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Histological assessment of gill pathology in two fish species (*Clarias gariepinus* and *Oreochromis niloticus*) From the Sanyati Basin in Lake Kariba, Zimbabwe

N. Mabika [1*], M. Barson [2, 3]

[1] *Department of Anatomy, University of Zimbabwe. PO Box MP 167, Mt Pleasant, Harare, Zimbabwe*

[2] *Department of Biological Sciences, University of Zimbabwe. PO Box MP 167, Mt Pleasant, Harare, Zimbabwe*

[3] *Department of Zoology, University of Johannesburg, Auckland Park campus, PO Box 524, Auckland Park, 2006 Johannesburg, South Africa*

Abstract

Histopathology is one of the bioindicators of pollutants in aquatic organisms. Two fish species, African catfish *Clarias gariepinus* and Nile tilapia *Oreochromis niloticus* were sampled by seine nets and fyke nets from the Sanyati basin, Lake Kariba, Zimbabwe. Gill tissues were dissected from the fish, preserved for histology, sectioned and stained in haematoxylin and eosin. The sections were observed under a light microscope. Histopathological lesions observed included hyperplasia of lamellae, lamellae fusion, aneurysm, epithelial lifting and curving of secondary lamellae. All these lesions were more prevalent in *Clarias gariepinus* than in *Oreochromis niloticus*, except for the lifting of the epithelium. These results indicate that the fish have been exposed to stressors, with *Clarias gariepinus* being more affected than *Oreochromis niloticus*. However the effects of these stressors have been mild to moderate. We therefore recommend a biomonitoring and toxicological monitoring framework for Lake Kariba to monitor the water quality and fish health, as to ensure sustainability of the fishery.

Keywords: Histological lesions, *Clarias gariepinus*, *Oreochromis niloticus*, Biomonitoring, Lake Kariba

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[*] Corresponding author. *E-mail address:* mabikan2@yahoo.co.uk

1. Introduction

Histopathological biomarkers can be sensitive indicators of subcellular stress in organisms exposed over short and long periods to a range of pollutants (Adams et al., 2000). These biomarkers embody tissue lesions arising as a result of previous or current exposure of the organism to one or more toxins (Velkova-Jordanoska and Kostoski, 2005). Well- documented lesions based on experimental data in liver, ovary, skeleton system and skin have been used as biomarkers to date (Hinton et al., 1985).

Gills are sensitive organs which are easily damaged by numerous pollutants, even at low concentrations (Karlsson, 1983). Since the gills perform various vital functions (respiration, osmoregulation and excretion) and have a large surface area in contact with the external environment, they are particularly sensitive to chemical and physical changes of the aquatic environment, thereby being the target organ in fish for pollutants carried out by water (Mallat 1985; Cerqueira and Fernandez, 2002). Consequently, the presence of toxic substances in an aquatic environment causes alterations in the vital functions carried out by the gills and alterations in the morphologic structure of these organs. Poleksic and Mitrovic-Tutundzi (1994) and Stentiford et al. (2003) have actually utilized the gills of fish as method for evaluating the presence of pollutants in natural environments. These field studies are more important components for the evaluation and understanding of the biological or ecological effects of chemical agents under natural conditions. The main advantages of such studies include the use of realistic exposures which will determine directly the effects observed, and the use of natural environments which preclude the necessity of extrapolation of the results for the ecosystem (Graney et al., 1995)

African catfish *Clarias gariepinus* is omnivorous, utilising a wide variety of aquatic and terrestrial foodstuffs through scavenging (Skeleton, 1993). The Nile tilapia *Oreochromis niloticus* feeds on algae, insects and zooplankton, becoming predominantly phytoplanktivorous as it grows bigger (Marshall, 2010). The catfish is endemic to the Zambezi River system while the Nile tilapia was introduced into the lake in the 1990's when aquaculture activities intensified in the lake (Marshall, 2010). Both species can live in different water bodies from lakes to rivers and sewage canals, and tissue and organ abnormalities resulting from environmental stress can be observed. These factors make them good indicators of chronic environmental stress, enabling them to reflect accumulative effects of both past and recent water quality conditions (Avenant-Oldewage, 2001).

Most studies in Lake Kariba have focused on chemical methods to assess the water quality (Mathiesen, 1985; Berger et al., 1992). These chemical methods allow determination of the extent of pollution but not that of its damage to living organisms (Rai et al., 2002). In Zimbabwe the use of bioindicators has been generally neglected. As a result, the use of fish species as monitoring tool to reflect the health status of an aquatic ecosystem has rarely been investigated. Few studies done in the Manyame system around the capital city of Harare explored the use of fish parasite communities as bioindicators (Madanire-Moyo and Barson, 2010), histological and parasitological responses to heavy metal pollution (Mabika, 2009), and a preliminary application of the fish health assessment index on the Manyame lakes (Taruvinga, 2011).

Economic activities in and around Kariba town and the lake are largely dominated by fisheries and aquaculture. Many of the inhabitants depend on subsistence fishing for livelihoods, while artisanal fishing cooperatives and aquaculture companies employ the bulk of the working population. It is therefore an understatement that there is urgent needs to understand and address fish health issues in Lake Kariba. Industrial activities around the Sanyati basin of the lake, which accommodates Kariba town and its Zambian neighbour Siavonga, include fish and crocodile farming and processing, boating and tourism, hydropower generation, among others, which contribute heavy metal, persistent organochlorines and chemical pollution to the basin. Domestic effluents from municipal sewers, as well as crocodile and fish farm organic effluents, all find their way into the waters of this basin. The resident fish species are therefore exposed to these stressors, and little research has been documented to determine the potential impact of the stressors on fish and public health.

The objective of this study was therefore to assess the extent of histological damage to fish gills from African catfish *Clarias gariepinus* and Nile tilapia *Oreochromis niloticus* in the Sanyati basin of Lake Kariba. This study could provide valuable information for fish biologists working in Lake Kariba and could provide useful information for biomonitoring programmes regarding the effects of pollutants on the health of fish populations within the Sanyati basin.

1.1. Study site

Lake Kariba is situated between Zimbabwe and Zambia around 17°S and 28°E. It is 320km long, has a maximum width of 40km, mean depth of 29.5m, maximum depth of 120m and a surface area at average water level 5 250km^2 . The lake is divided into five distinct hydrological basins of which the Sanyati basin (Figure 1) is the most northern and believed to be the most nutrient rich as the Sanyati river which drains a large part of Zimbabwe's agricultural land enters into the basin (Balon and Coche, 1974).

2. Materials and methods

Samples of *C. gariepinus* were collected by fyke nets whilst samples of *O. niloticus* were collected by seine netting in the Sanyati basin of Lake Kariba (16° 31'S, 028° 50' E, 496 m asl) in August 2011. They were all transported to laboratory in aerated tanks full of lake water. For each individual, the spinal cord was severed before the gills were excised keeping the filaments and rakers intact. Dissecting tools were rinsed in 70% alcohol, washed in distilled water and dried with paper towel between each dissection. The tissues were fixed in 10% buffered formalin for 48 hours.

The fixed tissues were dehydrated and infiltrated with molten wax on a processing machine. They were embedded in paraffin wax, sectioned into 5μm slices by a rotary microtome and stained using haematoxylin and eosin. The sections were examined and photographed using a Leica Leitz Labor Lux microscope fitted with a camera (Leica DX 0.32X) and an automatic light exposure unit (Leica Orthomate E).

2.1. Water quality

Surface water temperature and dissolved oxygen content were measured using a HACH oxygen meter, conductivity using a WTW conductivity meter and pH using a HACH pH meter.

3. Results

3.1. Water quality

The values of the basic physicochemical parameters are shown in Table 1. Water pH was close to neutral (7.11), whilst the mean temperature was 28.3°C. Conductivity was 128.40μS.cm^{-1} and dissolved oxygen was 4.20mgl^{-1}.

3.2. Histopathology

A total of 30 *C. gariepinus* were analysed. The standard lengths of the specimens varied from 335mm to 602mm. Thirty individuals of *O. niloticus* were also analysed. The standard lengths varied from 147mm to 240mm.The histological lesions observed in both fish species included; hyperplasia of lamellae, lamellae fusion, aneurysm, lifting of respiratory epithelium and curving of secondary lamellae (Table 2).

Hyperplasia (Figure 5) was the major lesion observed in both species which accounted for 77% in *C. Gariepinus* and 50% in *O. niloticus*. Lamella fusion (Figures 3 and 5), aneurysm (Figure 4) and curving of secondary lamellae (Figure 6) were all higher in *C. gariepinus* than in *O. niloticus*. Lifting of the respiratory epithelium (Figure 5) was the same in both species.

4. Discussion

It is very difficult to find an aquatic ecosystem which is free from pollutants. Hence most water bodies are subjected to pollutants and the only difference is in the magnitude of pollutants. The exposure of fish to various biotic and abiotic stressors during several years through their lifespan can cause remarkable anomalies especially hyperplasia and related lesions like fusion of adjacent lamella. The severity of damage depends on the concentration of toxicants and the period of exposure (Oliveira et al., 1996). These lesions after prolonged exposure can lead to fish mortality.

In this study the major lesion observed in both fish species was hyperplasia. Hyperplasia is a fish's response to ward off or block something that irritates its tissue whether externally or internally (Roberts, 2001). It may be an early response of the gills to harmful substances in the water or sediments. This observation also corroborates earlier work by Movahedinia et al. (2012) who also observed a high incidence of hyperplasia in two species of sturgeons.

Fusion of secondary lamellae, which is a result of hyperplasia, could cause a decrease in free gas exchange, thus affecting the general health of the fish (Skidmore and Tovel, 1972). Arrelano et al. (1999) reported lamellae fusion in copper treated specimens of *Solea senegalensis*. This could also imply that the fish collected in this study were also responding in a similar manner in Kariba. However, the fused lamellae were generally focal and moderate in both fish species.

Aneurysm results from accumulation of blood in secondary lamellae (Alazemi et al., 1996), to protectthe gill epithelia from infection and possible mechanical abrasion (Bagwart and Elahee, 2002). Some researchers have suggested that aneurysm might also be used as a sensitive and reliable biomarker of acute toxicant exposure to metals such as cadmium and copper (Alazemi et al., 1996; Monteiro et al., 2008). Epithelial lifting appears to be one of the initial reactions of a gill to a variety of pollutants (Smart, 1976; Mallat, 1985; Authman and Abbas, 2007). In this study the percentage of individuals affected was the same in both species. It may act as a protective measure by increasing the pollutant blood diffusion distance, impairing oxygen uptake, typical of an acute inflammatory response. However under these conditions the fish increase their rate of respiration by compensating for the low entrance of oxygen (Fernandes and Mazon, 2003).

Curving of the secondary epithelium observed in this study was an attempt by the fish to reduce available surface area to the stressors. A similar histopathological response was also recorded by Gabriel et al. (2007) when juvenile *C. gariepinus* were exposed to kerosene. The negative impact of such a response is that it reduces the available surface area for respiration and ionic exchange, consequently resulting in an internal hypoxic and toxic environment.

Sensitivity to pollutants, as well as pollution-induced histopathological features may vary within a wide range depending on the species (Bernet et al., 1999). This was observed in this study as *C. gariepinus* had a greater proportion of histopathological manifestations on the gills than *O.niloticus*.

In conclusion, results of this study indicate that *C. gariepinus* and *O. niloticus* in Lake Kariba are indeed responding to some stressors whose exact nature still needs to be determined. However, the extent of gill damage was generally mild to moderate except for hyperplasia which could be a cause of concern. Moyo (1997) established that massive mortalities of *Oreochromis macrochir* observed in Lake Chivero, Zimbabwe, were a result of hyperplastic gills caused by ammonia accumulation during Lake turnover. As current debate on impacts of climate variability on the aquatic environment rages, it may be opportune to compliment routine limnochemical monitoring of strategic water bodies such as Lake Kariba with routine biological monitoring programmes. Availability of such data will be invaluable for present and future sustainable management of aquatic resources.

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Figures and Tables

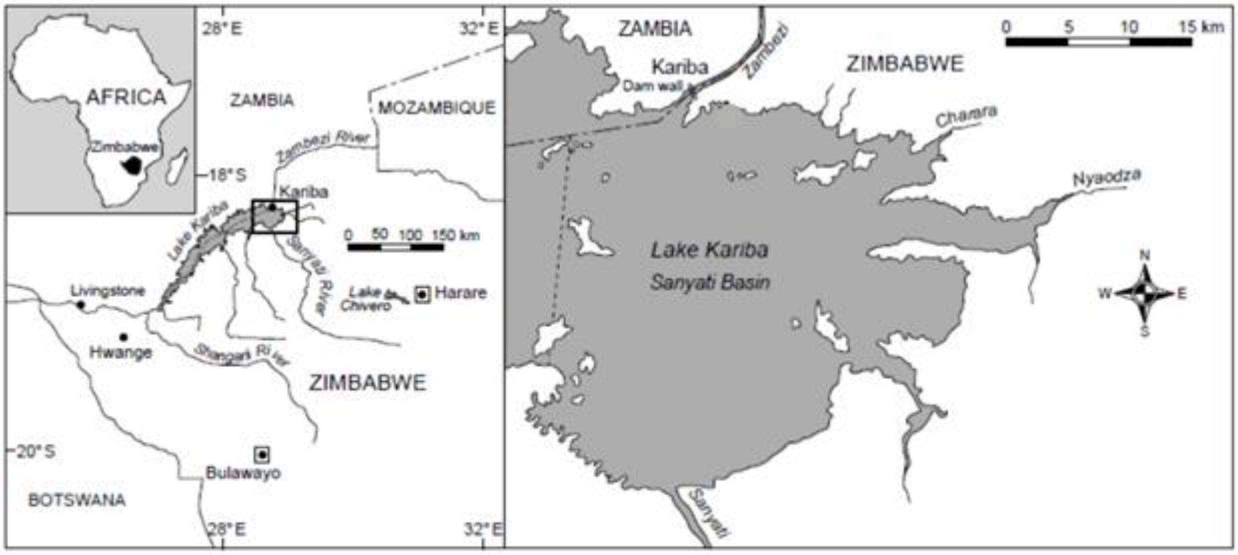

Figure 1. Map showing the Sanyati basin

Table 1. Basic physicochemical parameters

pH	7.11
Temperature (^{o}C)	28.30
Conductivity (µS.cm^{-1})	128.40
Dissolved Oxygen (mgl^{-1})	4.20

Table 2. Histopathological lesions and the number of fish affected in each species

Histopathological lesion	**Number affected in *C.gariepinus*(N=30)**	**Number affected in *O.niloticus*(N=30)**
Hyperplasia of lamellae	23 (77%)	15 (50%)
Lamellar fusion	10 (33%)	7 (23%)
Aneurysm	14(47%)	10(33%)
Lifting of respiratory epithelium	8(27%)	8(27%)
Curving of secondary lamellae	6(20%)	2(6%)

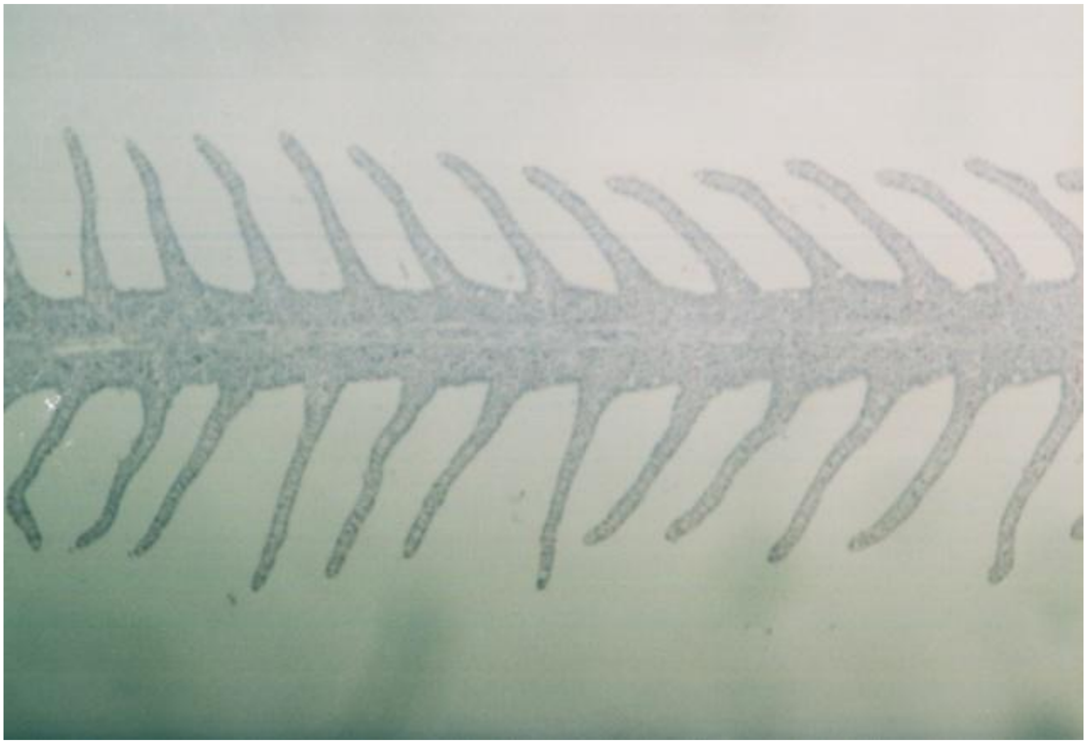

Figure 2. Normal fish gill

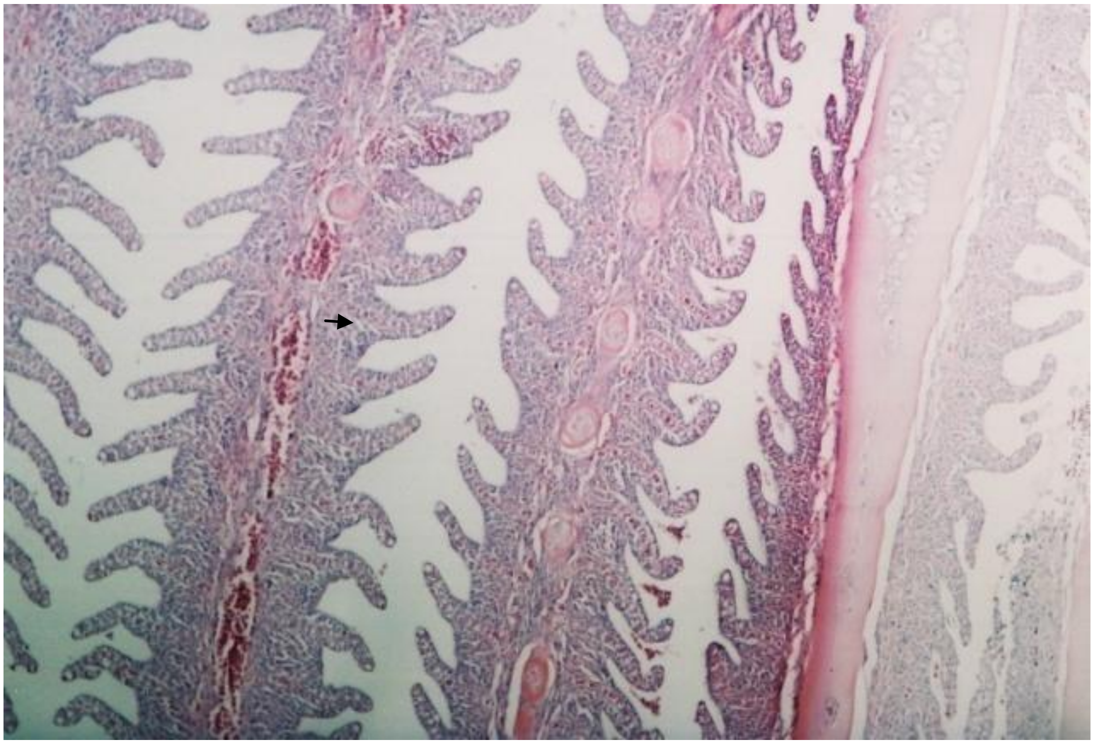

Figure 3. Focal lamellae fusion (arrow)

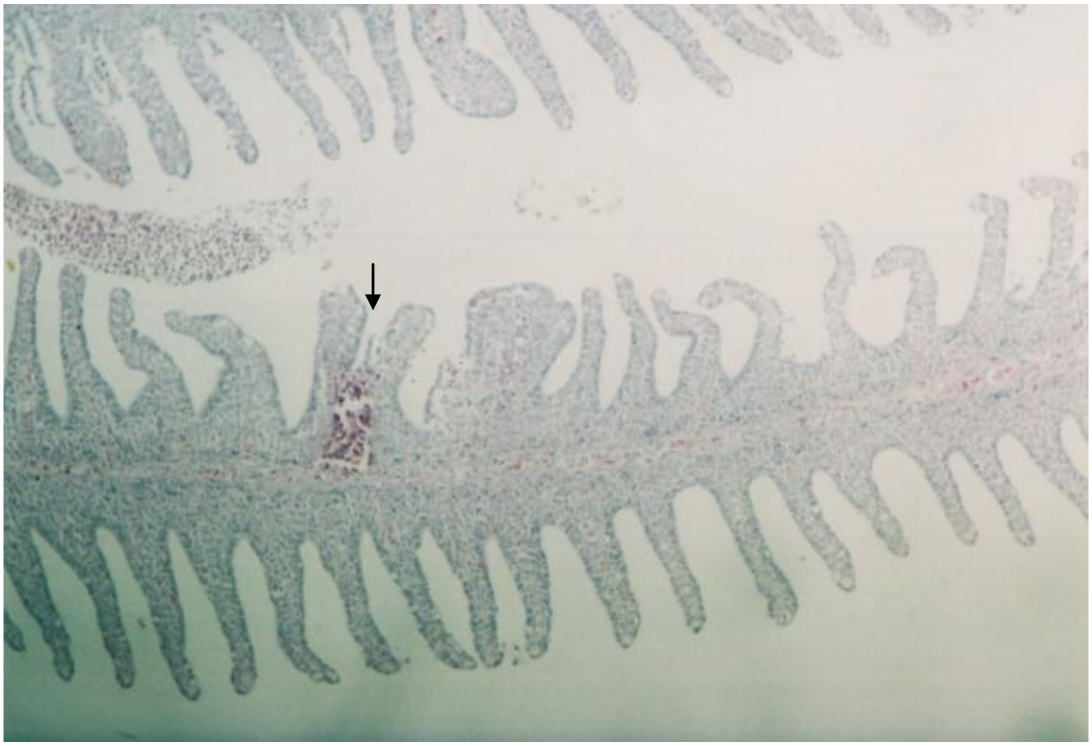

Figure 4. Aneurysm(arrow) of secondary lamellae

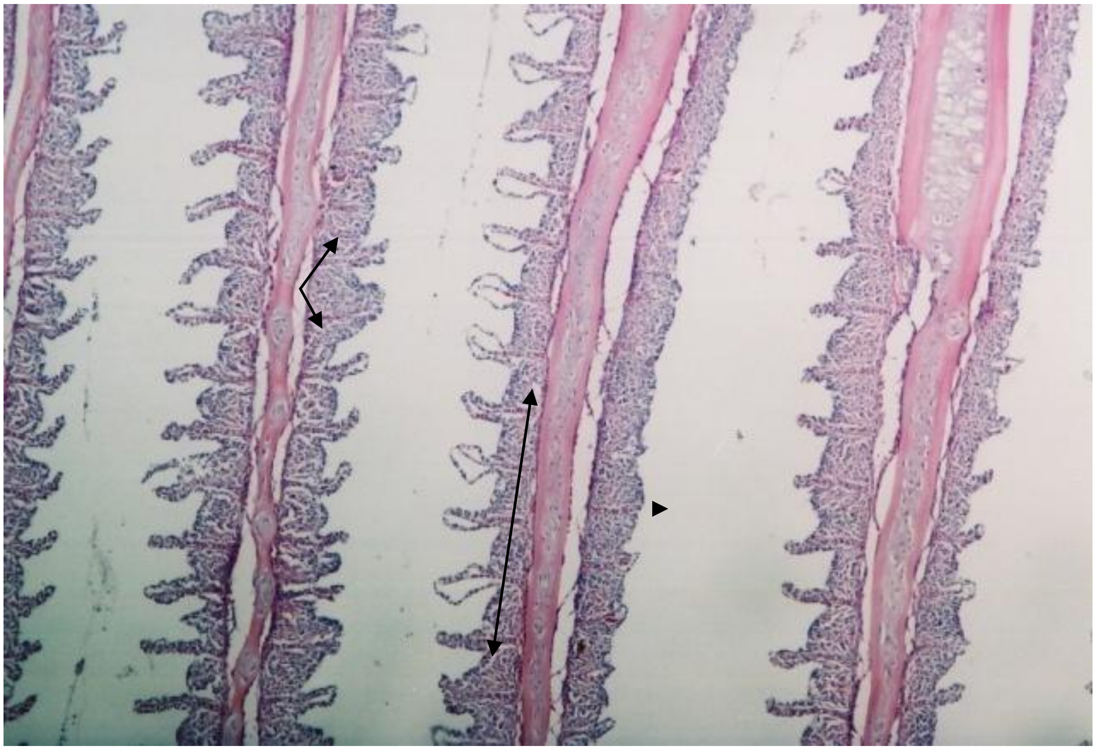

Figure 5. Epithelial lifting (arrows), hyperplasia(arrowhead) and lamellae fusion(double arrow)

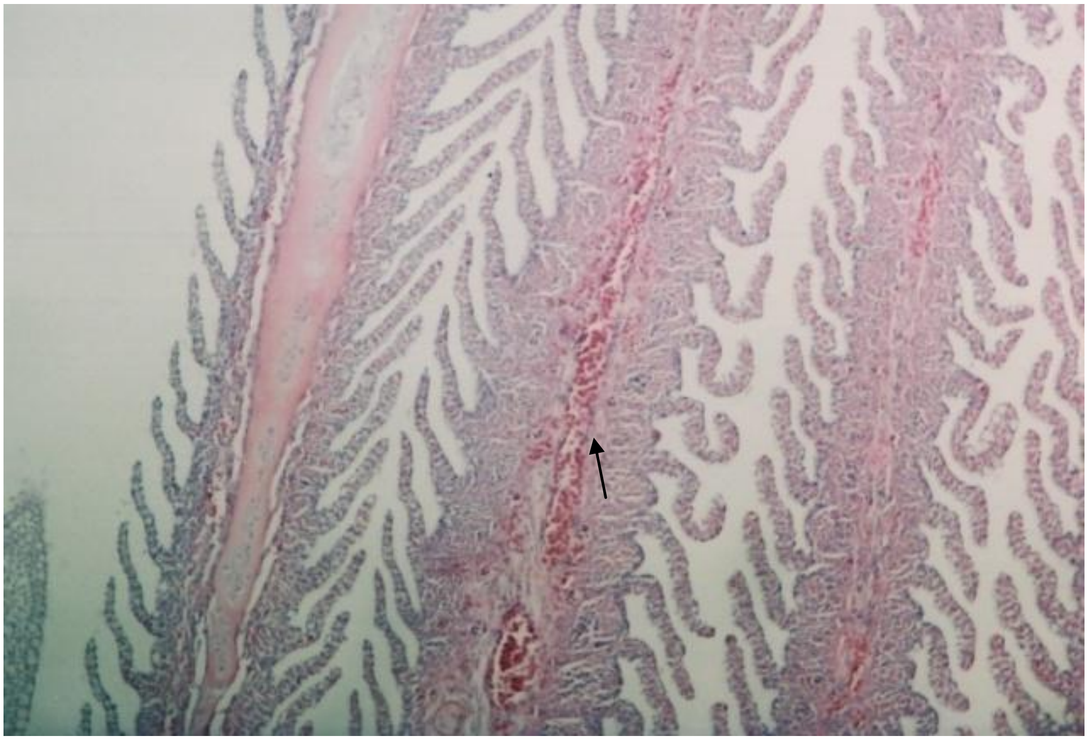

Figure 6. Curving (arrow) of secondary lamellae