The Impact of pH on Ionic Concentrations in Zebrafish Gills

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INTRODUCTION

As the global temperatures and atmospheric carbon dioxide levels continue to rise, aquatic systems are expected to experience considerable drops in pH, or an increase in dissolved hydrogen ion concentration.[1] These decreases in pH have been shown to cause potentially lethal physiological changes in fish, drastically reducing the functionality of their gills as the equilibrium concentrations of ions in their gill cells change with changes in hydrogen ion concentration.[2]

Zebrafish, *Danio rerio*, are one of the best studied freshwater fish. Zebrafish gills are comprised primarily of three types of ionocytes: H⁺-ATPase-rich cells (HR cells), Na⁺–K⁺-ATPase-rich cells (NaR cells), and Na⁺–Cl⁻ cotransporter cells (NCC cells), named for the ion transporters and enzymes they express.[3] As we are concerned with the impact of changing pH on ion transfer in these gill cells, we are primarily concerned with the HR cells, which are the only cells that pump hydrogen ions. Thus, we will generate a model of ion transport in gill HR cells and determine the constants for each relevant ion transporter using Michaelis Menten kinetics.

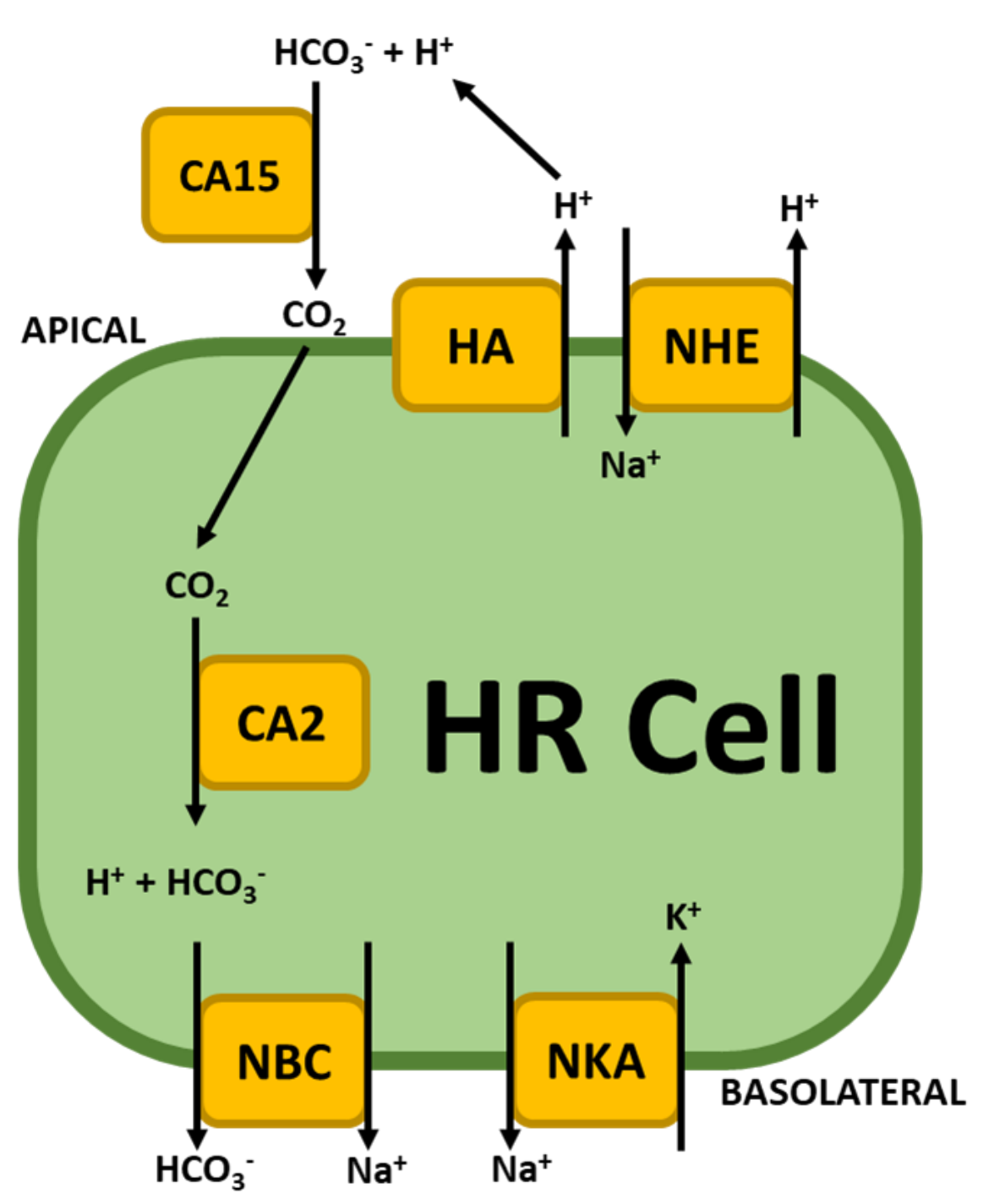

Figure 1: HR Cell Diagram[3]. Diagram of the relative positions of ion transporters in the HR and the directionality and chemical symbols of the ions they move.

- **HA:** An apically localized hydrogen ATPase which utilizes ATP hydrolysis to actively pump hydrogen ions from the cytoplasm to the extracellular environment.
- **NHE:** A sodium-hydrogen antiporter which transports sodium ions into the cell and hydrogen out of the cell in a one-to-one ratio.
- **NBC:** A sodium-bicarbonate cotransporter; different isoforms of this channel transport different ratios of sodium to bicarbonate (either 1:1, 1:2, or 1:3 ions) and thus we select the most common and median ratio of 1:2 for this model.[17]
- **NKA:** A sodium-potassium ATPase which utilizes ATP hydrolysis to pump two ions of sodium out of the cell and three ions of potassium into the cell, against both of their concentration gradients.[5]
- **CA2 and CA15: C**arbonic anhydrases which convert bicarbonate and hydrogen ions into carbon dioxide, transport carbon dioxide into the cell, and then convert the carbon dioxide back into bicarbonate and hydrogen ions, thus implicating the carbonic anhydrases in both bicarbonate and hydrogen ion transfer.

BOX MODEL

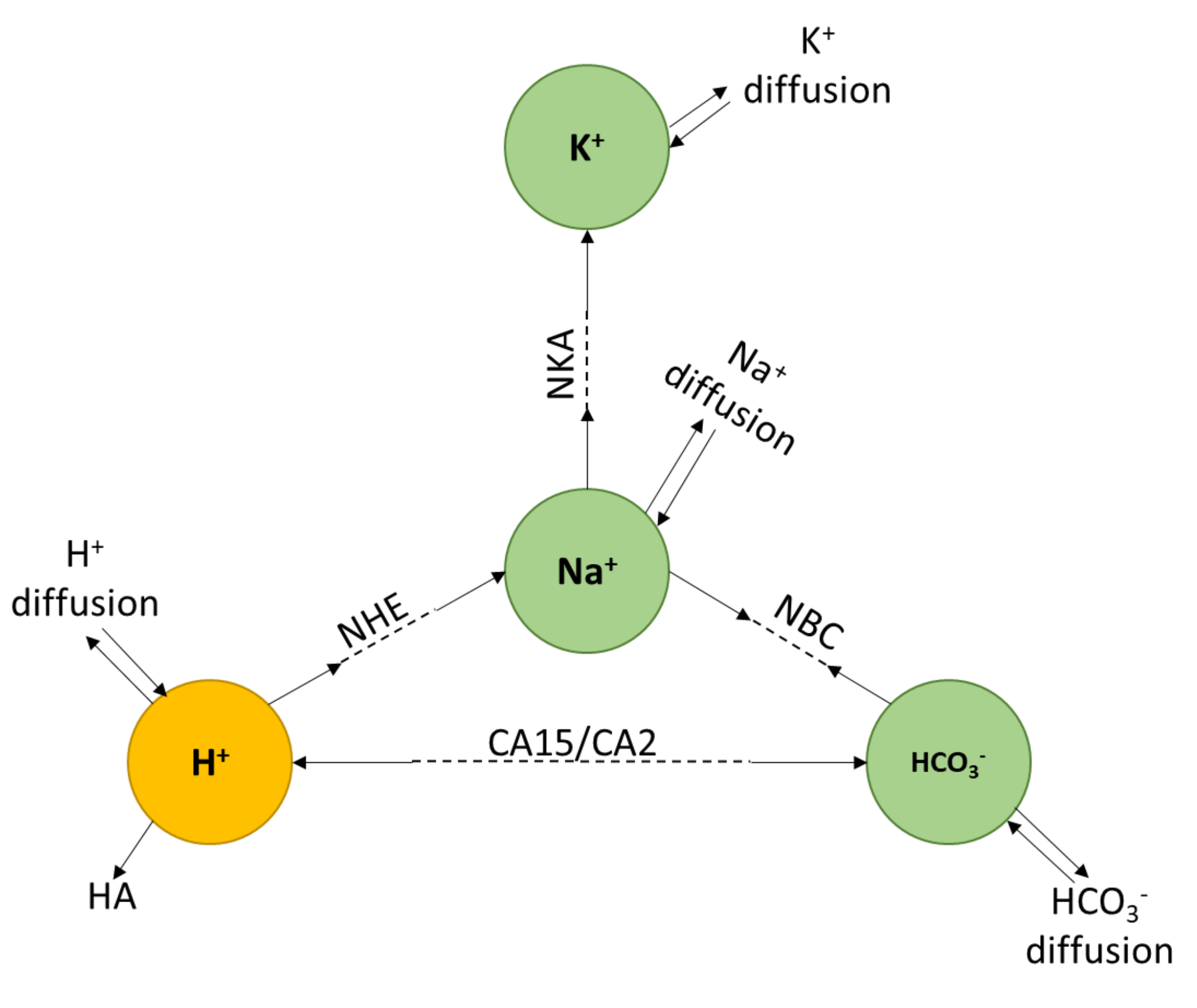

Figure 2: Ion Transport Box Model. This is a box model of the various transporters that move ions in and out of the HR gill ionocyte. Arrows pointing toward a compartment signify movement of that ion into the cell and arrows pointing away from the compartments signal ions moving out of the gill cell into either the surrounding water or being carried away by the bloodstream. This diagram shows the effect of different transport pumps on the concentration of Na⁺ within the cell.

EQUATIONS

Functional Forms

Drawing inspiration from the paper by Wallace and Tanenbaum, we define the functional forms of the transporters that move two ionic species as the product of Michaelis Menten equations:

$$V = \frac{V_{max}[S]}{K_{half} + [S]}$$

$$V = \frac{V_{max}[S_1][S_2]}{(K_{half_1}+[S_1])(K_{half_2}+[S_2])}$$

These are equations for the transporters using this functional form:

$$(NKA) = \frac{V_{NKA}[Na^+][K_E^+]}{(K_{Na_1}+[Na^+])(K_K+[K_E^+])}$$

$$(NHE) = \frac{V_{NHE}[Na_E^+][H^+]}{(K_{Na_2}+[Na_E^+])(K_H+[H^+])}$$

$$(NBC) = \frac{V_{NBC}[Na^+][HCO_3^-]}{(K_{Na_3}+[Na^+])(K_{HCO_3}+[HCO_3^-])}$$

$$(CA15\ and\ CA2) = \frac{V_{CA}\left[HCO_3^-{}_E\right][H_E^+]}{(K_{CA}+\left[HCO_3^-{}_E\right])(K_{CA}+[H_E^+])}$$

$$(HA) = \frac{V_{HA}[H^+]}{K_{HA} + [H^+]}$$

System of Equations

$$\frac{d[Na^+]}{dt} = -NBC + NHE - NKA + d_{Na} * ([Na_E^+] - [Na^+]$$

= - (Sodium ions removed from the cell by NBC) + (Sodium ions entering the cell through NHE) - (Sodium ions removed from the cell by NKA) + (Diffusion of sodium ions)

$$\frac{d[K^+]}{dt} = NKA + d_K * ([K_E^+] - [K^+]$$

= (Potassium ions entering the cell through NKA) + (Diffusion of potassium ions)

$$\frac{d[HCO_3^-]}{dt} = -NBC + CA + d_{HCO_3} * (\left[HCO_3^-{}_E\right] - [HCO_3^-]$$

= - (Bicarbonate ions removed from the cell by NBC) + (Bicarbonate ions added to the cell via CA15 and CA2 activity) + (Diffusion of bicarbonate ions)

$$\frac{d[H^+]}{dt} = -NHE + CA + d_H * ([H_E^+] - [H^+]$$

= - (Hydrogen ions removed from the cell by NHE) + (Hydrogen ions added to the cell via CA15 and CA2 activity) - (Diffusion of hydrogen ions)

RESULTS

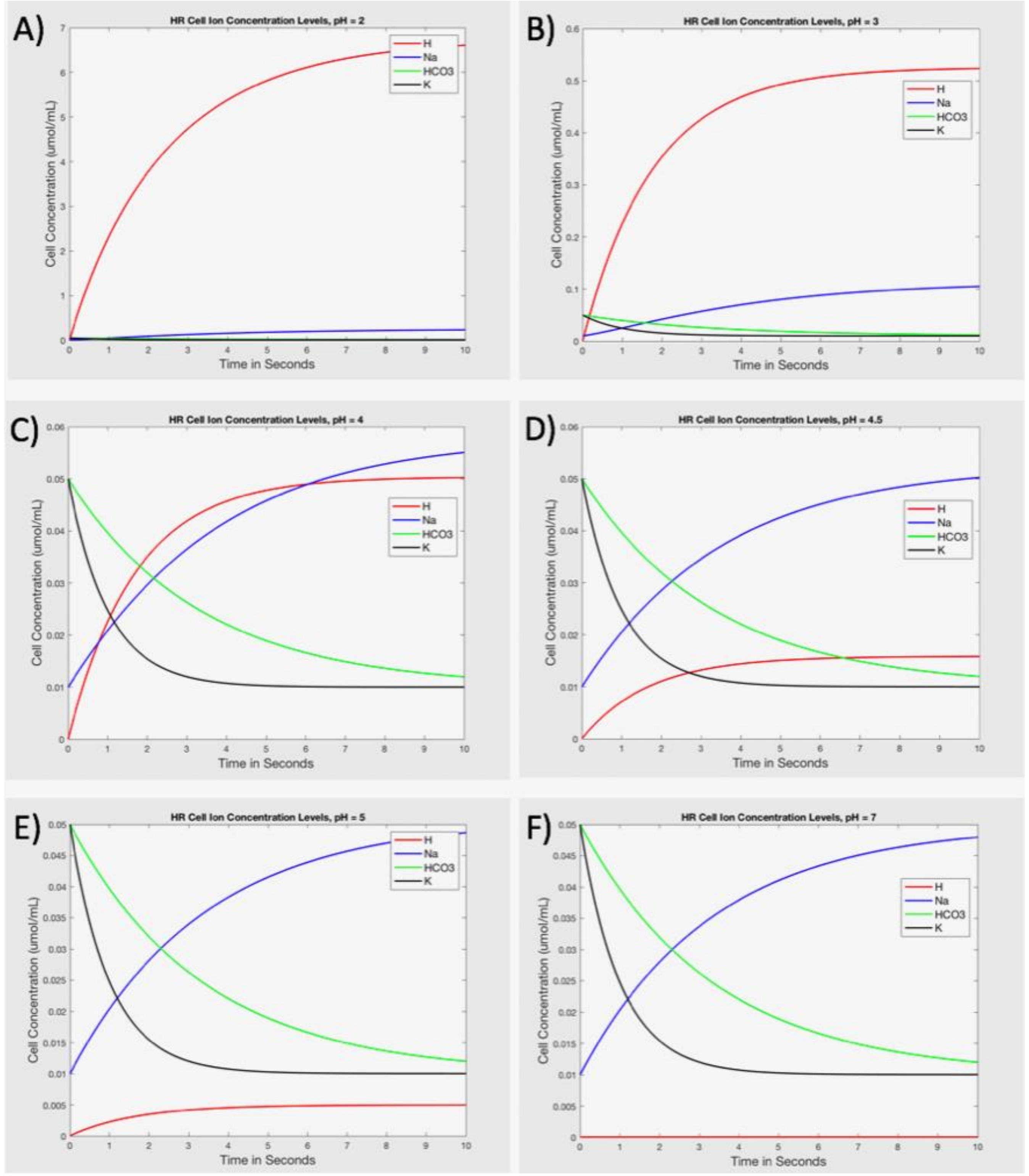

Figure 3: HR Cell Ion Concentration Levels with Varying pH. The above subplot is a compilation of results from modeling Na⁺, K⁺, HCO$_3^-$, and H⁺ ion concentrations within Zebrafish HR cells based on different external pH values. As pH increased from 2 to 7 all four ion concentrations experienced a variety of changes. At low pH levels, suggesting acidic external environments, the concentration of hydrogen ions within the cell was comparatively high, while the concentrations of the other ions remained much smaller. As pH increased and the external environment of our model became less acidic, the model experienced a smaller relative surge in hydrogen concentration. "C" pictures a pH of 4 in which the concentrations of different ions are relatively similar and maintained within the same order of 10 in micro-moles per milliliter. In "F", hydrogen ion concentration decreased to an almost trivial amount while the concentrations of the other ions remained at the same values as in "A"-"E". Our results most greatly reflect varying hydrogen ion concentration levels with respect to changes in pH. The large increase in hydrogen concentration between pH of 4 and 4.5 suggests that the viability of the Zebrafish becomes critically susceptible at an intermediate pH.

CONCLUSIONS

- Our model assumes that the processes of sodium and ion transport across cells through various pumps is similarly maintained across different species of animals. It is important to note that this demonstrated effect of extracellular acidity is not necessarily applicable to all types of aquatic species. Additionally, we based our models of the concentration of ions in cells on the Michaelis-Menten equations for lack of previous literature on the exact transport of these ions.
- Our model has shown that with drops in pH from 7 (pH neutral water) to 2 (lethal levels of acidity), intracellular sodium ion concentrations increased by a factor of five while intracellular bicarbonate and potassium concentrations remained the same.
- Intracellular hydrogen ions increased dramatically in response to the decline in pH; this significant cell pH decline will likely reach lethal levels, thereby killing the cells. Even if lethal pH levels are not attained, zebrafish gill cells will likely flood with water to offset the unnaturally elevated sodium concentrations, thereby inducing lysis in some cells and, in sufficiently acidic conditions, the fish will eventually die.
- Thus, there are two potential mechanisms by which pH drops can induce negative physiological changes to fish: by increasing internal hydrogen ions, or sodium ions to lethal levels.

FUTURE DIRECTIONS

- To further refine this model, we will first define parameters for the relative abundances of each of the ion channels on the HR ionocytes to more accurately reflect the ion transport rates.
- We will further validate the parameters for the Michaelis Menten functional forms by searching the literature for ion transport analyses in aquatic organism and finding other papers that confirm the values we currently are using. Alternatively, if we find different values for maximum pumping velocity and the Michaelis constant, we will implement those in our analysis.
- We will explicitly conduct an equilibrium analysis for the various external pH values we have considered and we will more thoroughly evaluate the implications of these equilibria.
- We will evaluate the impact of pH on the external dissolved ion concentrations using datasets from natural freshwater bodies to more accurately represent the external ion concentrations under the various pH conditions.

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