# CALCIUM BALANCE IN DEVELOPING FISH

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

## Introduction

Freshwater teleosts maintain their plasma $Ca^{2+}$ levels within a narrow limit (2-4 mM) in a wide range of external $Ca^{2+}$ concentrations. Gill mitochondria-rich (MR) cells (or chloride cells) are the major sites for $Ca^{2+}$ uptake from freshwater environments, and active $Ca^{2+}$ uptake in response to low ambient $Ca^{2+}$ is therefore a prerequisite for $Ca^{2+}$ homeostasis. Developing fish embryos or larvae, whose organ systems are under or poorly developed, have to face freshwater environments with a wide range of external $Ca^{2+}$ levels as their adults do. The present work was aimed to study the mechanisms how fish embryos and larvae maintain the body $Ca^{2+}$ balance in freshwater environments.

## Materials and methods

Fertilized eggs or hatched larvae of tilapia (*Oreochromis mossambicus*) goldfish (*Carassius auratus*), zebrafish (*Danio rerio*) and ayu (*Plecoglossus altivelis*) were incubated in low-$Ca^{2+}$ (0.02 mM), mid-$Ca^{2+}$ (0.2 mM), and high-$Ca^{2+}$ (2 mM) for different periods of time depending on experimental designs. For long-term acclimations, fertilized eggs were incubated with different levels of $Ca^{2+}$, and for the acute exposure experiments larvae with different ages were transferred from mid-$Ca^{2+}$ to low-$Ca^{2+}$ or low-$Ca^{2+}$ freshwater. Animals were sampled for the measurements of $Ca^{2+}$ fluxes and $Ca^{2+}$ contents by radio $^{45}Ca^{2+}$ tracing and atomic spectrometry, respectively.

**Ontogeny of $Ca^{2+}$ balance**

Tissue $Ca^{2+}$ content in tilapia remained at a constant level during the embryonic stages and showed dramatic changes after hatching; tissue $Ca^{2+}$ increased about 30-35 fold during 10 d after hatching. Both $Ca^{2+}$ influx and efflux in whole body of tilapia significantly increased following larval development. However, the extent of increase was much higher in influx (4.3 fold) than in efflux (2.3 fold), resulting in a 4.6-fold increase of $Ca^{2+}$ net uptake rate within 1 week after hatching. A continuous increase in body calcium content with larval development may be critical for the normal growth and development of larvae.

**Acclimation to low-$Ca^{2+}$ environment**

Upon acclimation to low-$Ca^{2+}$ environment, tilapia larvae not only increased $Ca^{2+}$ influx but also decreased $Ca^{2+}$ efflux, and thus resulted in an enhanced $Ca^{2+}$ net uptake. Developing larvae not only increased $Ca^{2+}$ influx but also decreased $Ca^{2+}$ efflux when they were acclimated to low-$Ca^{2+}$ environments. After acclimation for 8 d, influx and efflux of low-$Ca^{2+}$ group were about 106% and 43%, respectively, to those of high-$Ca^{2+}$ group. The enhanced calcium uptake capacity was characterized by 40-50 % decrease in $K_m$ and 7-25 % increase in $J_{max}$. These results suggest that tilapia larvae are able to modulate their $Ca^{2+}$ uptake mechanism to maintain normal level of body $Ca^{2+}$ content and growth under the environment with different levels of $Ca^{2+}$. Moreover, the sensitivity and response to low-$Ca^{2+}$ environments are age-dependent. Upon acute exposure to low $Ca^{2+}$, newly hatched (H0) larvae increased both $Ca^{2+}$ influx (from 24% to 67% of high-$Ca^{2+}$) and net uptake (from 5% to 69%) within 64 h, while 3-d-old (H3) larvae managed to reach the levels of the control within 38 h. Declining $Ca^{2+}$ efflux in H3 larvae occurred 14 h after exposure, much faster than did those in H0 larvae (38 h).

Table 1. Comparison of the kinetic parameters for $Ca^{2+}$ influx in 3-d-old tilapia larvae reared in high- and low-calcium artificial freshwater

| | Medium | $J_{max}$ (pmol $mg^{-1}$ $h^{-1}$) | $K_m$ (mmol $l^{-1}$) |
|---|---|---|---|
| Exp 1 | High $CaCl_2$ | 201.9±5.1 | 0.0285±0.0024 |
| | Low $CaCl_2$ | 251.8±7.8 | 0.0141±0.0014 |
| Exp 2 | High $CaSO_4$ | 228.5±6.0 | 0.0108±0.0008 |
| | Low $CaSO_4$ | 244.1±5.4 | 0.0067±0.0001 |

Fertilized eggs were incubated in high- or low-$Ca^{2+}$ media until 3 days after hatching. Mean±SD (N=4) was indicated. Significant difference was found between low and high calcium groups in each experiment (*t* test, $p<0.05$)

**Comparisons among different species**

Comparisons were made in the $Ca^{2+}$ influx regulation among goldfish, zebrafish and ayu when acclimated to low-$Ca^{2+}$ environment, goldfish larvae show the best compensation in $Ca^{2+}$ balance, a higher $Ca^{2+}$ influx than that in control (high-$Ca^{2+}$), while ayu was the worst in compensation, about 74% decrease in $Ca^{2+}$ influx. The species differences in the capacities for $Ca^{2+}$ balance may be associated with the differences in the development patterns and the inhabiting environments of fishes.

**Comparison with adults**

Compared with adults that merely maintain an almost constant level of internal $Ca^{2+}$ content, developing larvae must have a continuous increase in body calcium content with development. The net $Ca^{2+}$ uptake in developing larvae is about 2~7 fold higher than that in adults. The net uptake makes only a very minor, less than 0.01% of total content, contribution to increase the $Ca^{2+}$ content of adult, which already have a considerable pool of $Ca^{2+}$. However, it provides a significant contribution (13~19%) to larval $Ca^{2+}$ pool, which is still being developed. Moreover, developing larvae appear to regulate their $Ca^{2+}$ balance more rapidly and efficiently upon environmental challenge, implying that developing larvae may allow much less fluctuation in the internal hydro-mineral conditions, which may be critical for the development and survival of larvae.

Table 2. Comparison of $Ca^{2+}$ balance between developing larva and adult of tilapia

| | Adult | 1-d-old larva | 5-d-old larva |
|---|---|---|---|
| Body weight (mg) | 20,000 | 6.7 | 9.5 |
| Total content (nmole $mg^{-1}$) | $2\times10^2$ | 2.223 | 11.450 |
| Influx (nmole $mg^{-1}h^{-1}$) | 0.0279 | 0.0486 | 0.1572 |
| Efflux (nmole $mg^{-1}h^{-1}$) | 0.0081 | 0.0069 | 0.0133 |
| Net flux (nmole $mg^{-1}h^{-1}$) | 0.0198 | 0.0417 | 0.1439 |
| (Net flux÷total content) ×100% | 0.0099 | 18.76 | 12.57 |

Data of adult was from Flik et al. (1986).

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