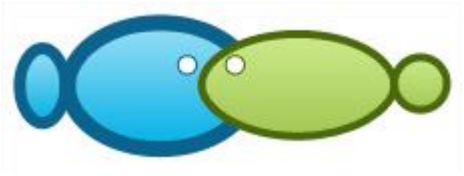

Histopathology of nephrocalcinosis in some ornamental fishes

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Abstract. Nephrocalcinosis is a chronic disorder which may be resulted in decrease in growth rate and finally mortality in severe condition. Nephrocalcinosis is defined as the presence of calcification in the kidney. A total number of 80 ornamental fishes of different species included of discus fish (*Symphysodon discus*), angelfish (*Pterohyllum* sp.), goldfish (*Carassius auratus*) and oscar fish (*Astronotus ocellatus*) were randomly collected from some aquarium shops. The clinical examination indicated the presence of excessive mucous secretion, detachment of the scales, exophthalmia. Also skin petechiae and haemoharrgia particularly at the base of the fins was observed. Histopathological examination revealed presence of basophilic deposits in tubular sections of kidney, distinct dilation of tubules and fibrosis around glomerulus with chronic glomerulitis and multiple microscopic calculi in tubules with different size can be pathognomonic for this disease and it is the best indicator for diagnosis of this disease.
Key Words: fish pathology, aquarium, aquaculture, basophilic deposits, urolithiasis, chronic glomerulitis.

Introduction. Ornamental fish breeding and culture is a fast-growing business in the world. But many potential stressors such as unsuitable management and environmental conditions affect ornamental fish and interact with natural defenses of fish, so they cannot effectively protect themselves against pathogens (Ruth 2002).

In fish, the kidney is a vital organ that receives the vast majority of post-branchial blood. Renal lesions may be expected to be good indicators of environmental stressors (Hinton & Laurén 1990).

Nephrocalcinosis (NC) is a chronic disease which is characterized by degenerative changes and deposition of calcium salts in kidney tissue (Schlotfeldt 1980; Bruno 1996). Bruno (1996) presented that nephrocalcinosis (urolithiasis) is the term used to describe the deposition of calcium and magnesium salts within the urethras and collecting ducts of farmed and wild fish. Grossly the kidney appears swollen and grey in color with an irregular surface and a white deposit in the urethras.

Renal calcification may occur at molecular, microscopic or macroscopic levels on different locations such as tubular cells, interstitial tissue or within the tubular lumen that leading to progressive amounts of renal damages (Sayer et al 2004).

Nephrocalcinosis was reported in rainbow trout (Schlotfeldt 1980; Mulcahy et al 1984; Ferguson 1989), other salmonidae (Landoblt 1975), Atlantic halibut larvae (Diamant et al 1991), and angelfish, *Pterophyllum scalare* (L.) (Sangmaneedet 1999).

In fish diseases, histopathology is increasingly being used as an indicator of environmental stressors and pathogens (Stentiford et al 2003). Therefore, histopathology can provide good information about changes which are occurring in tissues (Rodger 2010). The objective of this study was to assessment of histopathological alterations caused by the nephrocalcinosis in some species of ornamental fishes.

Material and Method. Eighty fish samples of four species, including discus (*Symphysodon discus*), angelfish (*Pterohyllum* sp.), goldfish (*Carassius auratus*) and

oscar fish (*Astronotus ocellatus*), were randomly obtained from some aquarium shops located in Abadan and Khorramshar cities in Iran. Collected fish clinically have shown some symptoms such as lethargy and anorexia. Fish were transferred to the fisheries laboratory in glass aquaria, supplied with chlorine-free tap water with continuous aeration. Clinical examination was performed then fish were euthanized by eugenol. After necropsy, kidney samples were carefully collected and were fixed in 10% formalin buffer. After fixation, the tissue samples were processed and then were embedded in paraffin and sectioned into five micrometers thickness and subsequently stained by haematoxylin-eosin (H&E). The sections were studied by light microscope and related pictures were captured (Gurr 1972).

Results and Discussion. In this study, clinical examination indicated the presence of various clinical signs including excessive mucous secretion, scales detachment, exophthalmia, petechia and hemorrhage particularly at the base of the pectoral fins.

The kidney of all fishes did not show any macroscopic lesions. The microscopic sections revealed the presence of basophilic deposits in tubular sections of kidney. The incidence of nephrocalcinosis in different fish species was revealed in Table 1.

Table 1

The incidence of nephrocalcinosis in goldfish (*Carassius auratus*), angel fish (*Pterohyllum* sp.), oscar (*Astronotus ocellatus*) and discus (*Symphysodon discus*)

Fish species	*Number of susceptible species*
Angel fish	6 (out of 20)
Gold fish	3 (out of 20)
Oscar	1 (out of 20)
Discus	1 (out of 20)
Total	11 (out of 80)

The most intensive lesions were found in *P. scalare*. Microscopically, basophilic deposits were observed in tubules and interstitial tissue. In two *P. scalare*, basophilic deposits were large particles which were occupied a part of interstitial tissue and renal tubules (Figure 1).

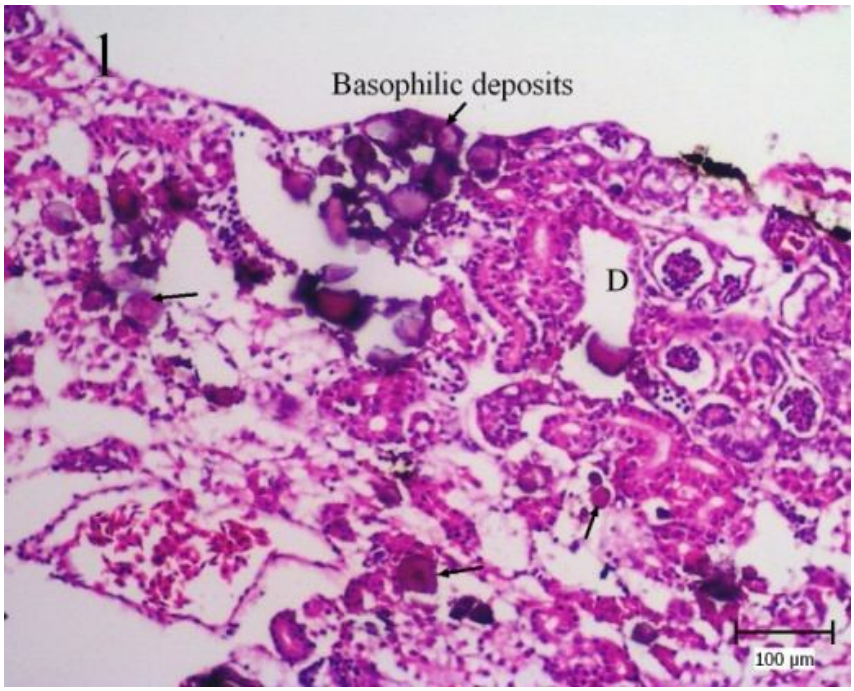

Figure 1. *Pterophyllum scalare* kidney. Demonstrating predominantly basophilic deposits (arrows) and enlargement of renal tubules (D) (H&E staining, Bar 100 µm) (original).

In some cases, tubules had distinct dilation and fibrosis around glomerulus with chronic glomerulitis (thickening of Bowman's capsule and adhesion of tufts to it) were observed (Figure 2).

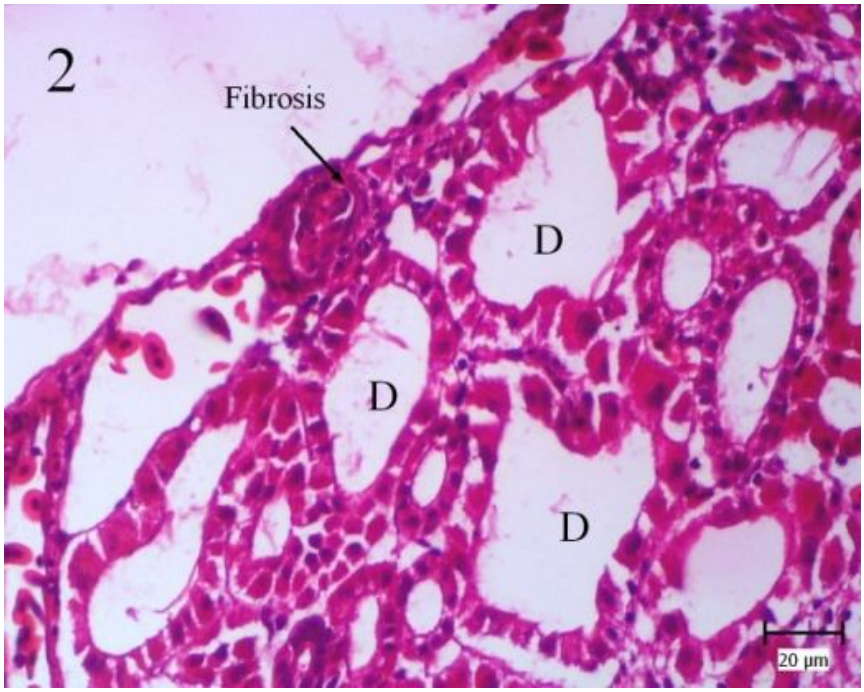

Figure 2. *Pterophyllum scalare* kidney. Note periglomerular fibrosis and occluded Bowman's space and dilation of tubules with the distended lumen (D) (H&E staining, Bar 20 µm) (original).

Microscopic calculi were seen in tubules and they were multiple with different size that the smallest one was around 26 µm (Figure 3). These were accompanied by other degenerative changes in the tubules such as cell swelling and necrosis of tubules.

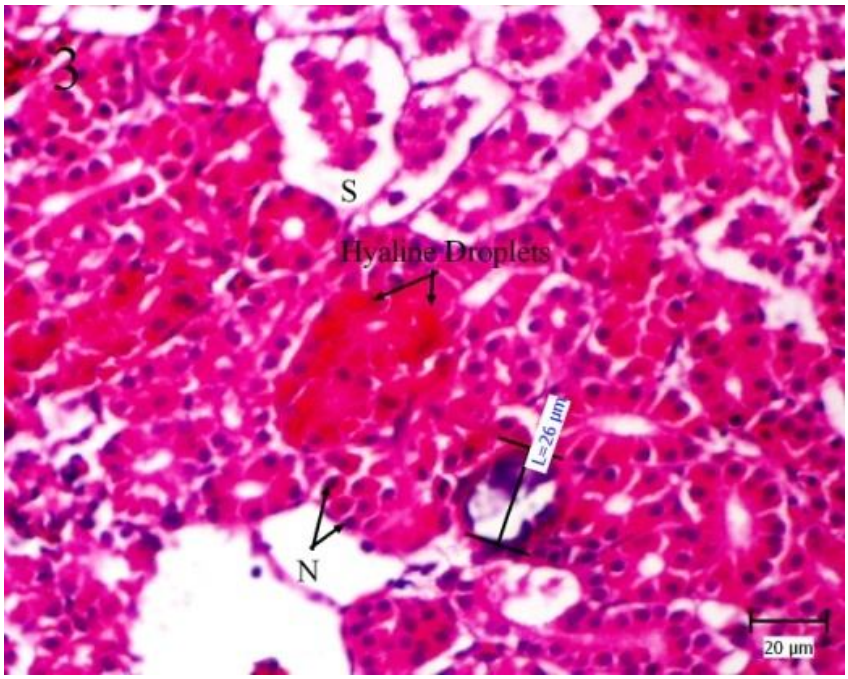

Figure 3. *Carassius auratus* kidney. The smallest microscopic calculi with basophilic characteristics which has been seen in this research. Cell swelling of epithelial cells (S), Tubular necrosis (N) and hyaline droplets were detected in proximal tubules (H&E staining, Bar 20 µm) (original).

Nephrocalcinosis is a granular deposit composed mainly from calcium phosphate (Smart et al 1979) may involve part of the kidney tubules and ducts, which become sclerotic (Harrison & Richards 1979).

Etiology of this disease is still uncertain and it is believed that it is related to dietary factors and or alteration in water physical and chemical parameters (Ferguson 1989). Bruno (1996) presented that there are two circumstances which are considered to cause or exacerbate nephrocalcinosis. Firstly, a prolonged exposure of fish to high levels of carbon dioxide at levels greater than 10 to 20 mg/L, and secondly a nutritional aspect where a magnesium deficiency and a selenium toxicity have been implicated. The severity and prevalence of nephrocalcinosis is considered to increase with increasing carbon dioxide levels, which may be particularly high in some ground waters or the bottom water of deep lakes and where water may be used intensively in aquaculture systems, e.g. where oxygen is added without removal of the accumulated respiratory carbon dioxide. Other factors including overcrowding and low water flow may also accentuate nephrocalcinosis. An excess of carbon dioxide in the blood of the fish leads to a metabolic acidosis which resulted in the precipitation of mainly calcium salts in the urine. The outcome may be significantly influenced by the diet used in aquaculture situations.

In the current study, the clinical examination of randomly collected ornamental fishes indicated presence of different general clinical signs including excessive mucous secretion, detachment of the scales, exophthalmia, body deformity and external hemorrhage which were seen and reported by Bruno (1996) as well. But these clinical signs were not specific to nephrocalcinosis. Although, nephrocalcinosis by interference with normal operation of kidney and other organs can caused abnormalities in fish physiology (Ferguson 1989) and subsequently, some abnormal clinical signs.

In the present study, nephrocalcinosis was found in *P. scalare* more severe than other species. It may be attributed to the different histopathological manifestations of the disease in studied fish. In *P. scalare*, predominantly basophilic deposits are mainly associated with the enlargement of renal tubules which appear to contain it in such a way as to minimize renal damage. The disease resulted in massive destruction of renal tissue due to tubular distension and obstruction, with associated necrosis, fibrosis and granuloma formation, which is occasionally found in intensive fish culture (Tixerant et al 1984; Paperna 1987).

Results of this study showed the histopathology of nephrocalcinosis with microscopic calculi with basophilic characteristics, which is a diagnostic feature of the disease and it may be, in fact, a secondary phenomenon occurring by accretion to necrotic cell debris or to abnormal metabolic products accumulated in the renal tissue. Mulcahy et al (1984) reported the pattern of calcium deposits in the kidney of freshwater and saltwater farmed rainbow trout. Therefore, nephrocalcinosis as a disease which may be initiated by a variety of etiological aspects which cause either necrosis of renal cells or deposition in the kidney. According to Eddy et al (1979), high carbon dioxide levels may interfere with normal kidney tubular function, resulting in alkaline tubular fluid and urine, which in turn favor the formation of calcium phosphate deposits (nephrocalcinosis).

A probable relation between prolonged increased levels of CO_2 in freshwater and an increased occurrence of nephrocalcinosis in some fish species has been demonstrated by some researchers (Smart et al 1979; Fivelstad et al 1999). It is also commonly observed in seabass and seabream with the same mechanism (Marino et al 2007) and with reduced conversion factor and reduced growth in Atlantic salmon (Fivelstad et al 1999). Unsuitable feeding, as another possible cause (Cowey et al 1977) was reported by Schlotfeldt (1980) that nephrocalcinosis in intensive culture is arising from a multiple factors, in which the complex of environmental conditions has a critical importance. Golomazou et al (2006) reported that nephrocalcinosis was found in all sampled fish and this was suspected to be related to *Myxobolus* sp. infection.

Conclusions. Finally this research has revealed that nephrocalcinosis may be occurred in some ornamental fish without any specific external signs but some histopathological results such as presence of basophilic deposits in tubular sections of kidney, distinct

dilation of tubules and fibrosis around glomerulus with chronic glomerulitis and multiple microscopic calculi in tubules with different size can be pathognomonic and it is the best indicator for diagnosis of this disease and also, the results of this study indicates that prevention from unsuitable environmental condition or intensive condition may be decreased prevalence of nephrocalcinosis.

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