

**THE DYNAMIC ROLE OF THE FISH GILL  
IN EXTREMELY ALKALINE ENVIRONMENTS**

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**Introduction**

Although salmonids are not usually subjected to extremely alkaline environments (pH > 9.0), they may have to withstand upward pH surges due to the photosynthetic processes of aquatic plants and algae (Murray and Zeibell 1984). There are also many permanently saline-alkaline lakes throughout Western North America, which has complicated efforts to stock these waters with salmonids such as the rainbow trout (*Oncorhynchus mykiss*) and other game fishes (Coleman and Johnson 1988; Wagner et al. 1997). A major complication of high pH exposure is that it leads to severe, sometimes lethal, reductions in plasma electrolytes (Yesaki and Iwama 1992). Thus, a major objective of this study was determine how ion uptake and loss across the rainbow trout gill was influenced by high pH exposure, and to determine how changes in the fine architecture of the gill allowed fishes to correct high pH induced ion disturbances. Highly alkaline environments also lead to pronounced blood acid-base disturbances (see Wilkie and Wood 1996 for review), which are frequently characterized by a combined respiratory alkalosis (reduced arterial P<sub>CO2</sub>) and metabolic acidosis (reduced arterial HCO<sub>3</sub><sup>-</sup> concentration). Thus, a second objective was to test the hypothesis that high pH-induced acid-base disturbances contributed to ion imbalances by interfering with gill mediated ion transport processes.

## Methods and Materials

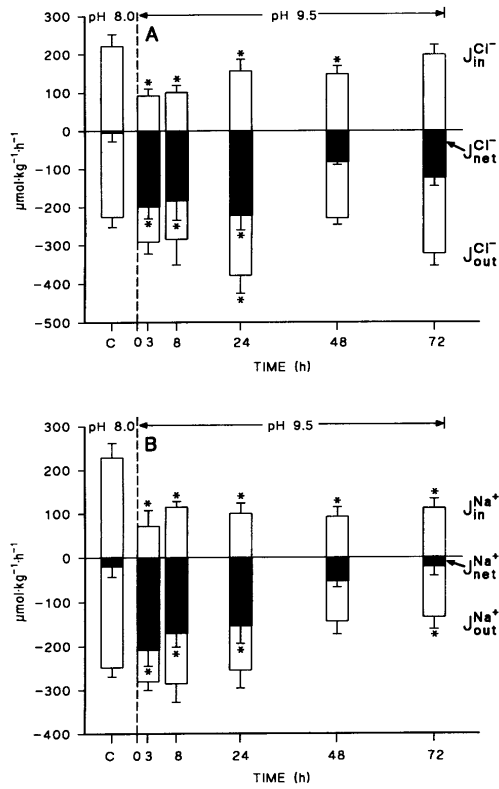
To determine how disturbances to internal ion balance took place in alkaline environments, rainbow trout (N = 6-8) were exposed to pH 9.5 for 72 h, and radiotracers ( $^{22}\text{Na}^+$ ,  $^{36}\text{Cl}^-$ ) were used to monitor ion movements across the gills. The blood acid-base status of rainbow trout was also used to determine how high pH-induced alterations to internal metabolic acid ( $\text{H}^+$ ) and base ( $\text{HCO}_3^-$ ) concentrations influenced  $\text{Na}^+$  and  $\text{Cl}^-$  transport. To achieve this goal, we used the 2-substrate approach described by Goss and Wood (1991) to determine how ion uptake is influenced by a fish's internal acid-base status. A detailed ultrastructural analysis of the gills, using scanning and transmission electron microscopy, was also conducted to relate changes in the gill's fine architecture to altered patterns of ion movement. Since the chloride cells and pavement cells of gills appear to be the respective sites involved with  $\text{Cl}^-$  and  $\text{Na}^+$  uptake (Perry 1997), we hypothesized that high pH induced reductions in ion uptake, and eventual recovery of ion balance, would be closely correlated to changes in the ultrastructure of these cells. A complete description of the methods used in these experiments can be found in Wilkie et al. (1999) and Laurent et al. (2000).

## Results and Discussion

Altered ion balance at high pH was due to initial 60-70 percent reductions in  $\text{Na}^+$  influx ( $J_{\text{in}}^{\text{Na}}$ ) and  $\text{Cl}^-$  influx ( $J_{\text{in}}^{\text{Cl}}$ ), which lead to net losses of both ions (Figure 1). The decrease in  $\text{Cl}^-$  influx at high pH was partially due to transient 50 percent decreases in maximal  $\text{Cl}^-$  uptake rate ( $J_{\text{max}}^{\text{Cl}}$ ), which reflected a decrease in the number of  $\text{Cl}^-$  transport sites (Table 1). The development of a metabolic acidosis (Table 2), characterized by lower plasma  $\text{HCO}_3^-$  concentration, likely contributed to reduced  $J_{\text{in}}^{\text{Cl}}$  by limiting  $\text{HCO}_3^-$  supply to  $\text{Cl}^-/\text{HCO}_3^-$  exchangers on the apical membrane of branchial chloride cells. This interpretation was supported by the 2-substrate analysis, which indicated that there was a *real* decrease in the number of  $\text{Cl}^-$  transport sites on the gill after 10 h at high pH (Figure 2A). A decrease in the fractional surface area of the most active, highly villous chloride cells (CCs) on the gill epithelium after 8 h (Figure 3), also contributed to the decrease in  $J_{\text{max}}^{\text{Cl}}$  at that time.

A complete recovery of  $J_{\text{max}}^{\text{Cl}}$  after 72 h at high pH (Table 1) likely accounted for the recovery of  $\text{Cl}^-$  influx (Figure 1A). This recovery was paralleled by a two-fold increase in the total fractional surface area of branchial CC's (Figure 3), suggesting that the gill

had actually increased its  $\text{Cl}^-$  transporting capacity.



**Figure 1.** Influx ( $J_{in}^{\text{Ion}}$ ; upward facing bars), outflux ( $J_{out}^{\text{Ion}}$ ; downward facing bars) and net movements ( $J_{net}^{\text{Ion}}$ ; shaded bars) of (A)  $\text{Cl}^-$  and (B)  $\text{Na}^+$  across rainbow trout gills during exposure to pH 9.5 for 72 h. Asterisks demonstrate statistical significance ( $P < 0.05$ ) from control measurements at pH 8.0.

**TABLE 1.** Changes in the kinetic parameters of unidirectional Cl<sup>-</sup> and Na<sup>+</sup> uptake during exposure of rainbow trout to pH 9.5 for 72 h. Means  $\pm$  1 SEM (N = 6 - 7). C = control (pH 8.0) conditions.

pH	Time (h)	Chloride	Sodium		
		Jmax ( $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ )	Km ( $\mu\text{mol}\cdot\text{L}^{-1}$ )	Jmax ( $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ )	Km ( $\mu\text{mol}\cdot\text{L}^{-1}$ )
<b>8.0</b>	<b>C</b>	358.2 $\pm$ 39.9	311.0 $\pm$ 84.8	481.5 $\pm$ 53.1	88.5 $\pm$ 13.3
<b>9.5</b>	<b>10</b>	172.6 $\pm$ 30.2*	405.6 $\pm$ 116.3	155.5 $\pm$ 14.9*	66.2 $\pm$ 19.8
	<b>24</b>	285.8 $\pm$ 31.9	391.9 $\pm$ 51.7	259.2 $\pm$ 33.2*	188.3 $\pm$ 49.0
	<b>72</b>	297.3 $\pm$ 37.8	335.6 $\pm$ 58.1	322.6 $\pm$ 13.5*	375.2 $\pm$ 85.4*

Asterisks (\*) indicate significant differences from control (pH 8.0) values ( $p < 0.05$ ).

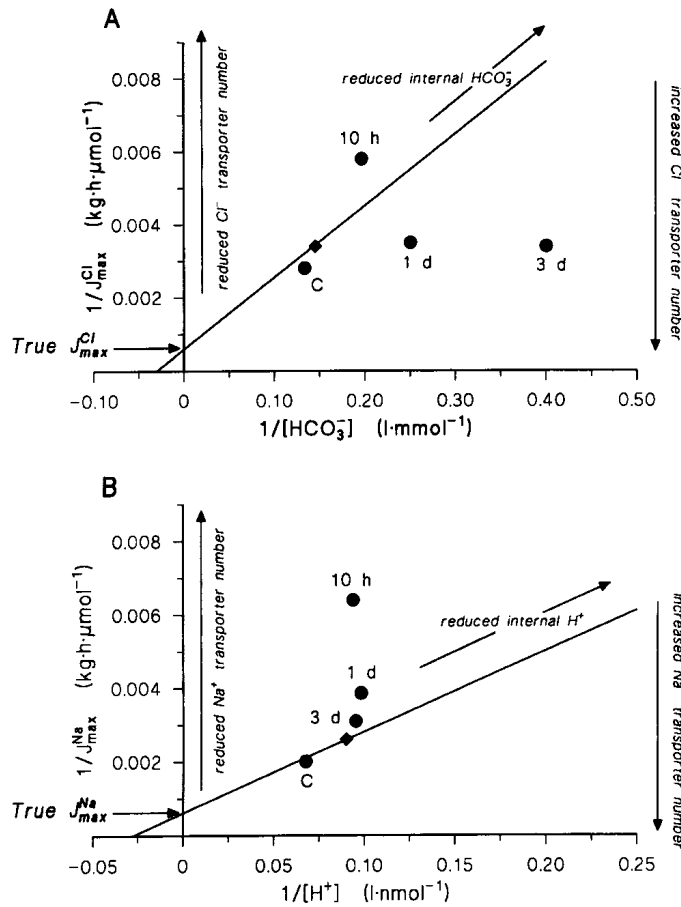
Data taken from Wilkie et al. (1999).

**TABLE 2.** Arterial Blood Acid-Base Status of Rainbow Trout at High pH (pH 9.5).

WATER	pH	Blood Acid-Base Status		
		[H <sup>+</sup> ] (nmolL <sup>-1</sup> )	P <sub>CO2</sub> (Torr)	[HCO <sub>3</sub> <sup>-</sup> ] (mmolL <sup>-1</sup> )
<b>pH 8.0</b>	7.83 $\pm$ 0.01	14.8 $\pm$ 0.3	3.07 $\pm$ 0.09	7.50 $\pm$ 0.30
<b>pH 9.5</b>				
<b>8 h</b>	7.97 $\pm$ 0.01*	10.7 $\pm$ 0.3*	1.43 $\pm$ 0.08*	5.04 $\pm$ 0.34*
<b>24 h</b>	7.99 $\pm$ 0.01*	10.2 $\pm$ 0.2*	1.12 $\pm$ 0.14*	4.08 $\pm$ 0.40*
<b>72 h</b>	7.98 $\pm$ 0.03*	10.5 $\pm$ 0.7*	0.68 $\pm$ 0.07*	2.58 $\pm$ 0.37*

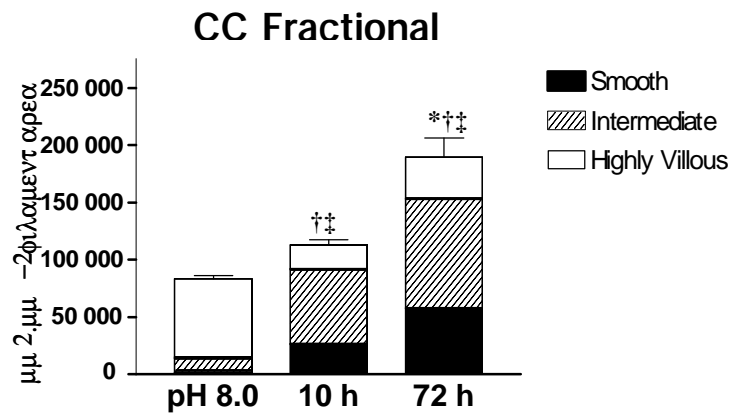
Asterisks (\*) indicate significant differences from pH 8.0 values ( $p < 0.05$ ).

Data taken from Wilkie and Wood (1991).



**Figure 2.** Two-substrate kinetic analysis of ion uptake across rainbow trout gills at pH 8.0 or pH 9.5, based on methods used by Goss and Wood (1991). The analyses indicate the relative roles that internal substrate (counterion) availability and transporter number play in altering the respective apparent  $J_{max}^{Cl}$  and apparent  $J_{max}^{Na}$  for (A)  $Cl^-$  influx and (B)  $Na^+$  influx by rainbow trout. Respective arterial plasma  $HCO_3^-$  ( $[HCO_3^-]_a$ ) and  $H^+$

( $[H^+]_a$ ) concentrations were taken from trout exposed to identical high pH conditions (Table 2; Wilkie and Wood 1991). The regression lines represent data collected by Goss and Wood (1991), following imposition of different acid-base disturbances on the rainbow trout. The control data points of Goss and Wood (1991; diamonds) are indicated. Circles are the inverse of the apparent  $J_{max}^{Cl}$  and  $J_{max}^{Na}$  estimates (presented Table 1) plotted against corresponding inverse measurements of  $[HCO_3^-]_a$  and  $[H^+]_a$ . Upward or downward deviations (vertical arrows) away from the regression line represent *true* changes in transporter number, while changes in internal substrate availability are reflected by movements along or in parallel to the regression line (diagonal arrows).



### Time at pH 9.5

**Figure 3.** Alterations in the fractional chloride cell (CC) apical surface area on the filamental epithelium of rainbow trout gills at pH 8.0 or exposure to pH 9.5. Chloride cells were placed into one of three chloride cell classes according to the density of microvilli on their apical surface. Accordingly, the proportion of chloride cells with a smooth (no villi; solid region), intermediately villous (hatched region) or highly villous (open region) apical surface are illustrated. Values of fractional chloride cell apical surface area are based on the CC numerical density and individual CC apical surface area (not shown). Asterisks (\*) indicate statistically significant differences from pH 8.0 values for entire chloride cell

populations, while daggers (H), double daggers (I), and stars (★) indicate statistically significant differences from pH 8.0 values for smooth, intermediate and highly villous CC's, respectively

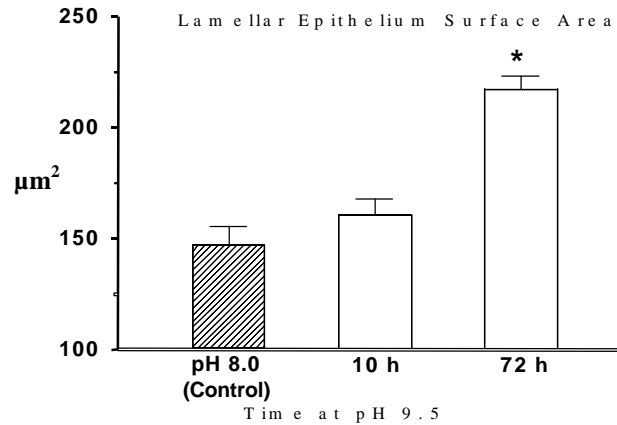
This observation was substantiated by the two-substrate analysis, which revealed an actual increase in  $\text{Cl}^-$  uptake capacity after 1 d and 3d (Figure 2A). Since  $\text{Cl}^-$  influx takes place via  $\text{Cl}^-/\text{HCO}_3^-$  exchange, greater branchial CC fractional surface area would also promote additional metabolic base excretion and therefore help to stabilize blood pH. Similar morphological observations in the Lahontan Cutthroat Trout of highly alkaline (pH 9.4) Pyramid Lake, Nevada (Wilkie et al. 1994), suggest that greater CC fractional surface area is an essential response that allows fishes to tolerate highly alkaline waters.

The 2-substrate analysis revealed that the respiratory alkalosis present in rainbow trout during high pH exposure (Table 2) limited  $\text{H}^+$  supply to the  $\text{Na}^+$  channel/proton transport system during the first 10 h of high pH exposure (Figure 2B). Thus, the respiratory alkalosis likely contributed to the reductions in  $J_{\text{in}}^{\text{Na}}$  commonly observed at high pH (Figure 1B). The restoration of net  $\text{Na}^+$  balance was partially explained by a progressive decrease in the  $\text{Na}^+$  permeability of the gill, as illustrated by significantly reduced  $\text{Na}^+$  outflux ( $J_{\text{out}}^{\text{Na}}$ ) after 72 h at high pH (Figure 1B). Although internal  $\text{H}^+$  supply continued to be limiting, the two-substrate analysis also revealed that there was a recovery of the *true*  $\text{Na}^+$  transport capacity of the gill by 3 d (Figure 2B). The interpretation that there was a *true* recovery of branchial  $\text{Na}^+$  transporting capacity was also supported by morphological examinations of the lamellar epithelium, which revealed that there was a greater density of microvilli on the pavement cells (Figure 4). As pavement cell membranes are where the putative  $\text{H}^+$ -pumps and possibly  $\text{Na}^+$  channels are located (Perry 1997), the 143 percent increase in the surface area of these cells likely contributed to the partial restoration of  $\text{Na}^+$  transport capacity that was observed after 3 d at high pH.

## Conclusions

Fish exposed to alkaline water experience acid-base disturbances that can directly impair ion uptake capability by limiting the supply of internal counterions such as  $\text{H}^+$  and/or  $\text{HCO}_3^-$ . Compensatory changes in gill architecture are essential, however, for allowing fishes such as the rainbow trout and the Lahontan cutthroat

*(Oncorhynchus clarki henshawi)* trout to correct and maintain internal ion and acid-base balance in extremely alkaline environments.



**Figure 4.** Changes in the gill lamellar pavement cell (PVC) surface area in rainbow trout subjected to pH 8.0 (hatched bars) or pH 9.5 (open bars) for 10 h or 72 h. PVC surface area estimates take into account the topographical relief due to micro-villi and ridges, and were determined using an arbitrary reference surface area of 100  $\mu\text{m}^2$  (10  $\mu\text{m}$  X 10  $\mu\text{m}$ ) which would represent a completely smooth surface. Asterisks (\*) indicate statistically significant differences from pH 8.0 values.

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