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The Effect of Calcium Concentration of Water on Chloride Cell Density in Gill of Brown Trout (*Salmo trutta* L.) Larvae

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Abstract: The effects of the calcium concentration on chloride cell (mitochondria-rich cell) density in the gills of brown trout (*Salmo trutta* L.) larvae were studied. For this purpose, animals were divided into 3 groups 2 weeks after hatching and each groups was exposed to different calcium concentrations of water for two weeks.
The results showed that the number of chloride cells in gill arch epithelia of larval fish increased with decreased calcium concentration.This increase was statistically significant in the gills of larvae kept in zero-Ca^{2+} freshwater (ZCWF) ($P< 0.001$) and of those kept in low-Ca^{2+} freshwater (LCFW) ($P< 0.05$) in comparision with the gills of larvae kept in normal freshwater (NFW).

Key Words: Brown trout, *Salmo trutta*, chloride cells, calcium concentration.

Kahverengi Alabalık (*Salmo trutta* L) Larvasının Solungaçlarındaki Klor Hücreleri Yoğunluğuna Sudaki Kalsiyum Konsantrasyonunun Etkisi

Özet: Bu çalışma, sudaki kalsiyum konsantrasyonunun kahverengi alabalık (*Salmo trutta* L) larvasının solungaçlarındaki klor hücrelerinin (mitokondrice zengin hücre) yoğunluğuna etkisinin belirlenmesi için yapılmıştır. Bu amaçla, larvalar yumurtadan çıktıktan iki hafta sonra üç gruba ayrıldı ve her bir grup iki hafta süre ile farklı kalsiyum konsantrasyonuna maruz bırakıldı.
Solungaç kemeri epitelindeki klor hücreleri sayısının sudaki kalsiyum konsantrasyonunun azalmasına paralel olarak arttığı sonucu elde edilmiştir. Klor hücrelerindeki bu artış normal tatlısuda tutulan larvalarla karşılaştırıldığında düşük kalsiyumlu suda tutulan larvaların solungaçlarında ($P<0.001$) ve düşük kalsiyumlu tatlısuda tutulan larvaların solungaçlarında ($P<0.05$) istatistiksel olarak önemli bulunmuştur.

Anahtar Sözcükler: Kahverengi alabalık, *Salmo trutta*, klor hücreleri, kalsiyum konsantrasyonu.

Introduction

Calcium is a very important element for animals because it necessary for a variety of functions such as bone and scale growth, muscle contraction, transmission of nerve impulses, hormone secretion and intracellular signaling. In addition, calcium plays a protective role for freshwater fish against osmotic and ionic grains and losses, and against most environmental toxicants. In freshwater animals, calcium concentrations in blood is 2-4 mmol l^{-1} in a wide range of external calcium concentrations (> 0.01 mmol l^{-1}), even when food is withheld for long periods (1, 2).

Altohough calcium enters the fish trough the gills, intestines and skin, the gills are a particularly important calcium uptake site. Chloride cells, whic have been implicated in active calcium uptake (3, 4, 5), are located in the gill epithelium and the body integument.

Chloride cells were originally described by Keys and Willmer, 1932 (6) in the gill of seawater-adapted teleosts. They called them "chloride secreting cells". These cells have been shown to be the site of $C1^-$ -secretion in the gill and skin of seawater-adapted teleosts (7). However, these cells are often termed chloride cells, even when found in freshwater-adapted teleosts, where a $C1^-$-secretory function is unlikely and a $C1^-$-uptake function is uncertain (8, 9). Chloride cells are rich in mitochondria.

There is considerable indirect evidence to implicate the chloride cells as the site of branchial calcium uptake. Morphological evidence has provided indirect evidence that chloride cells are involded in calcium uptake in the gill of fresh water teleosts (4, 10). Perry and Wood, 1985 (3) found excellent correlation between lamellar chloride cell number and calcium uptake in rainbow trout adapted to low external calcium concentrations. They also showed that calcium uptake in rainbow trout in fresh water occurred at equal rates through the gills and general body surface. In contrast, Marshall et al., 1992 (11) have reported that the contribution of extrabranchial calcium transport via the skin of juveniles is small, and this is reflected in differences in the absolute numbers of chloride cells in gills and skin.

Several recent studies have shown that there are close inverse relationships between calcium concentration of water and chloride cell density in teleosts (3, 11, 12, 13, 14, 15). All this work has been on juvenile or adult teleosts. There is a lack of studies on larval teleosts in this particular subject. The present study was therefore underaken to determine the effects of calcium concentration on chloride cell density in the gills of larval brown trout.

Materials and Methods

Brown trout (*Salmo trutta*) eyed ova were obtained from a commercial hatchery (Trent Fish Culture Ltd., Mercaston, Derbyshire). They were hatched in a tank at the University of Nottingham, Department of Life science, in aerated and dechlorinated flowing freshwater.

Two weeks after hatching, animals were divided into three groups. Each group was transferred to a 10-liter tank supplied wit zero-Ca freshwater (ZCFW: deionised water supplemented with nominal 0.7 mM Na^+ and zero Ca^{2+}), low-Ca freshwater (LCFW: deionised water supplemented with nominal 0.7 mM Na^+ and 0.01 mM Ca^{2+}) and normal freshwater (NFW: 0.7-1.0 mM Na^+ and 0.9-1.4 Ca^{2+}). The experiment tanks were kept in a constant teperature room (8.0 ± 1°C) with a lighting regime of a 12 h photoperiod. Animals were exposed to these experimental conditions for two weeks prior to sampling for examination.

Water samples for analysis of calcium and sodium concentrations were taken from all tanks once a day and they were preserved by addition of HNO_3 (BDH'AnalaR), ca. 5.0 mol 1^{-1}, to yield a final concentration of 0.05 mol 1^{-1}. Water samples and standard solutions for calibration of analytical equipment were stored in darkness, at 2-8°C, in polyethylene containers. Ca^{2+} and Na^+

concentrations were determined by atomic absorption spectrophotometry (Pye Unicam, model SP9, combined with SP9 computer).

After two weeks, five animals from each experimental medium were sampled. They were lightly anahesthetised and their gills were dissected. Dissected tissues were immediately rinsed with trout Ringer solution (130 mM NaCl, 2.55 mM KCl, 1.56 mM $CaCl_2.2H_2O$, 0.93 mM $MgSO_4.7H_2O$, 13 mM $NaHCO_3$, 3.36 mM $NaH_2PO_4.2H_2O$, 0.30 NH_4Cl and 5.5 mM glucose) modified from Richman et al., 1987 (16). Rinsed samples were incubated in oxytgenated trout Ringer solution with 8 mg l^{-1} DASPMI (dimethylaminostyrylmethylpyridiniumiodine), a which. Mitochondrion-specific stain which stains only active mitochondria (12, 17, 18) for 30 min. Following the incubation period, the tissue was rinsed several times with trout Ringer solution to remove remaining dye and then placed on a glass slide and covered with a coverslip for microscopic examination. Prepared tissues were examined by epifluorescent microscope (Leitz "Diavert"). Active chloride cell density was measured by counting of the number of DASPMI-stained cells in five randomly chosen fields at a magnification of 40 (total counted area: 7.81 mm^2) for each of five individuals per group and then the mean chloride cell density was calculated. Statiscal analyses were performed by Mann-Whitney *U* test.

Results

Calcium concentrations (mmol l^{-1}) determined in the experiment tanks were as follows: not detected in zero-Ca^{+2} freshwater (ZCFW) tank, 0.011±0.002 in low -Ca^{+2} freshwater (LCFW) tank and 1.35±0.05 in normal freshwater (NFW) tank. Chloride cell counts were made on the gill arch epithelium of animals. It was observed that large numbers of chloride cells were located at the base of gill filaments and on the interlamellar regions of filaments. The effect of calcium concenration of water on chloride cell density is given in Figure 1. At the end of two weeks exposure, density of chloride cells (DASPMI-stained cells) significantly increased in gill arch epithelium of larvae adapted to ZCFW ($P<0.01$) and adapted to LCFW ($P<0.05$) compared that of larvae kept in NFW.

Discussion

It has been found that the chloride cell density in the gill lamellae of adult rainbow trout (3), in the skin of the Juvenile Nile tilapia (13) and in cleithrum skin of the adult rainbow trout (11) shows an increase with decreasing external calcium concentration. In addition, McCormick et al., 1992 (13) found that there was a correlation between chloride cell density and the rate of calcium influx in low external calcium. Similarly using SEM, Brown, 1992 (19) found that freshwater-adapted adult trout have a greater total number of choride cells in the gill epithelium than do trout adapted to sea water. The results of present study show that the same general conclusion is true of larvae: the number of chloride cells in the gill arch epithelia of larval fish increased with decreasing external calcium concentration. Although there is a close inverse relationship between chloride cell density and external calcium concentration, chloride cell density may be influenced by all gradients, and not just the need to take up calcium. There are probably three different factors causing the increase in chloride cells at lower calcium:

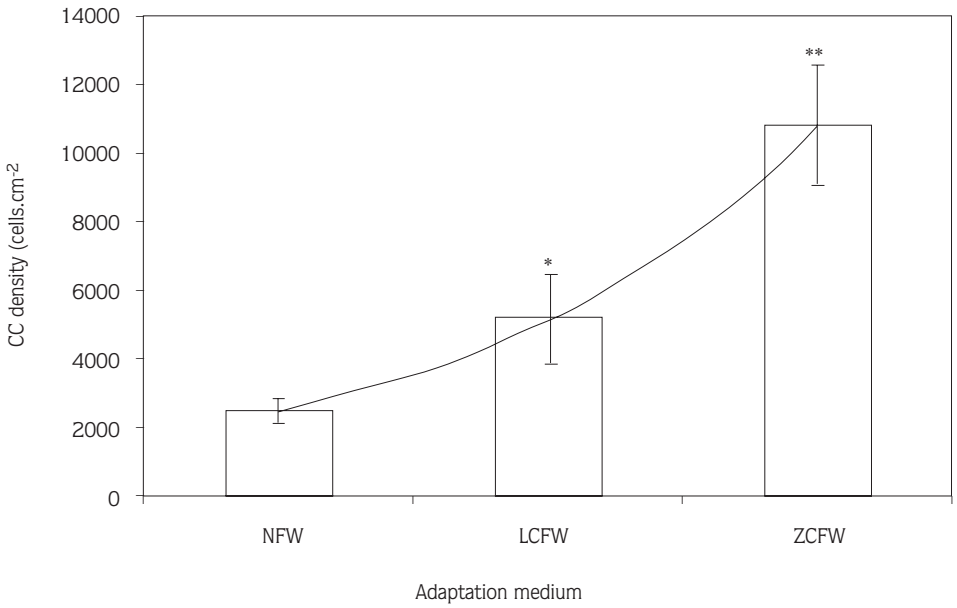

Figure 1. Effect of adaptation of brown trout (*Salmo trutta* L.) larvae to zero-Ca^{2+} freshwater (ZCFW), low-Ca^{2+} freshwater (LCFW) and normal freshwater (NFW) medium on chloride cell (CC) density in gill arch epithelium. Gill arch epithelium were stained with DASPMI and examined as detailed in text. Significant difference from larvae adapted to NFW (**P < 0.05; ** P<0.01*, Mann-Whitney *U* test).

(i) The need for a greater ability to take up calcium against a greater gradient,

(ii) the same point for sodium, chloride, etc.,

(iii) the greater permeability of the gill membrane at low calcium concentration hence a greater loss of ions and the need for activity is needed to replace them.

In conclusion, it is generally agreed that there is an inverse correlation between external calcium concentrations and chloride cell density, which is demonstrated for brown trout larvae in the present study. In addition chloride cell distribution in juvenile and adult fish is well documented (3, 11, 12, 13, 15, 20, 21, 22) because it is comparatively easy to find numerous chloride cells in sections of gill filaments ad integument of juvenile and adult teleots. However, because of the difficulties involded in studying larval fish, the chronology of appearance desity and location of chloride cells not well documented. The results described here in hep to fill this gap in our knowledge.

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