Journal of Environmental Biology
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April 2007, 28(2) 385-393 (2007)
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Calcium homeostasis in low and high calcium water acclimatized *Oreochromis mossambicus* exposed to ambient and dietary cadmium

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(Received: November 12, 2005 ; Revised received: June 15, 2006 ; Accepted: July 30, 2006)

Abstract: *The effects of cadmium administered via ambient water (10 μg/l) or food (10 μgCd/fish/day) on plasma calcium, corpuscles of Stannius and bony tissues of Oreochromis mossambicus acclimated to low calcium (0.2 mM) and high calcium (0.8 mM) water were studied for 2, 4, 14 and 35 days. In low calcium water acclimated fish, ambient cadmium induced significant hypocalcemia, while the structure and morphometry of type-1 and type-2 cells of corpuscles of Stannius were not affected on day 2 and 4. Subsequently on day 14 and 35, recovery of plasma calcium to normal levels was observed followed by a decrease in corpuscles of Stannius index (CSI), cell size, volume of granular endoplasmic reticulum ($p<0.05$) of type-1 cells in both, fish exposed to ambient or dietary cadmium. The type-2 cells were not affected. In high calcium water acclimated fish both, ambient and dietary cadmium caused a significant reduction of plasma calcium levels on day 2 and 4. In these fish, there was a significant transient increase in the size of corpuscles of Stannius on day 4, followed by recovery on day 14 and 35. Ultrastructural observations of corpuscles of Stannius revealed that cadmium did not cause any cellular damage on type-1 and type-2 cells during 35 days exposure. In low or high calcium water acclimatized tilapia exposed to ambient or dietary cadmium had no effect on the calcium and phosphate composition of the scales, operculum and vertebrae. Thus, it is unlikely that recovery of hypocalcemia was due to the dissolution of calcium from bony tissues. This study also revealed that cadmium does not mediate stimulation of the corpuscles of Stannius gland, and that high Ca^{2+} water had a protective effect against ambient and dietary cadmium.*

Key words: *Calcium, Cadmium, Corpuscles of Stannius, Oreochromis mossambicus*

Introduction

Cadmium is present in natural waters as a result of industrial processes and other anthropogenic contamination (Rand, 1995). Cadmium is known to cause severe toxic effects on aquatic organisms (Sorensen, 1991; Calow, 1998). Most reports on the effects of cadmium on teleost are from fish exposed to high concentrations of the metal, whereas information on the chronic exposure of fish to low cadmium levels is rather scanty. Cadmium induced hypocalcemia has been reported in carp (Koyama and Itazawa, 1977), flounder (Larsson *et al.,* 1981) and rainbow trout (Reader and Morris, 1988). The disturbance of calcium homeostasis suggests that the calcium endocrine mechanisms that regulate the calcium balance may be affected by cadmium. The pituitary gland, the inter-renals and the corpuscles of Stannius are likely the most important endocrine glands in calcium metabolism (Wendelaar Bonga and Pang, 1991). Many workers investigating the function of the corpuscles of Stannius in fish from different aquatic environments have attributed a function to these glands in the endocrine control of calcium metabolism (Hanssen *et al.,* 1989). In several species of teleost, two cell types (type-1 and type-2) have been reported in the corpuscles of Stannius (Krishnamurthy, 1976; Wendelaar Bonga *et al.,* 1976). Type-1 cells were found to respond to changes in ambient water calcium concentrations (Urasa and Wendelaar Bonga, 1985; Hansen *et al.,* 1989), whereas the function of Type 2 cells remains unknown. Although the role of corpuscles of Stannius is well established, the response of these glands to stress induced by heavy metals such as cadmium is not known.

Water as well as food form important sources of cadmium for fish, since the metal may progressively concentrate through trophic level transfer along the aquatic food chain, and it has been reported that cadmium entering the body along both the routes can disturb osmo-ionic balance in tilapia (Wendelaar Bonga and Lock, 1992). Although the mechanism involved in the recovery of cadmium induced hypocalcemia is not well understood, however, it is suggested that this could be achieved by mobilization of calcium from the bone (Muramoto, 1981). The calcium and phosphate content of cellular bone in carp decreased after exposure to ambient and dietary cadmium (Koyama and Itazawa, 1977; Muramoto, 1981). Hence, the stored calcium in the bony tissues could be mobilized by the endocrine hormone(s). In this study the effect of ambient and dietary cadmium on plasma calcium, the ultrastructural and morphometric observations of type-1 and type-2 cells of the corpuscles of Stannius and the calcium / phosphate composition of bony tissues of *Oreochromis mossambicus* were investigated. In our previous study it has been shown that the effect of ambient and dietary cadmium on the gills of tilapia was greater in fish adapted to low calcium water than in high calcium water (Pratap and Wendelaar Bonga, 1993).

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These findings clearly demonstrate the protective role of high calcium waer against cadmium toxicity. To evaluate the protective role of high calcium water the effect of ambient and dietary cadmium on calcium homeostasis in *Oreochromis mossambicus* was studied in fish acclimated to water with high (0.8 mM) and low (0.2 mM) calcium levels.

Materials and Methods

Laboratory stock of male *Oreochromis mossambicus* ranging from 12 - 14 cm in total length and 22.0 ± 4.2 g in weight (mean ± SD) was used in the present study. The fish were maintained in 100 liter aquaria (0.55 mM Ca^{2+}) with circulating filtered water (Eheim pumps 1021) at 28°C and a daily 12 hr light and dark photoperiod. The water pH ranged from 7.4 to 7.6.

Experimental design: The fish were divided into six groups of which three were kept in low calcium water (0.2 mM Ca^{2+}) and three in high calcium water (0.8 mM Ca^{2+}). Artificial freshwater was prepared according to Flik *et al.* (1985). Acclimation of fish to low and high calcium water, maintenance, preparation and administration of ambient cadmium (10 µg Cd l^{-1}) or dietary cadmium (10 µg Cd $fish^{-1}$ day^{-1}) were as reported earlier (Pratap and Wendelaar Bonga, 1993). Food was prepared as a mixture containing tetramin tropical fish food (85%), gelatin (10%) and agar (5%), dissolved in warm distilled water. Cadmium was blended into this mixture. Samples of food were digested in HNO_3 followed by cadmium analysis in atomic absorption spectrophotometer (Thermo Jarell Ash, USA). The measured concentration did not deviate more than 2% of the calculated cadmium concentration. Groups 1, 2, 5 and 6 shown below were fed cadmiumfree food.

The experimental set-up and cadmium exposure were as follows:-

(a) group 1: low Ca^{2+} water (0.2 mM) containing 10 µg Cd^{2+} l^{-1}
(b) group 2: high Ca^{2+} water (0.8 mM) containing 10 µg Cd^{2+} l^{-1}
(c) group 3: low Ca^{2+} water (0.2 mM), 10 µg Cd^{2+} $fish^{-1}$ day^{-1} via the food
(d) group 4: high Ca^{2+} water (0.8 mM) 10 µg Cd^{2+} $fish^{-1}$ day^{-1} via the food
(e) group 5: low Ca^{2+} water (0.2 mM) control
(f) group 6: high Ca^{2+} water (0.8 mM) control

Sampling and analytical procedure: Fish from each tank were sampled after 2, 4, 14 and 35 day. They were anesthetized in 0.2% 2-phenoxyethanol. The blood was collected from caudal vessel in heparinized microhaematocrit capillaries. After centrifugation the plasma was stored at -20°C. The total plasma calcium was determined spectrophotometrically by cresolphthalein complexon method (Sigma diagnostics, U.S.A.). The opercular bone from both sides, vertebrae and scales from anterior dorsal part of the body were collected. All bony tissues were dried overnight at 70°C and digested in teflon beakers with 1:1 mixture of concentrated nitric acid and perchloric acid. The resultant clear solution was diluted with double distilled water. Calcium concentrations of bony tissues were analysed by inductively coupled plasma atomic emission spectrometer (ICP AES, Plasma IL 200, Thermo Electron, U.S.A.). After dissection the long (l) and short (s) axis of both the corpuscles of Stannius were measured under a dissecting microscope, equipped with an eyepiece micrometer (Olympus, OSM, magnification x40). The corpuscles of Stannius index (CSI) was calculated as CSI = $(l_1 + S_1)$ $(l_2 + S_2).4^{-1}$. The corpuscles of Stannius were prepared for electron microscopy as described by Wendelaar Bonga and Van der Meij (1989).

Morphometric analysis of corpuscles of Stannius: For morphometric evaluation of the corpuscles of Stannius, several micrographs for each fish were made in a Phillips EM200 electron microscope with a final magnification of 8,100x, representing a sampling area of 130 mm^2. The electron micrographs were morphometrically analysed using Kontron digiplan integration equipment with an X-Y magnetostriction tablet. About 20 cells of type-1 and type-2 cells per fish were measured. The cell and nuclear areas (µm^2) and the cytoplasmic organelles (mitochondria, granular endoplasmic reticulum, the golgi zone and the secretory granules) were expressed as the fractional percentage of the cytoplasmic volume. The data for the experimental groups and their respective controls were tested for significance by analysis of variance (ANOVA) and statistical significance set at 5 % level.

Results and Discussion

Plasma calcium: In groups 1, 2, 3 and 4 exposure to ambient or dietary cadmium caused transient significant hypocalcemia (Fig. 1 and 2). Reduced plasma calcium levels were observed after 2 and 4 days in low (group 1) and high (group 2) calcium water acclimated fish exposed to ambient cadmium. When cadmium was administered *via* the food, significant hypocalcemia was observed after 2 and 4 day in low calcium water acclimated fish (group 3) and a similar effect was apparent only after 4 days in fish from high calcium water (group 4). After 14 days, the plasma calcium levels in all the groups had recovered to normal and thereafter no changes occurred after the 35 days exposure to ambient or dietary cadmium.

Corpuscles of Stannius structure and morphometry: In *Oreochromis mossambicus* the corpuscles of Stannius are a pair of small oval glandular bodies situated dorso caudally to the mesonephric kidney. Each gland consists of lobules of varying sizes. The gland cells are radially arranged, forming a pseudolumen in the middle of each lobule. Within each lobule, two cell types are present, type-1 and type-2 (Fig. 3 to 6). The type-1 cells are more numerous than type-2 cells and have large electron dense spherical secretory granules enclosed by a limiting membrane. Each type-1 cell in tangential section appears long and slender with an oval nucleus at the basal part and the secretory granules mainly located in the apical end of the cytoplasm. Large arrays of granular endoplasmic reticulum are

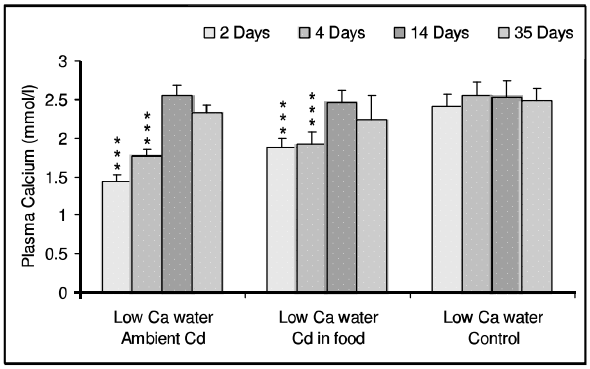


Fig. 1: Effect of ambient and dietary cadmium on plasma calcium (mmol. l^{-1}) in *Oreochromis mossambicus* acclimatized to low calcium water (*** p< 0.001)

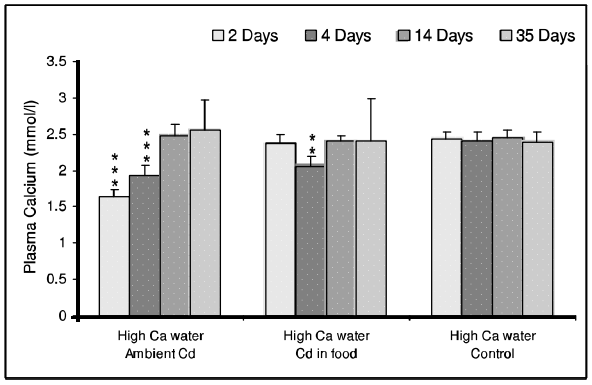


Fig. 2: Effect of ambient and dietary cadmium on plasma calcium (mmol. l^{-1}) in *Oreochromis mossambicus* acclimatized to high calcium water (** p< 0.01, *** p< 0.001)

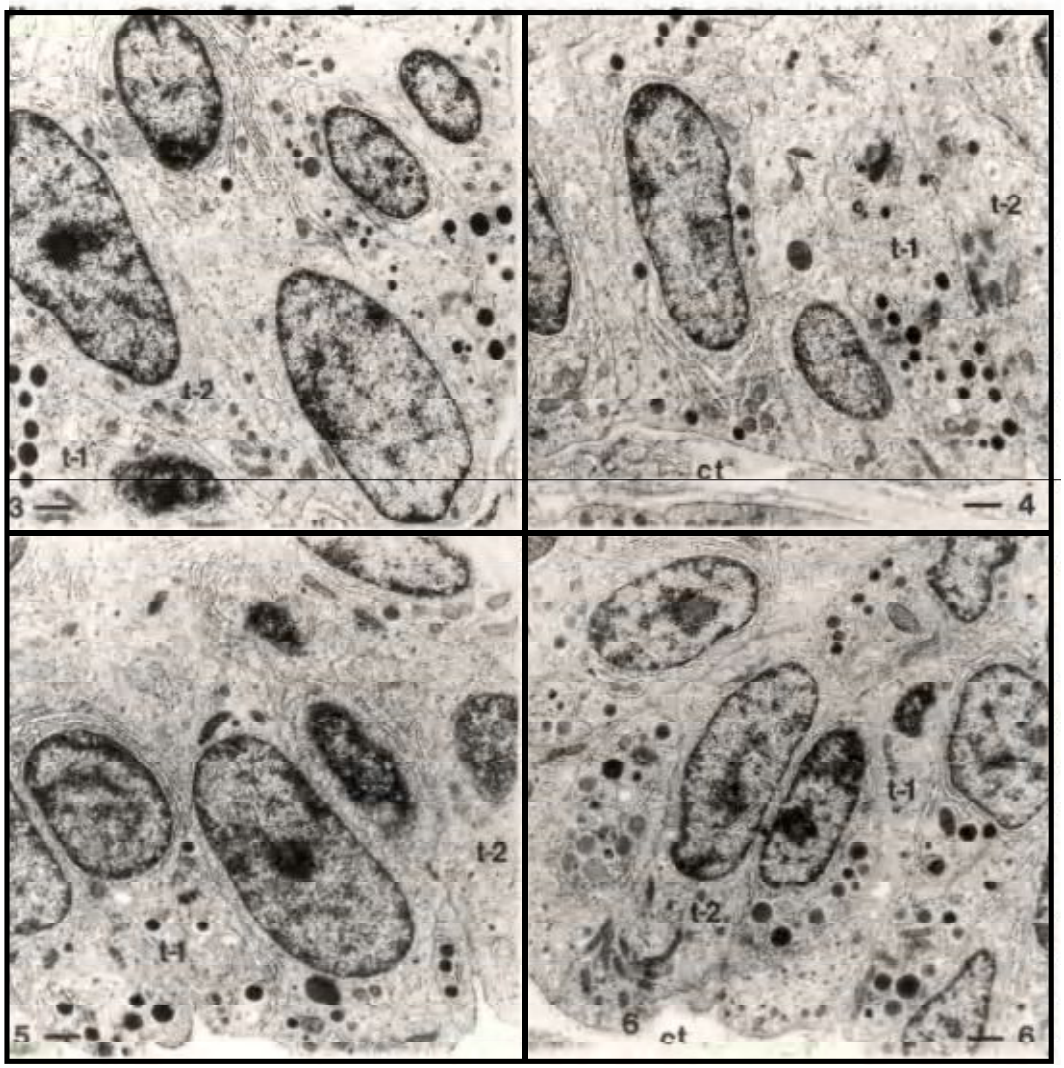


Fig. 3-6: Ultrastructure of the corpuscles of Stannius of *Oreochromis mossambicus* acclimatized in low calcium water (Fig. 3-4), or high calcium water (Fig. 5-6), exposed to ambient cadmium; t-1: type-1 cell; t-2: type-2 cell; ct – connective tissue; bars represent 1µm. (Fig. 3) low calcium control; (Fig. 4) 4 days cadmium; (Fig. 5) high calcium control; (Fig. 6) 14 days cadmium

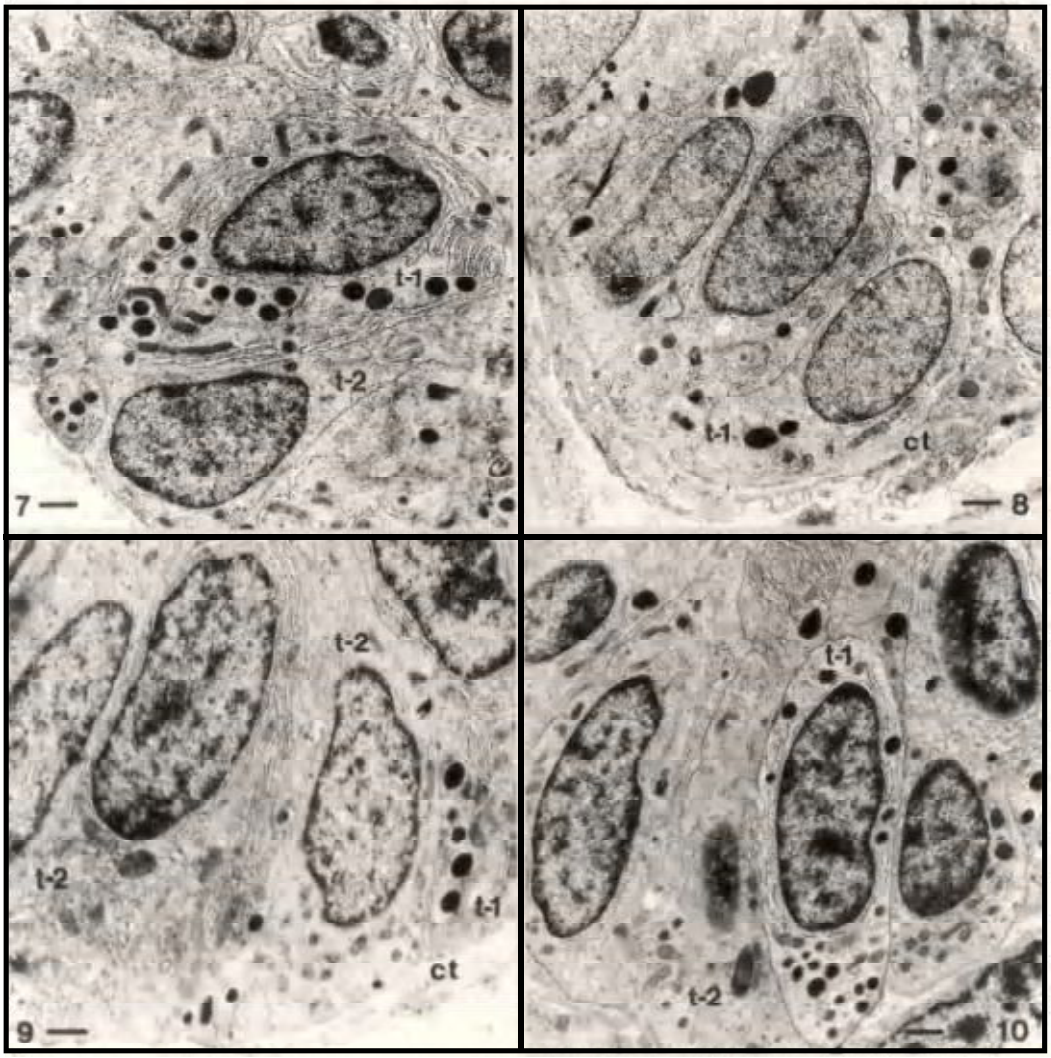


Fig. 7-10: Ultrastructure of the corpuscles of Stannius of *Oreochromis mossambicus* acclimatized in low calcium water (Fig. 7-8) or high calcium water (Fig. 9-10), exposed to dietary cadmium; t-1: type-1 cell; t-2: type-2 cell; ct – connective tissue; bars represent 1μm. (Fig. 7) 2 days cadmium; (Fig. 8) 4 days cadmium; (Fig. 9) 14 days cadmium; (Fig. 10) 35 days cadmium

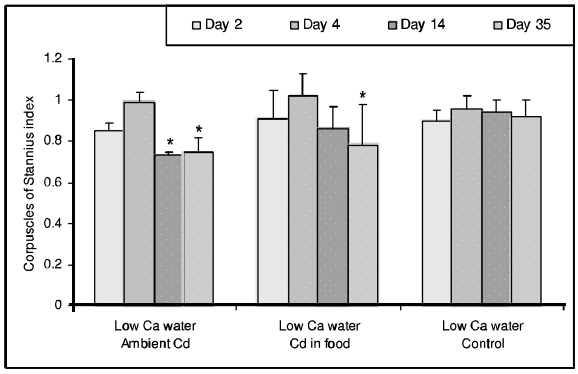


Fig. 11: The corpuscles of Stannius index (CSI) of low calcium water acclimatized *Oreochromis mossambicus* exposed to ambient and dietary cadmium (* $p<0.05$)

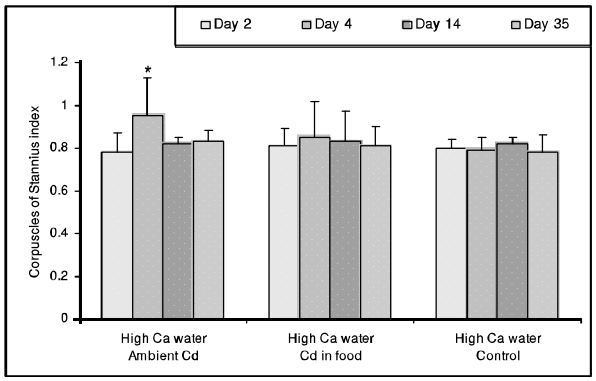


Fig. 12: The corpuscles of Stannius index (CSI) of high calcium water acclimatized *Oreochromis mossambicus* exposed to ambient and dietary cadmium (* $p < 0.05$)

found in the middle and apical regions of the cytoplasm. The Golgi zones are present around the nucleus or, more often, in the apical nuclear region. The type-2 cells are found between the type-1 cells and have relatively small isolated strands of granular endoplasmic reticulum distributed evenly in the cytoplasm. The nucleus is located either in the middle or in the apical region of the cell. The Golgi areas are well developed in both cell types. The ultrastructural observations of the type-1 and type-2 cells of the corpuscles of Stannius showed that, ambient and dietary cadmium did not cause any cellular damage during the 35 days exposure period (Fig. 7 to 10). Similarly, in control fish acclimated to low calcium and high calcium water; both cell types did not show any significant structural differences.

Morphometric analysis of type-1 cells of the corpuscles of Stannius in electron micrographs of fish adapted to low calcium water showed that the cell size and the percentage volume of granular endoplasmic reticulum was significantly reduced by 23% ($p<0.05$) and 21% ($p<0.05$) after 35 days exposure in both, ambient and dietary cadmium (Table 1). No differences were observed in cell size or volume of organelles of type-1 cells in fish from high calcium water exposed to ambient or dietary cadmium. Morphometric differences were not observed in type-2 cells of the corpuscles of Stannius in fish acclimatized to low calcium or high calcium water and exposed to ambient and dietary cadmium for 35 days (Table 2).

Corpuscles of Stannius index (CSI): The size of the corpuscles of Stannius, as reflected by the corpuscles of Stannius index (CSI), is given in Figs. 11 and 12, for fish adapted to low calcium and high calcium water and exposed to ambient and dietary cadmium. In the control groups, there was no significant difference in the CSI between fish adapted to low calcium and high calcium water during the 35 days experimental period. In tilapia from low calcium water, a significant decrease in the CSI by 22% ($p<0.001$) was observed after 14 and 35 days exposure to ambient cadmium. Fish adapted to the same low calcium water but fed cadmium food showed a significant decrease in the CSI by 15% ($p<0.001$) after 35 days. The CSI of fish adapted to high calcium water had increased by 20% ($p<0.05$) after 4 days exposure to

Table - 1: Morphometric analysis of electron micrographs of type-1 cells of corpuscles of Stannius in *Oreochromis mossambicus* adapted to low calcium water (0.2 mM Ca^{2+}), or high calcium water (0.8 mM Ca^{2+}) and exposed to ambient or dietary cadmium. Values are expressed as means ± SD of 5 fish per group

	Days	Ambient cadmium		Dietary cadmium		Controls	
		Low Ca^{2+} water	High Ca^{2+} water	Low Ca^{2+} water	High Ca^{2+} water	Low Ca^{2+} water	High Ca^{2+} water
Cell	2	23.6 ± 3.3	22.0 ± 5.4	23.9 ± 7.0	22.5 ± 6.3	23.7 ± 6.7	19.6 ± 5.9
μm^2	4	22.9 ± 8.6	21.3 ± 7.7	21.2 ± 6.8	20.1 ± 4.8	19.9 ± 6.2	21.7 ± 3.5
	14	18.5 ± 4.5	17.1 ± 6.8	19.0 ± 5.0	18.9 ± 4.2	21.9 ± 7.5	20.6 ± 6.3
	35	16.8 ± 3.1*	18.1 ± 4.7	16.9 ± 3.0*	19.5 ± 3.1	22.0 ± 5.2	22.2 ± 5.6
Nucleus	2	6.4 ± 1.7	7.2 ± 2.3	7.1 ± 2.3	7.9 ± 3.3	7.3 ± 3.5	7.8 ± 4.8
μm^2	4	6.9 ± 2.3	7.0 ± 2.4	7.2 ± 2.4	8.8 ± 2.1	6.8 ± 2.3	8.8 ± 1.9
	14	7.6 ± 2.9	6.0 ± 2.8	6.9 ± 1.9	8.0 ± 3.3	8.7 ± 2.4	8.5 ± 3.2
	35	6.8 ± 3.0	6.2 ± 2.4	7.2 ± 1.6	8.8 ± 1.6	7.6 ± 3.2	7.6 ± 1.9
Endoplasmic	2	19.7 ± 3.9	18.9 ± 6.2	24.8 ± 7.1	22.5 ± 5.8	19.0 ± 5.5	20.7 ± 3.9
reticulum	4	19.5 ± 4.3	21.9 ± 5.5	21.2 ± 5.0	23.6 ± 5.9	20.4 ± 5.1	21.6 ± 4.7
volume %	14	17.6 ± 4.8	18.5 ± 5.7	18.4 ± 3.2	18.1 ± 6.8	22.4 ± 5.4	20.0 ± 3.9
	35	16.7 ± 3.2*	17.8 ± 4.9	16.4 ± 3.0*	20.4 ± 5.7	21.3 ± 4.3	19.8 ± 2.6
Mitochondria	2	5.9 ± 2.0	3.9 ± 1.4	5.4 ± 1.6	4.0 ± 2.0	4.9 ± 2.1	4.7 ± 1.9
volume %	4	5.0 ± 2.6	4.1 ± 1.9	4.4 ± 1.7	5.3 ± 2.1	5.5 ± 1.8	4.8 ± 1.8
	14	4.4 ± 1.2	4.8 ± 1.4	4.5 ± 1.6	4.1 ± 1.8	5.4 ± 1.9	4.0 ± 2.1
	35	4.6 ± 1.0	4.6 ± 1.7	4.5 ± 1.7	4.7 ± 1.6	4.2 ± 1.2	4.9 ± 2.5
Golgi	2	7.9 ± 1.2	7.9 ± 1.1	7.3 ± 2.1	7.9 ± 1.4	8.3 ± 1.3	7.8 ± 1.5
volume %	4	7.7 ± 1.7	8.2 ± 1.9	7.7 ± 1.9	8.7 ± 1.3	8.1 ± 1.8	8.7 ± 1.2
	14	6.8 ± 2.2	7.8 ± 1.8	8.9 ± 1.6	8.4 ± 2.1	7.9 ± 2.4	8.9 ± 1.8
	35	6.7 ± 1.7	7.2 ± 1.5	6.9 ± 1.8	8.0 ± 1.8	7.5 ± 2.1	7.7 ± 2.3
Granules	2	4.1 ± 2.1	4.0 ± 1.8	4.2 ± 2.9	4.6 ± 2.2	4.4 ± 1.4	5.1 ± 2.9
volume %	4	5.7 ± 2.3	5.7 ± 2.7	4.1 ± 2.5	4.9 ± 2.0	5.1 ± 2.5	4.6 ± 2.7
	14	4.0 ± 1.9	5.4 ± 2.1	4.4 ± 2.7	4.4 ± 2.6	4.2 ± 2.1	4.9 ± 2.6
	35	4.1 ± 1.7	4.3 ± 2.2	4.9 ± 2.7	4.3 ± 1.3	4.8 ± 2.3	4.4 ± 2.1

Levels of significance (ANOVA) *$p<0.05$

Table - 2: Morphometric analysis of electron micrographs of type-2 cells of corpuscles of Stannius in *Oreochromis mossambicus* adapted to low calcium water (0.2 mM Ca^{2+}) or high calcium water (0.8 mM Ca^{2+}) and exposed to ambient or dietary cadmium. Values are expressed as means ± SD of 5 fish per group

	Days	Ambient cadmium		Dietary cadmium		Controls	
		Low Ca^{2+} water	High Ca^{2+} water	Low Ca^{2+} water	High Ca^{2+} water	Low Ca^{2+} water	High Ca^{2+} water
Cell μm^2	2	22.6 ± 4.8	21.6 ± 6.7	20.8 ± 3.1	21.6 ± 6.2	20.3 ± 4.4	18.3 ± 3.6
	4	17.7 ± 6.3	16.8 ± 4.5	19.2 ± 6.3	20.0 ± 6.1	18.3 ± 5.5	17.3 ± 4.5
	14	19.1 ± 6.1	17.2 ± 6.9	18.5 ± 3.8	19.0 ± 4.2	19.7 ± 4.3	21.1 ± 5.1
	35	18.3 ± 5.1	20.4 ± 6.5	17.2 ± 5.0	19.0 ± 3.9	17.8 ± 5.2	18.4 ± 3.5
Nucleus μm^2	2	7.1 ± 2.3	8.2 ± 2.8	7.6 ± 2.2	7.5 ± 1.7	8.6 ± 2.2	8.7 ± 2.2
	4	6.9 ± 2.0	6.7 ± 2.4	7.8 ± 2.0	8.8 ± 2.7	8.5 ± 2.5	8.4 ± 2.0
	14	7.4 ± 3.0	7.2 ± 3.1	6.7 ± 2.2	7.6 ± 2.7	8.8 ± 3.6	7.9 ± 3.4
	35	7.6 ± 3.5	8.2 ± 3.6	6.8 ± 2.4	7.8 ± 3.5	7.7 ± 3.5	7.5 ± 2.0
Endoplasmic reticulum volume %	2	17.8 ± 3.4	17.1 ± 3.8	18.5 ± 3.3	21.6 ± 5.1	19.0 ± 3.7	18.2 ± 4.6
	4	20.5 ± 4.0	21.9 ± 5.7	21.5 ± 5.2	22.6 ± 4.6	20.0 ± 4.9	21.8 ± 6.5
	14	18.9 ± 6.2	17.7 ± 6.6	18.7 ± 3.3	18.7 ± 5.4	21.3 ± 5.7	19.5 ± 2.9
	35	21.0 ± 4.9	22.6 ± 5.2	20.8 ± 4.9	21.2 ± 4.8	21.8 ± 5.5	20.5 ± 3.6
Mitochondria volume %	2	5.0 ± 2.4	5.1 ± 1.9	4.9 ± 2.7	4.5 ± 2.0	5.7 ± 2.2	5.8 ± 2.5
	4	5.0 ± 2.7	5.1 ± 2.1	4.3 ± 1.9	4.9 ± 2.2	4.7 ± 1.7	4.6 ± 2.2
	14	5.1 ± 1.8	4.6 ± 2.0	4.1 ± 1.3	4.7 ± 2.4	5.4 ± 2.3	4.8 ± 1.7
	35	4.6 ± 1.9	5.8 ± 2.3	5.7 ± 2.5	5.3 ± 2.3	4.6 ± 1.4	5.3 ± 2.6
Golgi volume %	2	6.8 ± 1.6	6.6 ± 1.6	6.7 ± 1.5	7.1 ± 1.9	7.0 ± 1.6	7.1 ± 1.2
	4	8.3 ± 1.5	8.6 ± 2.1	8.3 ± 1.7	8.4 ± 1.5	8.0 ± 2.0	8.1 ± 2.1
	14	7.8 ± 2.2	7.1 ± 2.0	8.2 ± 1.5	7.7 ± 1.5	8.6 ± 1.7	7.4 ± 1.4
	35	8.5 ± 1.7	7.2 ± 1.7	7.7 ± 2.0	8.2 ± 2.2	7.8 ± 2.3	8.5 ± 1.6

Table - 3 : Calcium and phosphate content (mmol. $g.^{-1}$ dry weight) of the scales, operculum and vertebrae in low calcium water adapted *Oreochromis mossambicus* exposed to ambient or dietary cadmium for 2, 4, 14 and 35 days. Values are expressed as means ± SD, N = number of fish

Tissue	N	Days	Low calcium water					
			Ambient Cd^{2+}		Dietary Cd^{2+}		Controls	
			Ca^{2+}	PO_4	Ca^{2+}	PO_4	Ca^{2+}	PO_4
Scales	10	2	3.13 ± 0.21	2.25 ± 0.15	3.16 ± 0.19	2.29 ± 0.17	3.27 ± 0.25	2.40 ± 0.11
	10	4	3.08 ± 0.17	2.19 ± 0.17	3.01 ± 0.16	2.34 ± 0.19	3.22 ± 0.34	2.19 ± 0.22
	10	14	3.32 ± 0.24	2.39 ± 0.35	3.47 ± 0.34	2.19 ± 0.15	3.37 ± 0.29	2.16 ± 0.05
	5	35	3.29 ± 0.21	2.49 ± 0.12	3.10 ± 0.11	2.29 ± 0.18	3.28 ± 0.27	2.33 ± 0.14
Operculum	10	2	4.09 ± 0.11	2.33 ± 0.15	4.09 ± 0.34	2.43 ± 0.23	4.25 ± 0.21	2.23 + 0.19
	10	4	4.13 ± 0.14	2.79 ± 0.21	4.19 + 0.23	2.76 + 0.31	4.36 ± 0.29	2.57 ± 0.37
	10	14	4.49 ± 0.21	2.77 ± 0.08	4.55 ± 0.30	2.51 ± 0.13	4.73 ± 0.47	2.63 ± 0.26
	5	35	4.30 ± 0.19	2.86 ± 0.26	4.58 ± 0.31	2.61 ± 0.15	4.49 ± 0.23	2.77 ± 0.15
Vertebrae	10	2	3.24 ± 0.11	2.51 ± 0.22	3.32 ± 0.27	2.26 ± 0.23	3.45 ± 0.24	2.32 ± 0.26
	10	4	3.52 ± 0.31	2.39 ± 0.19	3.42 ± 0.19	2.52 ± 0.18	3.21 ± 0.34	2.51 ± 0.32
	10	14	3.19 ± 0.22	2.46 ± 0.23	3.39 ± 0.32	2.34 ± 0.21	3.38 ± 0.21	2.46 ± 0.35
	5	35	3.41 ± 0.17	2.39 ± 0.24	3.40 ± 0.22	2.37 ± 0.19	3.49 ± 0.29	2.37 ± 0.28

Table - 4: Calcium and phosphate content (mmol. g^{-1} dry weight) of the scales, operculum and vertebrae in high calcium water adapted *Oreochromis mossambicus* exposed to ambient or dietary cadmium for 2, 4, 14 and 35 days. Values are expressed as means ± SD, N = number of fish

Tissue	N	Days	High calcium water					
			Ambient Cd^{2+}		Dietary Cd^{2+}		Controls	
			Ca^{2+}	PO_4	Ca^{2+}	PO_4	Ca^{2+}	PO_4
Scales	10	2	3.63 ± 0.31	2.51 ± 0.20	3.31 ± 0.43	2.44 ± 0.32	3.71 ± 0.40	2.64 ± 0.25
	10	4	3.77 ± 0.12	2.63 ± 0.13	3.66 ± 0.67	2.51 ± 0.16	3.67 ± 0.12	2.54 ± 0.13
	10	14	3.08 ± 0.32	2.13 ± 0.16	2.98 ± 0.27	2.26 ± 0.16	3.24 ± 0.23	2.12 ± 0.14
	5	35	3.25 ± 0.21	2.41 ± 0.17	3.12 ± 0.16	2.39 ± 0.14	3.27 ± 0.25	2.40 ± 0.16
Operculum	10	2	4.70 ± 0.18	2.31 ± 0.12	4.54 ± 0.22	2.23 ± 0.32	4.73 ± 0.22	2.26 ± 0.14
	10	4	4.37 ± 0.32	2.46 ± 0.28	4.52 ± 0.19	2.62 ± 0.42	4.57 ± 0.31	2.78 ± 0.34
	10	14	4.19 ± 0.16	2.71 ± 0.11	4.45 ± 0.25	2.51 ± 0.13	4.29 ± 0.34	2.65 ± 0.16
	5	35	4.09 ± 0.68	2.84 ± 0.51	4.72 ± 0.16	2.68 ± 0.11	4.79 ± 0.39	2.77 ± 0.15
Vertebrae	10	2	3.41 ± 0.12	2.31 ± 0.19	3.42 ± 0.31	2.32 ± 0.23	3.62 ± 0.14	2.45 ± 0.11
	10	4	3.64 ± 0.31	2.49 ± 0.22	3.39 ± 0.28	2.39 ± 0.41	3.44 ± 0.21	2.44 ± 0.22
	10	14	3.84 + 0.13	2.64 ± 0.18	3.53 ± 0.33	2.71 ± 0.33	3.58 ± 0.45	2.37 ± 0.39
	5	35	3.58 ± 0.21	2.56 ± 0.11	3.36 ± 0.27	2.64 ± 0.28	3.30 ± 0.34	2.41 ± 0.29

ambient cadmium. Similarly in fish fed cadmium food an increase by 14% ($p>0.05$) of the CSI was observed after 4 days in high calcium water. On further exposure for 14 and 35 days to ambient cadmium and dietary cadmium, the CSI of fish in high calcium water was not different from control fish.

Calcium and phosphate composition of bony tissues: The mean concentration of calcium and phosphate of the operculum, vertebrae and scales are given inTables 3 and 4. There were no significant differences in the calcium and phosphate content of bony tissues from low and high calcium water acclimated controls. After 2, 4, 14 and 35 days exposure to ambient or dietary cadmium, the calcium and phosphate levels in the bony tissues were not significantly different from the low and high calcium water controls. The calcium content of the opercular bone was significantly greater ($p<0.001$) than the vertebrae and scales (Table 3 and 4).

Short term exposure to ambient or dietary cadmium caused significant hypocalcemia. The magnitude of hypocalcemia was more pronounced in low calcium water acclimated fish compared to high calcium water. Similar cadmium transient hypocalcemia has also been observed in flounder (Larsson *et al*., 1981), rainbow trout (Giles, 1984) and carp (Yamawaki *et al*., 1986) exposed to cadmium. After 14 days recovery of plasma calcium levels in both, fish exposed to ambient or dietary cadmium was apparent. The biochemical and physiological mechanisms responsible for this restoration of plasma calcium is rather unclear. Although cadmium binding proteins such as metallothioneins (MT) play an important role in the detoxification process and acclimation (Klaverkamp and Duncan, 1987), it is possible that calcium dissolution from the bony tissues occurs during hypocalcemia. Bone demineralization in carp and *Girella punctata* exposed to ambient or dietary cadmium has been attributed to the recovery of hypocalcemia (Muramoto, 1981; Kuroshima 1987). In this study there is no evidence of such a mechanism is taking place in tilapia, since Ca^{2+} and PO_4^{-2} composition of the vertebrae and scales had not changed during the 35 days cadmium exposure. Verbost *et al*. (1987) have shown that Ca^{2+} uptake mechanism across the gills is inhibited by Cd^{2+}. Therefore, it is possible that Ca^{2+} uptake across the body from water or food is sufficient for recovery of transient hypocalcemia. This probably explains why loss of Ca^{2+} in the bony tissues was not observed. Cadmium taken up by fish from water or food is particularly redistributed to the gills and intestine and subsequently to the liver and kidneys (Pratap and Wendelaar Bonga, 2004). The Ca^{2+} imbalance induced by Cd^{2+} suggests that the endocrine regulation of calcium is affected. The corpuscles of Stannius are perhaps the most important calcium regulating endocrine glands (Wendelaar Bonga and Pang, 1991; Van der Heijden *et al*., 1999). The corpuscles of Stannius are located on the dorso caudal kidney and cadmium may exert its toxic effects.

Various studies have shown that the corpuscles of Stannius are involved in calcium homeostasis in fish (Flik *et al.,* 1990; Wendelaar Bonga and Pang, 1991). Stanniectomy caused increased net branchial inflow of Ca^{2+} followed by hypercalcemia in eels maintained in freshwater and seawater (Van der Heijden *et al.,* 1999). Removal of corpuscles of Stannius caused hypercalcemia in killifish maintained in calcium rich seawater, but not in calcium poor freshwater or artificial seawater. Transplants of corpuscles of Stannius or administration of stannius homogenate, corrected hypercalcemia in stainniectomized fish in high calcium water or caused hypocalcemia in intact fish (Pang *et al.,* 1973). A significant hypercalcemia and increased activity of type-1 cells of the corpuscles of Stannius was observed in the eels transferred from freshwater to seawater, but transfer to distilled water from

seawater caused a significant fall in plasma calcium levels (Hanssen *et al.,* 1991). These studies clearly demonstrate that corpuscles of stannius is involved in calcium homeostasis.

The ultrastructural observations presented in this study on the corpuscles of Stannius of cadmium exposed fish did not reveal any structural damage in type-1 and type-2 cells. However, the morphometric analysis showed that a significant decrease in the cell size and volume of granular endoplasmic reticulum of type-1 cells occurred after 35 days in low calcium acclimatized tilapia exposed to ambient and dietary cadmium. In addition, the corpuscles of Stannius index (CSI) of low calcium water adapted tilapia decreased significantly after 14 and 35 days exposure to ambient cadmium, and in fish fed cadmium food for 35 days. This suggests that the activity of the gland cells of the Stannius corpuscles was reduced by cadmium at least in fish from low calcium water.

Cadmium can enter the body *via* the gills or the gut (Part and Svanberg,1981; Segner and Black, 1985) and it can cause structural alterations in several tissues such as gills, kidneys and liver (Dallinger *et al.,* 1987). The present study shows that in tilapia exposed to ambient or dietary cadmium, the metal also affects the activity of the Stannius of corpuscles. In our previous study, cadmium caused significant gill damage in tilapia (Pratap and Wendelaar Bonga, 1993). These studies showed that the effect of both, ambient and dietary cadmium was greater in fish from low Ca^{2+} water than in fish from high Ca^{2+} water. This has been attributed to the protective effect of high water calcium. For several teleost species water hardness has been shown to reduce cadmium toxicity (Carroll *et al.,* 1979; Pascoe *et al.,* 1986). In the high Ca^{2+} water adapted tilapia exposed to ambient or dietary cadmium, cellular damage in type-1 cells of the corpuscles of Stannius was not observed. The increase of the corpuscles of Stannius index noticed in fish from high calcium water can be attributed to an increase in the number of cells since there was no significant difference in cell size or volume of organelles. The results of this study are therefore consistent with the earlier results and support the conclusion that high Ca^{2+} water levels protect against the toxic effects of cadmium (Pratap and Wendelaar Bonga, 1993). The type-2 cells of the corpuscles of Stannius did not respond to ambient or dietary cadmium in tilapia adapted to high or low calcium water. High activity of type-2 cells was observed in sticklebacks, trout and killifish when transferred from freshwater to seawater (Wendelaar Bonga *et al.,* 1976, 1980; Meats *et al.,* 1978). The type-2 cells in tilapia were not affected by cadmium nor by changes in the calcium content of the ambient water. The control tilapia acclimatized to low Ca^{2+} and high Ca^{2+} water did not show any significant differences with respect to the volume of the granular endoplasmic reticulum, Golgi complex, mitochondria and secretory granules of type-1 and type-2 cells of the corpuscles of Stannius. Cohen *et al.* (1975) found increased granular endoplasmic reticulum, dilated golgi system and depletion of granules in the corpuscles of Stannius of killifish in seawater, while these organelles were inactive in fish from calcium deficient seawater. The type-1 cells of the corpuscles of Stannius have been shown to become activated upon transfer of fish from freshwater to seawater (Wendelaar Bonga *et al.,* 1976; Meats *et al.,* 1978). This activation has been attributed to the high calcium content of seawater: The high secretory activity could be prevented when the fish were transferred to low calcium seawater or freshwater. Aida *et al.* (1980) found degranulation in corpuscles of Stannius of salmon after incubation in media containing 3 or 6 mM Ca^{2+} and after treatment with calimycin (A23187) in the presence of 1.5 mM Ca^{2+}. These authors also found that media containing only 1.5 mM Ca^{2+} or lower did not induce degranulation. Similarly, in the corpuscles of Stannius of rainbow trout, Wagner *et al.* (1989) found no stimulation of stanniocalcin release after lowering the Ca^{2+} concentration of the media. However, in rainbow trout and eels, Hanssen *et al.* (1991), found a basal release of stanniocalcin when the external calcium concentrations or the plasma calcium variations were within or below the physiological range (1.0-1.5 mM Ca^{2+}). This did not cause any obvious degranulation of the corpuscles of Stannius nor influence stanniocalcin release. These observations indicate, that the difference in the calcium concentration between high and low calcium water used in this study, was not sufficient to influence the activity of the corpuscles of Stannius of tilapia. From this study it is evident that cadmium induced hypocalcemia is not mediated by activation of corpuscles of Stannius and restoration to normal plasma calcium is not due to mobilization of calcium from acellular bone of tilapia.

References

Aida, K., R.S. Nishioka and H.A. Bern: Degranulation of corpuscles of Stannius of coho salmon *Oncorhynchus kisutch* in response to ionic changes *in vitro*. *Gen. Comp. Endocrinol.,* **41**, 305-313 (1980).

Calow, P.: Handbook of Ecotoxicology. Publ. Blackwell Science, USA (1998).

Carroll, J.J., S.J. Ellis and W.S. Oliver: Influence of hardness constituents on the acute toxicity of cadmium to brook trout (*Salvelinus fontinalis*). *Bull. Environ. Contam. Toxicol.,* **22**, 575-581 (1979).

Cohen, R.S., P.K.T. Pang and N.B. Clark: Ultrastructure of corpuscles of Stannius of the killifish *Fundulus heteroclitus* and its relation to calcium regulation. *Gen. Comp. Endocrinol.*, **27**, 413-423 (1975).

Dallinger, R., F. Prosi, H. Segner and H. Back: Contaminated food and uptake of heavy metals by fish: A review and a proposal for further research. *Oecologia,* **73**, 91-98 (1987).

Flik, G., J.C. Fenwick, Z. Kolar, N. Mayer Gostan and S.E. Wendelaar Bonga: Whole body calcium flux rates in cichlid teleost fish *Oreochromis mossambicus* adapted to freshwater. *Am. J. Physiol*., **249**, 432-437 (1985).

Flik, G., T.J.M. Schoenmakers, J.A. Groot, C.H. van Os and S.E. Wendelaar Bonga: Calcium absorption by fish intestine: the involvement of ATP and sodium dependent calcium extrusion mechanisms. *J. Membr. Biol.,* **113**, 13-22 (1990).

Giles, M.: Electrolyte and water balance in plasma and urine of rainbow trout (*Salmo gairdneri*) during chronic exposure to cadmium. *Can. J. Fish Aquat. Sci.,* **41**, 1678-1685 (1984).

Hanssen, R.G., F.P. Laferber, G. Flik and S.E. Wendelaar Bonga: Ionic and total calcium levels in the blood of the European eel *Anguilla anguilla*: Effects of stainnectomy and stanniocalcin replacement therapy. *J. Exp. Biol.,* **141**, 177-186 (1989).

Hanssen, R.G., E.M. Aarden, W.P. Van der Venne, P.K.T. Pang and S.E. Wendelaar Bonga: Regulation of the secretion of the teleost fish hormone

stanniocalcin: Effects of extracellular calcium. *Gen. Comp. Endocrinol.*, **83**, (1991).

Klaverkamp, J.F. and D.A. Duncan: Acclimation to cadmium toxicity by white suckers, cadmium binding capacity and metal distribution in gill and liver cytosol. *Environ. Toxicol. Chem.*, **6**, 275-289 (1987).

Koyama, J. and Y. Itazawa: Effects of oral administration of cadmium on fish I. Analytical results of the blood and bones. *Bull. Jap. Soc. Sci. Fish*, **43**, 523-526 (1977).

Krishnamurthy, V.G.: Cytophysiology of corpuscles of stannius. *Int. Rev. Cyto.*, **46**, 177-246 (1976).

Kuroshima, R.: Cadmium accumulation and its effects on calcium metabolism in *Girella punctata* during a long term exposure. *Nippon Suisan Gakkaishi*, **53(3)**, 445-450 (1987).

Larsson, A., B.E. Bengtsson and C. Haux: Disturbed ion balance in flounder, *Platichthys flesus* L., exposed to sublethal levels of cadmium. *Aquat. Toxicol.*, **1**, 19-35 (1981).

Meats, M., P.M. Ingleton, I. Chester Jones, H.O. Garland and C.J. Keyon: Fine structure of the corpuscles of Stannius of the trout *Salmo gairdneri* : Structural changes in response to increased environmental salinity and calcium ions. *Gen. Comp. Endocrinol.*, **36**, 451-461 (1978).

Muramoto, S.: Vertebral column damage and decrease of calcium concentrations in fish exposed experimentally to cadmium. *Environ. Pollut.* (Ser. A), **24**, 125-133 (1981).

Pang, P.K.T., R.K. Pang and W.H. Sewyer: Effects of removal of stannius corpuscles and replacement therapy on serum calcium levels of the Killi fish *Fundulus heteroclitus. Endocrinol.*, **93**, 705-710 (1973).

Part, P. and O. Svanberg: Uptake of cadmium in perfused rainbow trout (*Salmo gairdneri*) gills. *Can. J. Fish Aquat. Sci.*, **38**, 917-923 (1981).

Pascoe, D., S.A. Evans and J. Woodworth: Heavy metal toxicity to fish and the influence of water hardness. *Arch. Environ. Contam. Toxicol.*, **15**, 481-487 (1986).

Pratap, H. B., S.E. Wendelaar Bonga: Effect of waterborne and dietary cadmium on pavement cells, chloride cells and Na^+-K^+ ATPase activity in the gills of the freshwater teleost *Oreochromis mossambicus* at normal and high calcium levels in ambient water. *Aquat. Toxicol.*, **26**, 133-150 (1993).

Pratap, H. B., S.E. Wendelaar Bonga: Mineral composition and cadmium accumulation in *Oreochromis mossambicus* exposed to waterborne cadmium. *Bull. Environ. Contam. Toxicol.*, **72(4)**, 741-749 (2004).

Rand, G.M.: Fundamentals of aquatic toxicology effects: Environmental fate and risk assessment. Taylor and Francis, USA (1995).

Reader, J.P. and R. Morris: Effects of aluminium and pH on calcium fluxes, and effects of cadmium and manganese on calcium and sodium fluxes in brown trout (*Salmo trutta*). *Comp. Biochem. Physiol.*, **91(C)**, 449-457 (1988).

Segner, H. and H. Black: Importance of contaminated food for uptake of heavy metals in rainbow trout, *Salmo gairdneri. Natuurwissench.*, **72**, 379-380 (1985).

Sorensen, E.M.: Metal poisoning in fish. CRC Press, Boca, Raton, Fl. USA (1991).

Urasa, F.M. and S.E. Wendelaar Bonga: Stannius corpuscles and plasma calcium levels during the reproductive cycle in the cichlid teleost fish, *Oreochromis mossambicus. Cell. Tiss. Res.*, **241**, 219-227 (1985).

Van der Heijden, A.J.H., P.M. Verbost., M.J.C. Bijvelds., W. Astma., S.E. Wendelaar Bonga and G. Flik: Effects of seawater and stainniectomy on branchial Ca^{2+} handling and drinking rate in eel (*Anguilla anguilla*). *J. Exp. Biol.*, **202**, 2505-25011 (1999).

Verbost, P.M., G. Flik, R.A.C. Lock and S.E. Wendelaar Bonga: Cadmium inhibition of Ca^{2+} uptake in rainbow trout gills. *Am. J. Physiol.*, **253**, 216-221 (1987).

Wagner, G.F., B. Gellersen and H.G. Friesen: Primary culture of teleocalcin cells from rainbow trout corpuscles of Stannius, regulation of teleocalcin secretion by calcium. *Mol. Cell. Endocrinol.*, **62**, 31-39 (1989).

Wendelaar Bonga, S.E., J.A. Greven and M. Veehuis: The relationship between ionic composition of the environment and secretory activity of the endocrine cell types of stannius corpuscles in the teleost *Gasterosteus aculeatus. Cell. Tiss. Res.*, **175**, 297-312 (1976).

Wendelaar Bonga, S.E., J.C.A. Van der Meij and P.K.T. Pang: Evidence for two secretory cell types in the stannius bodies of the teleost *Fundulus heteroclitus*, and *Carassius auratus. Cell. Tiss. Res.*, **212**, 295-306 (1980).

Wendelaar Bonga, S.E. and J.C.A. Van der Meij: Degeneration and death by apoptosis and necrosis of the pavement and chloride cells in the gills of the teleost *Oreochromis mossambicus. Cell. Tiss. Res.*, **255**, 235-243 (1989).

Wendelaar Bonga, S.E. and P.K.T. Pang: Control of calcium regulating hormones in the vertebrates, parathyroid hormone, calcitonin, prolactin and stanniocalcin. *Int. Rev. Cytol.*, **128**, 139-213 (1991).

Wendelaar Bonga, S.E. and R.A.C. Lock: Toxicants and osmoregulation in fish. *Neth. J. Zool.*, **42**, 478-493 (1992).

Yamawaki, K., W. Hashimoto, K. Fuji, J. Koyama, Y. Ikeda and H. Ozaki: Hemochemical changes in carp exposed to low cadmium concentrations. *Bull. Jap. Soc. Sci. Fish*, **52**, 459-466 (1986).