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MECHANISMS OF ACID EXTRUSION BY TWO MARINE FISHES: THE TELEOST, *OPSANUS BETA*, AND THE ELASMOBRANCH, *SQUALUS ACANTHIAS*

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SUMMARY

1. Rates of efflux of H^+ and ammonia from a marine teleost and an elasmobranch were measured.

2. Hypercapnia stimulated H^+ efflux from both species, stimulated ammonia efflux from the elasmobranch, and inhibited ammonia efflux from the teleost.

3. In both species the H^+ and ammonia efflux were predominantly across the branchial epithelium. In Na^+-free sea water, the H^+ efflux from both species was completely abolished and the ammonia efflux was inhibited by approximately 50%.

4. Injection of an acid load stimulated H^+ efflux, which continued for 2–5 h until more than the injected acid load was excreted. It therefore appears that injection of an acid load also produces a metabolic acid load which must be excreted.

5. The H^+ efflux from mineral/metabolic acidotic fish is entirely branchial and dependent upon external Na^+.

6. The data support the conclusion that marine teleosts and elasmobranchs possess branchial Na^+/NH_4^+ and Na^+/H^+ ionic exchange mechanisms and that Na^+/H^+ exchange plays a major role in the response to acidosis in both groups.

7. The possible evolution of these ionic exchange systems is discussed.

INTRODUCTION

In recent years it has become apparent that marine teleosts, and possibly marine elasmobranchs, excrete blood NH_4^+ in exchange for sea water Na^+ (Evans, 1977, 1980*a*, *b*). In freshwater fish this branchial ionic exchange system provides for the uptake of Na^+ required for existence in their hypo-osmotic environment, as well as for nitrogen (and presumably acid) excretion. However, in the marine environment the resulting Na^+ influx is obviously maladaptive for ion regulation. We have no information on the magnitude of the net influx of Na^+ presented by Na^+/NH_4^+ exchange in the elasmobranchs, but in at least one teleost, *Opsanus beta*, it presents a

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Na^+ load that is 10% of the total net influx of this ion, twice the load presented by oral ingestion (Evans, 1980*c*).

Investigations of freshwater teleosts indicate that, in addition to Na^+/NH_4^+ exchange, Na^+/H^+ exchange may also play a significant role in Na^+ balance (Evans, 1980*a*, *b*). No direct evidence for Na^+/H^+ exchange exists for marine teleost fishes; however, its presence in the elasmobranchs is suggested by the finding that the Na^+ influx into *Scyliorhinus canicula* can be stimulated by the injection of HCl (Payan & Maetz, 1973) and inhibited by acidifying the external sea water (Bentley, Maetz & Payan, 1976). In addition, Evans, Kormanik & Krasny (1979) recently demonstrated that acid (but not ammonia) efflux from the skate, *Raja erinacea*, is dependent upon external Na^+.

The importance of branchial extrusion pathways in acid/base regulation by both teleosts and elasmobranchs is presently under active investigation (see reviews by Cameron, 1978, and Heisler, 1980). Cameron & Wood (1976) found that compensation for the post-operative fall in blood pH of two Amazonian teleosts was made by an increased extrarenal efflux of acid; however, Wood & Caldwell (1976) showed that trout (*Salmo gairdneri*) responded to a mineral acid load with an increase in the renal excretion of acid which could account for the entire acid load within 72 h. More recently, Cameron (1980) has found that extrarenal mechanisms predominate in pH compensation in the catfish, *Ictalurus punctatus*, after the production of either hypercapnic or mineral acidosis. In addition, Kobayashi & Wood (1980) demonstrated that renal excretion could amount for only 2% of the lactate load and 6% of the proton load 72 h after the infusion of lactic acid into *S. gairdneri*. Branchial compensatory mechanisms also appear to be of major importance in elasmobranch pH regulation because Heisler, Weitz & Weitz (1976) found that hypercapnia stimulated extrarenal H^+ efflux from *S. canicula*, with less than 1% of the acid load appearing in the urine (Heisler, 1980). The excretion of acid by this species after HCl influsion or exercise is also approximately 99% via branchial pathways (Heisler, 1980). In addition King & Goldstein (1979) showed that only 7% of a mineral acid load injected into *Squalus acanthias* was excreted renally within the first 72 h after injection of the HCl. These data corroborate an earlier study (Cross *et al.* 1969) that found that an increased renal excretion of acid plays a vanishingly small (less than 1%) role in the compensation of hypercapnic acidosis by *S. acanthias*.

While it appears that pH regulation in freshwater teleosts and marine elasmobranchs is branchial, there is no direct evidence that it is via Na^+/NH_4^+ and/or Na^+/H^+ exchange. Both DeRenzis & Maetz (1973) and Cameron (1976) demonstrated that alteration of blood pH of freshwater fish (via injection of acid or base, or production of hypercapnia) was accompanied by alteration in the relative influxes of Na^+ and Cl^-, presumably because they were linked to H^+ (or NH_4^+) and HCO_3^- extrusion, respectively. However, alteration of blood pH could have had secondary effects on permeability or transepithelial electrical potentials which could have resulted in the observed alterations in isotopic influxes. In addition, Kerstetter & Mize (1976) found that acute metabolic acidosis in *S. gairdneri* did not affect Na^+ or Cl^- influxes measured over a short time-span (15 min). More recently Perry *et al.* (1981) have found that hypercapnia in the same species does not alter the uptake of either ion measured for 1 h.

after production of hypercapnia for 24 h. Importantly, the role of branchial ionic exchange systems in pH regulation by marine teleost fishes has not been investigated, and it is possible that alternative pathways exist to avoid increased Na^+ (and Cl) influxes secondary to pH regulation.

The purpose of the present investigation was to determine: (1) the rate of H^+ and ammonia efflux from a marine teleost and elasmobranch, (2) the effect of either hypercapnia or mineral-acid loading on these effluxes, (3) the relative importance of branchial vs renal efflux, and (4) the role of external Na^+ in these effluxes.

MATERIALS AND METHODS

Toadfish (*Opsanus beta*) were collected by commercial shrimp fishermen in Biscayne Bay, Florida, and maintained in plastic aquaria containing filtered sea water (500 mM-Na^+) at room temperature (23–25 °C). Fish (10–30 g) were used in experiments within 2 weeks of capture and were not fed. Dogfish (*Squalus acanthias*) 'pups' were removed from sacrificed females that had been caught by hook and line in Frenchman's Bay, Maine, and maintained in running sea water (450 mM-Na^+) at 13–17 °C in wooden aquaria. 'Pups' are near-term embryos at the end of an approximately 2-year gestation period (Woodhead, 1979). They are able to survive in sea water for periods over 41 days (Gilbert, 1958) and display the major hallmarks of elasmobranch osmoregulation (Kormanik & Evans, 1978; Evans & Mansberger, 1979; Evans & Oikari, 1980). The 'pups' (40–60 g) had large yolk sacs and were therefore not fed. Except for the initial normocapnic to hypercapnic experiments, the yolk sac was ligated and removed before all experiments.

Net H^+ effluxes were measured using a technique modified from that of Heisler *et al.* (1976). Net H^+ efflux is defined in the present study exclusive of ammonia effluxes, with the assumption that ammonia is excreted predominately as NH_4^+ and does not release a proton at the pH's used in the present experiments. Total acid efflux would therefore be the sum of the net H^+ efflux and the ammonia efflux. The net H^+ efflux is best correlated with 'titratable acidity', but the latter term is not used here because back titrations were not performed. Individual fish were placed in aerated sea water, 100 ml for toadfish, 200 ml for dogfish, in a small plastic container. Experimental baths containing toadfish were maintained at room temperature (23–25 °C) while those containing shark 'pups' were placed in running sea-water troughs at 13–17 °C. At time zero, and at various time intervals thereafter, a 5 ml sample of the experimental bath was removed, bubbled with 1% CO_2 (in oxygen, from compressed gas cylinders, Air Products) and its pH recorded with a Radiometer PHM 62 pH meter attached to a Radiometer titragraph recorder. In this way the pH could be recorded to the nearest 0·002 units. The pH was noted only after it had been relatively stable (drift less than 0·02 pH units/h) for at least 2 min. The sample was then either frozen for ammonia analysis or returned to the experimental bath. As Heisler *et al.* (1976) have proposed, since the P_{CO_2} of the experimental sample is maintained constant during the recording of the pH, according to the Henderson–Hasselbalch equation, the net change in bath pH is dependent only upon acid or base addition. I have empirically determined that bubbling with 1% CO_2 immediately (less than

30 s) blows of any respiratory CO_2 and results in a relatively stable pH of the sample (drift of 0·02 pH units/h in sea water). The pH of samples of the experimental baths was always compared with the pH of samples from a control bath, treated in the same manner as any experimental bath, but containing no fish. The pH differential between the control and experimental baths at any time can be converted to acid or base fluxes since the volume of the bath, the weight of the fish (determined to the nearest 0·1 g at the end of an experiment) and the buffering capacity of the solutions are known. Buffering capacity of the control was determined at the beginning of the experiment and that of experimental baths at the termination of the experiment. In both cases the buffering capacity was measured by the addition of known amounts of HCl or NaOH (to a 5 ml sample) while recording the resulting change in pH. A linear regression of the data gives a slope of μM acid change per μM acid/base added. The final buffering capacity of the experimental solutions was determined over the pH range observed during the course of the experiment because the fish could change the buffering capacity, the control solution could drift spontaneously (probably due primarily to temperature changes, especially at MDIBL), and the buffering capacity depended on the pH range used. H^+ fluxes were calculated by converting the pH differential between the control and the experimental bath to hydrogen ion concentration, and correcting for the average buffering capacity for that time period and pH range, the volume of the sample, and the weight of the fish. The calculation also took into account removal of any samples for ammonia analysis. Computation of the rate of H^+ flux (μM $100\,g^{-1}\,h^{-1}$) was performed on the University of Miami Univac 1100 computer using a program written by J. B. Claiborne. Ammonia concentrations of defrosted samples were determined using either the technique of Harwood & Kuhn (1970) or Solarzano (1969).

Experimental solutions were either bubbled with compressed air or 5% CO_2 (in air) produced by a Wostoff gas mixing pump connected to a tank of liquid CO_2. To ascertain the role of renal/rectal pathways in acid and ammonia efflux, a washed rubber condom was placed over the tail and secured, anterior to the anus, with loops of cotton thread. The condom contained 10 ml of sea water, and at the end of 1 h the condom was removed and the pH of its contents and a sample of the experimental bath were determined as above. The sample was then frozen and saved for ammonia analysis. Mineral acidosis was produced in some experiments by injecting 50 μM $100\,g^{-1}$ of HCl under MS 222 anaesthesia (0·01%). The acid was injected intraperitoneally as 0·5 $\mu l\,g^{-1}$ of 1 N-HCl, normally over 1–2 min. The role of external Na^+ in H^+ and ammonia efflux was determined by monitoring efflux in control sea water for a specific time period, rinsing the fish (for approximately 30 s) in Na^+-free artificial sea water (formulated according to Evans and Cooper, 1976, with 2·5 mM-$KHCO_3$ and 7·5 mM-KCl, and choline chloride as the Na^+ substitute) made to conform to the osmolarity of the ambient sea water (Miami *v.* MDIBL), and then transferred into Na^+-free artificial sea water for 1 h. At the end of that experimental period the fish were transferred to another container of control sea water to test for reversibility of 'Na^+-free effect'.

In some experiments the transepithelial electrical potential (TEP) was measured as previously described (Evans & Cooper, 1976).

All data are expressed as $\bar{X} \pm$ S.E. (N) and statistical significance between means was tested with Student's t test (using paired data when appropriate).

Table 1. *Effect of hypercapnia on H^+ and ammonia efflux from the toadfish and dogfish*

Species	Normocapnic		Hypercapnic	
	H^+	Ammonia	H^+	Ammonia
Opsanus beta	36 ± 18 (6)	26 ± 4·1 (11)	101 ± 6·4 (20)**	13 ± 3·8 (11)*
Squalus acanthias	−0·7 ± 2·3 (9)	1·7 ± 0·5 (9)	12 ± 4·2 (9)*	4·4 ± 0·8 (9)**

Fluxes are in μM 100 g^{-1} h^{-1}, $\bar{X}$ ± S.E. (no. of animals). A negative flux indicates a net excretion of base. Animals were maintained normocapnic (bubbled with air) for 2–3 h before hypercapnia was produced. Fluxes under normocapnic conditions are calculated as means for the entire period, those for hypercapnia as means for hour 3–5 of hypercapnia. * $P < 0.05$; ** $P < 0.01$ when compared to normocapnia data.

RESULTS AND DISCUSSION

Hypercapnic acidosis

(1) *Normacapnic acid and ammonia efflux*

Under normocapnic conditions both *O. beta* and *S. acanthias* excrete small amounts of ammonia and little or no net amounts of H^+ (Table 1). The excretion rate of ammonia for *S. acanthias* is similar to the value of 6–7 μM 100 g^{-1} h^{-1} found for another elasmobranch *Raja erinacea* (Evans, 1977). In the present study, the reason for the low rate of ammonia excretion by the dogfish 'pups' may have been that they were still manufacturing protein for rapid growth. Since both *O. beta* and *S. acanthias* excrete more ammonia than H^+ under normocapnic conditions, it appears that protein catabolism provides the major pathway for the excretion of both nitrogen and protons under these conditions.

(2) *Effect of hypercapnia*

Production of hypercapnia by bubbling 5% CO_2 into the experimental medium produced a significant stimulation of net H^+ excretion from both species (Table 1). The onset of compensatory H^+ extrusion by both species was gradual (unpublished results) so the data presented in Table 1 were obtained 3–5 h after introduction of 5% CO_2. This delay in the onset of H^+ excretion stimulated by hypercapnia has also been demonstrated in two freshwater teleosts (Cameron & Wood, 1976) and in the elasmobranch *S. canicula* (Heisler *et al.* 1976) and presumably parallels the time course of blood pH decline and activation of acid excretory mechanisms. Hypercapnia inhibited ammonia efflux from *O. beta*, and stimulated it slightly from *S. acanthias*. Whether the inhibition of ammonia efflux from the toadfish indicates competition between H^+ and NH_4^+ for a common carrier remains to be seen, but it is clear that in both species compensation of hypercapnic acidosis is predominantly via H^+, rather than ammonia excretion. To ascertain the possible role of changes of electrical potential in the stimulation of H^+ efflux from hypercapnic fish the transepithelial electrical potential (TEP) was measured at least 2 h after the onset of hypercapnia in both species. Hypercapnic *O. beta* maintained a TEP of −8·5 ± 1·8 mV (four fish, blood relative to medium), only 1–2 mV more negative than that described for normocapnic individuals previously (Evans & Cooper, 1977; Evans, 1977). Hypercapnic *acanthias* maintained a TEP of −2·4 ± 0·5 mV (4), no different from that of

Table 2. *Branchial* v. *renal/rectal excretion of H^+ and ammonia by the toadfish and dogfish*

Species	Branchial efflux		Renal/rectal efflux	
	H^+	Ammonia	H^+	Ammonia
Opsanus beta (6)	110±8	11±1	−23±5	0·4±0·04
Squalus acanthias (6)	21±7	5±1	−3±1	1±0·2

Fluxes are in μM 100 g^{-1} h^{-1}, $\bar{X}\pm$S.E. (no. of animals). A negative flux indicates a net excretion of base. All data are on hypercapnic animals (5 % CO_2).

Table 3. *Effect of removal of external Na^+ on excretion of H^+ and ammonia by the toadfish and dogfish*

Species	Sea water		Na^+-free sea water		Sea water	
	H^+	Ammonia	H^+	Ammonia	H^+	Ammonia
Opsanus beta	173±40 (8)	15±1 (18)*	−28±9·2	6±1*	150±39	14±1*
Squalus acanthias (6)	21±7	10±2	−3±8	6±1	47±5	4±1

Fluxes are in μM 100 g^{-1} h^{-1}, $\bar{X}\pm$S.E. (no. of animals). A negative flux indicates a net excretion of base. All data on hypercapnic animals (5 % CO_2) except that from Evans (1977).

* Data from Evans (1977).

normocapnic individuals (−2·7±0·6 mV, seven fish). Thus, it is clear that changes in the TEP cannot account for any of the changes in H^+ (or ammonia) efflux in the present experiments. Since hypercapnia produced measurable H^+ effluxes the subsequent studies of the role of branchial *v.* renal efflux and the role of external Na^+ were made on fish which had been hypercapnic for at least 3 h.

(3) *Role of branchial* v. *renal/rectal efflux*

In both species, branchial pathways predominated over renal/rectal pathways for the extrusion of H^+ from hypercapnic individuals (Table 2); indeed, both species appeared to excrete a net amount of base into the sea water surrounding the renal/rectal openings. These data are the first demonstration of the importance of branchial H^+ extrusion by a hypercapnic marine teleost and support the recent findings (see introduction) that branchial pathways are the major site of H^+ extrusion by marine elasmobranchs and freshwater teleosts, and earlier studies (Smith, 1929; Fromm, 1963) which showed that ammonia was also excreted mostly via the gills in freshwater and marine fishes.

(4) *Effect of Na^+-free sea water*

Transfer of hypercapnic toadfish and dogfish to Na^+-free artificial sea water resulted in a cessation of H^+ excretion and, in many cases, a net excretion of base (Table 3). Thus, it is clear that H^+ extrusion by both species is completely dependent upon external Na^+. The observation that many individuals started to excrete base in Na^+-free sea water may indicate that Na^+/H^+ exchange normally masks a baseline base extrusion (linked to external Cl^-?); more data are needed to support this interesting proposition. The ammonia extrusion by hypercapnic dogfish was reduced by some

40% in Na^+-free sea water, supporting the previous finding that low Na^+ (2·5 mM), K^+-free artificial sea water reduces the ammonia efflux from *O. beta* by some 50% (Table 3, data from Evans, 1977). Thus it appears that elasmobranchs, like teleosts (Evans, 1977), excrete a considerable portion of their ammonia via pathways other than Na/NH_4 exchange. Indeed, the present data are the only direct demonstration of Na^+/NH_4^+ exchange in an elasmobranch; it has been previously found that ammonia efflux from *R. erinacea* was not sensitive to external Na^+ concentrations (Evans *et al.* 1979). One might propose that changes in the TEP could play a role in the Na^+-free effect in these experiments. However, hypercapnia did not affect significantly the TEP of either species in sea water (see above) and the TEP of normocapnic individuals of both species only becomes 3–7 mV more negative (blood relative to sea water) in Na^+-free (and K^+-free) sea water, far less than that TEP change necessary to account for the reduction in efflux of either H^+ or NH_4^+ shown in Table 3 (Evans & Cooper, 1976; Kormanik & Evans, 1978).

Return to Na^+-containing sea water restimulated both the H^+ and ammonia efflux in *O. beta*, but only restored the acid efflux in *S. acanthias*. The reasons for the apparent irreversibility of Na^+/NH_4^+ exchange in the latter species are unknown.

It is interesting to consider some reasons for the apparent discrepancy between my data and those of Perry *et al.* (1981). Their finding that production of hypercapnia in *S. gairdneri* did not alter the influx of either Na^+ or Cl^- supports the conclusion that alterations of Na^+/H^+, Na^+/NH_4^+ or Cl^-/HCO_3^- exchanges do not play a measurable role in hypercapnic-acidosis compensation in this species. Their data are especially surprising in light of their concurrent finding that addition of amiloride to the external bath inhibited Na^+ uptake and resulted in a pronounced acidosis, while addition of SITS (4-acetamido-4′-iso-thiocyanatostilbene-2,2′ disulphonic acid) inhibited Cl^- uptake and resulted in a significant decrease in plasma H^+ concentration. Thus, their work indicates that Na^+/H^+ or Na^+/NH_4^+ and Cl^-/HCO_3^- exchange systems are resident in the branchial epithelium *S. gairdneri*, can control blood pH, but do not seem to function in response to hypercapnic acidosis. However, they did find that addition of more Na^+ (3 mM) to the normally very dilute Vancouver tap water (40 μM) resulted in less acidosis and a more rapid blood pH recovery after hypercapnia. In addition, similar increases in external Na^+ concentration resulted in alkalosis under normocapnic conditions. It seems evident therefore that they did not see a change in the Na^+ influx under hypercapnic conditions because the transport system rate of turnover was limited by exceedingly low levels of one of its substrates, i.e. Na^+. It may be that under normal conditions the limitation of the Na^+ influx dictates that the response to acidosis is shifts in intracellular *v.* extra-cellular buffers (bicarbonate and non-bicarbonate) so that cytoplasmic pH is carefully controlled despite the lack of fine regulation of blood pH. Such a system has been described for the freshwater, airbreathing teleost *Synbranchus marmoratus* (Heisler, 1980). This fish is found in South American fresh water with extremely low Na^+ and Cl^- concentrations (3 μM) and responds to hypercapnia with an increased blood P_{CO_2}, but little increase in HCO_3 concentration. The result is a fall in blood pH of some 0·6 units for at least 5 days. The usual pattern in other species (Heisler, 1980) is an initial fall in blood pH which is compensated for by an increased blood HCO_3 concentration within

a few hours (interestingly, it apparently takes some 50 h for this compensation in *S. gairdneri*; Janssen & Randall, 1975). Perry *et al.* (1981) found that hypercapnic *S. gairdneri* in low-Na water also had an increased blood P_{CO_2}, but a relatively low blood pH (0·29 pH units below the control) secondary to only a slight rise in blood HCO_3 concentration. When the fish were placed into 3 mM-Na^+ solutions, the blood HCO_3 concentrations were significantly increased and the blood pH was controlled at levels only slightly (but significantly) below the control (0·06 pH units) after 24 h. It therefore appears that fish species living in solutions of low Na^+ and Cl^- content may have to rely on mechanisms other than branchial ionic exchanges to maintain blood pH relatively constant in the face of some environmental perturbations. This is obviously not a problem for marine species.

Mineral acidosis

(1) *Time course*

Injection of a small mineral acid load (50 μM-HCl 100 g^{-1}) into *O. beta* and *S. acanthias* resulted in a stimulation of the H^+ efflux as outlined in Table 4. The efflux of ammonia was not measured in these experiments because preliminary studies showed that animals which had been anaesthetized with MS-222 excreted inordinately high, and variable, amounts of ammonia, presumably as a result of the metabolism of this amine-containing anaesthetic. The toadfish responded to an injected acid load within 30 min and continued to excrete H^+ for 2 h after the injection. The dogfish did not begin to excrete acid until the second 30 min after the injection and continued to excrete acid until 4 or 5 h after the injection. This difference in time course presumably results from differences in buffering capacities, sensitivity to blood pH changes, and activation of extrusion mechanisms in the two species; however, there are no data to allow a determination of the limiting factors involved. In both cases the amount of H^+ finally excreted by the injected fish exceeded the injected acid load. Thus, it appears that, despite the use of an anaesthetic, the stress of mineral acid loading produced a mixed mineral/metabolic acidosis. In support of this, control injections of Ringer's solution (under MS-222 anaesthesia) produced measurable H^+ efflux in both species (unpublished results). Cameron (1980) found that the catfish, *Ictalurus punctatus*, responded to an injected acid load (100 μM-HCl 100 g^{-1}) with an extrarenal efflux of acid; however, after 2 h only some 20–30% of the injected load had been excreted. Since the pH of the blood of *I. punctatus* had returned to normal levels after 2 h, Cameron proposed that the other 70–80% of the injected acid load had been buffered in either intracellular or bone compartments. It is clear from the data in Table 4 that both *O. beta* and *S. acanthias* are able to excrete the injected acid load within 2–4 h after the injection.

(2) *Role of branchial* v. *renal/rectal efflux*

Table 5 shows clearly that all of the H^+ efflux subsequent to mineral acid loading is via branchial pathways. This dominance of branchial *v.* renal/rectal pathways corroborates most recent data on freshwater teleost species (see Introduction), but is the first description of the importance of branchial acid extrusion by a marine teleost made acidotic by an injection of a mineral acid load.

Table 4. *Effect of acid injection on excretion of H^+ by the toadfish and dogfish*

	(h post acid injection)					
	0–0·5	0·5–1	1–2	2–3	3–4	4–5
Opsanus beta (6)	54±11	45±27	15±7	0·8±9·7	0·4±13	—
Squalus acanthias (6)	6·6±8·9	44±5	18±7	15±2·9	4·2±1·7	6·0±3·9

Fluxes are in μM 100 g^{-1} h^{-1}, $\bar{X}\pm$S.E. (no. of animals). Acid load was 50 μM/100 g, injected I.P. under MS 222 anaesthesia (0·01 %).

Table 5. *Effect of acid injection on excretion of H^+ by the toadfish and dogfish: branchial* v. *renal/rectal excretion*

Species	Branchial efflux	Renal/rectal efflux
Opsanus beta (9)	146±17	−9·6±6·2
Squalus acanthias (6)	57±10	0·2±0·4

Fluxes are μM 100 g^{-1} h^{-1}, $\bar{X}\pm$S.E. (no. of animals). Fluxes from *O. beta* were measured during the first 30 min after the injection of the acid load. Branchial acid effluxes from *S. acanthias* were measured from 30–60 min after injection, renal/rectal effluxes were measured for the first 60 min after injection.

Table 6. *Effect of acid injection on excretion of H^+ by the toadfish and dogfish: effect of Na^+-free sea water*

			Sea water (h post acid injection)			
Species	Sea water	Na^+-free sea water	1–2	2–3	3–4	4–5
Opsanus beta (9)	146±17	−9·6±6·2	50±6·8	28±13	—	—
Squalus acanthias (6)	57±10	−10±8·2	—	23±4·0	56±11	11±3·0

Fluxes are in μM 100 g^{-1} h^{-1}, $\bar{X}\pm$S.E. (no. of animals). *O. beta* were placed into sea water for 30 min, Na^+-free sea water for 30 min and thence into sea water. *S. acanthias* were placed into sea water for 1 h (H^+ effluxes measured during the second 30 min), Na^+-free sea water for 1 h and thence into sea water.

(3) *Effect of Na^+-free sea water*

When HCl-injected fish were transferred into Na^+-free sea water the efflux of H^+ fell to below zero, so the fish were actually excreting net amounts of base (Table 6). When the fish were subsequently transferred back into Na^+-containing sea water the efflux of acid resumed and continued at a greater rate for a longer period than the control time-course in Table 4. This prolongation of the time of H^+ excretion subsequent to HCl injection was presumably because the experimental fish were unable to excrete acid (they actually excreted base) during the period in Na^+-free sea water. Thus, it is clear that both species require external Na^+ in order to excrete a mixed mineral/metabolic acid load across their branchial epithelium.

In summary, both the marine teleost *Opsanus beta* and the marine elasmobranch *Squalus acanthias* respond to either a hypercapnic or mineral/metabolic acidosis by excreting H^+ via the branchial epithelium. We have no data on HCl loaded fish but at least in hypercapnic acidosis, excretion of ammonia plays an insignificant role in this response. The excretion of H^+ by both species requires the presence of Na^+ in the external sea water – suggesting strongly that branchial Na^+/H^+ exchange plays a dominant role in this response to acidosis. This is the first direct evidence for branchial

Na^+/H^+ exchange in either a marine teleost or elasmobranch, and supports the conclusion that marine fishes, like their freshwater ancestors, excrete unwanted H^+ and ammonia across the branchial epithelium in exchange for external Na^+. The role of Na^+/H^+ and Na^+/NH_4^+ exchange in the Na^+ balance of these (and other) marine fish remains to be determined. One might propose that Na^+/H^+ and Na^+/NH_4^+ exchange in these marine forms is a remnant of freshwater ancestory (Evans, 1975); however, recent evidence (Evans, 1980*d*) indicates that H^+ (and possibly ammonia) efflux from the agnathan hagfish *Myxine glutinosa* is also dependent upon external Na^+. Since this line of agnathan evolution has apparently never entered fresh water (Hardisty, 1979) it appears that Na^+/H^+ and Na^+/NH_4^+ exchange may have evolved before the vertebrates entered fresh water, as a vehicle for H^+ and ammonia extrusion, rather than during entry into fresh water as a device for regulating blood Na^+ concentrations at levels above those in fresh water.

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