± 0·09	7·65 ± 0·04
Δ^{+}_{ECFV} 30 min post-infusion (*μ*equiv/kg)	783 ± 42	287 ± 91
Branchial H^+ excretion on day 0, i.e. basal acid production (*μ*equiv/kg.h)	28 ± 13	26 ± 21
Net H^+ excretion by +2 h (*μ*equiv/kg)	765 ± 177	191 ± 180
Net H^+ excretion by +11 h (*μ*equiv/kg)	2050 ± 480	231 ± 619
Time to clear acid load	5 ± 2 h	16 ± 5 h

30 min post-infusion there was still a substantial blood acidosis (pH > 0·5 below normal, Table 1) with 3/4 of the acid load (787 *μ*equiv/kg) remaining in the ECF. By 2 h, however, blood acid-base balance was nearly restored with the net excretion of 765 *μ*equiv/kg of H^+ at the gills. Despite this recovery of near-normal status, excretion continued at an elevated rate such that by +11 h the amount cleared was about twice the amount infused (Table 1).

The origin of the differences in the handling of the acid load between the two groups is unknown. It cannot be ascribed to infusion site as arterial and venous sites were about equally distributed between two groups (see Fig. 1), or to rate of acid infusion (this was kept low and constant after an initial fatality). There was a difference in body weight between the two groups (Table 1), the smallest fish buffering the load largely intracellularly. However, weight differences were not pronounced and, in any case, overlapped. This factor may nevertheless be important as marked size differences in acid-base regulation have been reported in the dogfish *Scyliorhinus stellaris* (Heisler, Weitz & Weitz, 1976; Heisler, Neumann & Holeton, 1980). The size effect in this species also remains unexplained.

Regardless of the origin of these differences they do point out two important features of the H^+ excretion mechanism. Firstly, a pronounced and relatively persistent extracellular acidosis of at least 30 min duration is required to activate branchial H^+ excretion. In those animals which had no major disturbance by this time (group 2, Table 1), branchial H^+ excretion was also low. Secondly, the mechanism, once activated, continues to excrete acid beyond the point at which the acid-base disturbance is corrected (compare Fig. 3 and Fig. 1). A similar overshoot in acid excretion occurred in rainbow trout in freshwater following 10 min severe activity (Heisler, 1980). Here, blood pH depression averaged 0·4 units, there was a marked stimulation in H^+ transfer to the environment (partly attributable to enhanced ammonia excretion) and by 6 h post-exercise there was a definite alkalosis, a pattern very similar to group 1 animals. No similar blood alkalosis was observed in the present study but it may have been missed in the gap between the 4 h and the 24 h sample (Fig. 1). Also not seen was any subsequent undershoot in H^+ excretion. However, a small reduction persisting for a long time would be very difficult to detect given the inherent variability of net H^+ uptake/excretion under resting conditions (Fig. 3).

In contrast to this pattern of rapid H^+ excretion, the freshwater catfish *Ictalurus punctatus* (Cameron, 1980) showed a response to acid infusion (1000 μequiv/kg) more similar to group 2 animals (Table 1). By +2 h post-infusion in the catfish, only 20–30% of the infused load was cleared by the gills and there was little subsequent excretion. This period was also the time over which blood pH was depressed. In these animals the load must have entered intracellular compartments and eventually was slowly cleared from the body.

Whether these interspecific differences are related to differences in methodology, environment, or nature of the acidosis is uncertain, but the present data do emphasize that the capacity for branchial H^+ excretion in marine teleosts is at least as great as in freshwater teleosts. Similar mechanisms of branchial acid excretion have been proposed for both groups, i.e. Na^+/H^+ or NH_4^+ and Cl^-/HCO_3^- exchanges and there is now substantial evidence supporting their existence (e.g. Maetz, 1973; DeRenzis & Maetz, 1973; Cameron, 1976; Evans, 1980). Marine teleosts, in contrast to freshwater forms, will exacerbate the salt load to their body fluids by the operation of these exchanges. Assuming 1:1 stoichiometry in Na^+/H^+ or NH_4^+ exchange the additional Na^+ influx over the 11 h period following acid infusion would have averaged 240 μequiv/kg.h in group 1 animals with values as high as 1200 μequiv/kg.h over short periods. Given that resting values for total Na^+ influx (i.e. branchial plus intestinal) in marine teleosts may be between 10000–20000 μequiv/kg.h (cf. Maetz, 1974), then the additional Na^+ uptake required for acid-base regulation may, in fact, impose only a minor load on ionoregulatory mechanisms. This conclusion is supported by the large overshoot in acid excretion which occurred in a majority of animals, i.e. the marine teleost may tolerate a minor disturbance in salt balance in order rapidly to correct a major extracellular acidosis.

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