Pathologic Gill Lesions in Two Edible Lagoon Fish Species, *Mulloidichthys flavolineatus* and *Mugil cephalus*, from the Bay of Poudre d’Or, Mauritius

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**Key words:** yellowstripe goatfish, *Mulloidichthys flavolineatus*, flathead mullet, *Mugil cephalus*, gill lesions, marine pollution, Mauritius

**Abstract**—This paper reports on a preliminary study carried out on gill tissues of two edible lagoon fish species, the yellowstripe goatfish (*Mulloidichthys flavolineatus*) and the flat head mullet (*Mugil cephalus*) from the Bay of Poudre d’Or, Mauritius, which is known to be contaminated with toxicants and sediments. Both light and scanning electron microscopic studies of the processed gills of these two fish species showed marked histological alterations. The pathologic lesions of the gills included ballooning dilatations or lamellar hyperemia; hypertrophy of the filament and lamellar cells; hyperplasia of the filament and lamellar cells, and deformities of gill arches, filaments and lamellae.

**INTRODUCTION**

The rapid increase in both human population and the number of industries in Mauritius has resulted in large amounts of industrial effluents and wastes being discharged into the sea around the island, where they degrade the flora and fauna of the marine ecosystem. To date, relatively little is known about the effects of various toxicants on the edible lagoon fish of Mauritius. Owing to their direct and continuous contact with the environment, fish gills, which are organs for respiratory gas exchange, osmoregulation, excretion of nitrogenous waste products and acid-base regulation, are directly affected by contaminants.

The study reported here is the first investigation of histological alterations due to environmental contaminants in the gills of two edible fish species, namely the yellowstripe goatfish, *Mulloidichthys flavolineatus*, and the flat head mullet, *Mugil cephalus*, collected from the wild populations in the bay of Poudre d’Or in Mauritius.

**MATERIALS AND METHODS**

**Study area**

The Bay of Poudre d’Or (latitude 20º 03’ S longitude 57º 40’ E) with an area of 25 km² (Fig.1), is located in the northern district of Rivière du Rempart some 19 km from Port Louis, the capital of the Republic of Mauritius. The bay is the discharge point for effluents from textile industries and has approximately 0.3 % (75,000 m²) of affected area at 1 km away from the shoreline. Mangroves border the muddy shore of the bay. The industrial effluents have produced a substrate of rubble and silt, which co-exist with a few coral species, algae, holothurians, and two species of fish, the flat head mullet (*Mugil cephalus*) and the yellowstripe goatfish (*Mulloidichthys flavolineatus*).
Fish sampling and analyses

Live samples of 15 each of *Mulloidichthys flavolineatus* and *Mugil cephalus* caught using basket traps from the muddy shore of the bay between March and May 2001, were bought from local fishermen. The fish were killed and transported on ice to the laboratory. The length and weight of the fish were measured. Seawater analysis for pH, temperature, salinity and dissolved oxygen was carried out using a portable water electronic multichounder probe (Horiba® model U-10, Japan).

Gill sampling and morphometric studies

The gills from the two species of fish were excised keeping the filaments and rakers intact, rinsed in seawater, fixed in 4% neutral formol-calcium for 48 h at 4 °C and processed for paraffin embedment under vacuum using an automatic tissue processor and wax dispenser. Thin sections (7 µm) cut by means of a rotatory microtome were dehydrated and stained with Harris haematoxyllin-eosin (H&E) stain, periodic acid Schiff’s reagent (PAS) and Papanicolaou’s stain as per the method of Bancroft & Cook (1994). The sections were examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus C35 AD4), a camera (Olympus C40 AB-4) and an automatic light exposure unit (Olympus PM CS5P). For SEM study, gill filaments from both species were fixed in 2.5 % glutaraldehyde buffered with 0.1M sodium cacodylate, pH 7.3 for 4 hours at 4 °C and post fixed for 1 hour in 1 % osmium tetroxide in cacodylate buffer, pH 7.3. The fixed gill tissues were then dehydrated in graded ethanol and critical-point dried in ethanol-hexamethyldisilazane (4:1 v/v) as per the method of Braet et al. (1997). The dried specimens were mounted on aluminium stubs, sputter-coated with gold/palladium in Polaron® model SC7616 sputter and examined in a Leo® 430 scanning electron microscope at 25 kV. The gill filaments (n=30) of both the species were measured with an Olympus calibrated graticule, for determining (i) thickness of the epithelium of primary lamellae; (ii) thickness of secondary lamellae; (iii) thickness of the epithelium of the secondary lamellae, and (iv) distance between adjacent secondary lamellae following the method of Hughes & Perry (1976). Regions of gill filaments from both fish species
showing equally spaced secondary lamellae, intact cellular layers and no sign of fusion between neighbouring lamellae were considered to be normal and used as controls.

Any statistical significance of difference between mean values was tested at 95% of confidence level using the student’s t test. Analyses were performed using the Statistical Package for the Social Sciences (SPSS version 7.5.1, 1996).

RESULTS

During the sampling period, the selected physical and chemical variables of the sea water were always close to: temperature 22 ºC; salinity 29.9 ‰; total dissolved solids 46,400 mg/l; pH 7.8; dissolved oxygen 4.7 mg/l.

The mean fork length of *Mulloidichthys flavolineatus* was 14.3 ± 0.56 cm with a mean mass of 101 ± 6.2 g; whilst, for *Mugil cephalus*, the mean fork length was 18.2 ± 0.76 cm with an average mass of 111.0 ± 3.01 g.

**Morphometry**

The results of the morphometric analysis are given in Table 1. Exposure to environmental contaminant(s) had led to significant tissue alterations in the gills of both fish species from the Bay of Poudre d’Or. For instance, the mean length of the secondary lamellae underwent significant decrease; while, the mean thickness of the (i) venous sinus; (ii) secondary lamellae, and (iii) epithelium of the primary lamellae was considerably increased. However, the mean thickness of the epithelial lining of the secondary lamellae in *Mul. flavolineatus* showed an increase and that of *Mug. cephalus* was unaffected. In addition, the mean distance between the adjacent secondary lamellae was found to be within the

<table>
<thead>
<tr>
<th>Measurements (µm)</th>
<th><em>Mug. cephalus</em> (n =15)</th>
<th><em>Mul. flavolineatus</em> (n =15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Altered</td>
</tr>
<tr>
<td>Length of secondary lamellae</td>
<td>95.0 ± 6.16</td>
<td>66.29 ±11.04* (P &lt; 0.05)</td>
</tr>
<tr>
<td>Distance between adjacent secondary lamellae</td>
<td>11.0 ± 2.58</td>
<td>11.94 ± 1.43</td>
</tr>
<tr>
<td>Thickness of venous sinus</td>
<td>6.3 ± 0.13</td>
<td>18.2 ± 1.58** (P &lt; 0.01)</td>
</tr>
<tr>
<td>Thickness of epithelium of primary lamellae</td>
<td>2.3 ± 0.28</td>
<td>4.72 ± 1.86* (P &lt; 0.05)</td>
</tr>
<tr>
<td>Thickness of secondary lamellae</td>
<td>5.69 ± 1.97</td>
<td>11.30 ± 2.04** (P &lt; 0.01)</td>
</tr>
<tr>
<td>Thickness of epithelium of secondary lamellae</td>
<td>2.4 ± 0.08</td>
<td>2.91 ± 0.5</td>
</tr>
<tr>
<td>Separation of epithelial tissue</td>
<td>66 %</td>
<td>45 %</td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>56.67 %</td>
<td>79 %</td>
</tr>
<tr>
<td>Club-shaped deformity</td>
<td>63.3 %</td>
<td>66 %</td>
</tr>
<tr>
<td>Mean number of nodules per filament</td>
<td>29.75 ± 6.09</td>
<td>29.17 ± 5.12</td>
</tr>
<tr>
<td>Average nodule sizes</td>
<td>Length: 95.32 ± 6.47</td>
<td>Length: 48.95 ± 5.90</td>
</tr>
<tr>
<td></td>
<td>Width: 80.06 ± 3.03</td>
<td>Width: 47.39 ± 9.43</td>
</tr>
</tbody>
</table>
PLATE I. Figs 1 and 2. Light micrographs of gill filaments of yellowstripe goatfish, (*Mulloidichthys flavolineatus*) and flat head mullet (*Mugil cephalus*) from the Bay of Poudre d’Or showing slight histological changes in the primary and secondary lamellae [H & E x100]. Fig. 3. Cellular hyperplasia in the interlamellar region with marked outgrowth (arrows) at the tip in *Mug. cephalus* [H & E x200]. Fig. 4. Cellular infiltration in the secondary lamellae (SL) with interlamellar hyperplastic (HPL) lesion in *Mul. flavolineatus* [H & E x400]. Fig. 5. The ballooning dilatation (BD) or club deformation at the tip of the secondary lamellae of *Mul. flavolineatus* is a common feature encountered [H & E x400]. Fig. 6. Gill of *Mul. flavolineatus* showing cellular proliferation (CP) accompanied by fusion of the secondary lamellae thus resulting in obliteration of the space between secondary lamellae. Large congestion of blood spaces (arrow) by blood corpuscles indicates circulatory anomalies [H & E x1000]. Fig. 7. Dilatation of the venous central sinus in *Mug. cephalus* with marked cellular hyperplasia, fusion, shortening of the secondary lamellae and necrosis [H&E x400]. Fig. 8. Epithelial lifting at the base of the secondary lamellae in *Mug. cephalus* showing oedematous condition, cellular infiltration, namely macrophage and other white cell types. Mucous cell, chloride cell (CC) and pavement cells (PC) are also seen [H&E x100]
normal limit in both fish species. In general, the most common tissue modification was the displacement of the epithelial layer of the secondary lamellae from the underlying connective tissue (Plates I and II). The epithelial lifting (Fig. 8) is often accompanied by oedematous condition. Cellular hyperplasia was an important feature in both the species of fish (Figs 4, 5, 8, 9). Numerous club-shaped deformations (also known as ballooning dilatation) at the apical end of the secondary lamellae (Figs 5, 6, 12–14) ascribed to cell accumulation were predominant in both the species. Circulatory anomalies and haemorrhage were also manifested in both species of fish as indicated by severe congestion of blood spaces by erythrocytes (Figs 10 and 11). Other major changes observed were deformities and stunting of the secondary lamellae (Figs 3 and 7). Cellular damage along with circulatory anomalies gave rise to the most severe gill lesion, known as teleangiectasia. Of particular interest was the presence of different leukocytes particularly in both the lamellae and the gill raker (Figs 8 and 16) coupled with the presence of mucin (Fig. 15), depicting an inflammatory reaction. The SEM studies (Plate III, Figs. 17–22) revealed abnormalities in the structure of the gills of the two fish species, as described in the figure captions.

**DISCUSSION**

Fish gills are very sensitive to changes in the composition of the environment and are an important indicator of waterborne toxicants. Consequently, injury to gill epithelium is a common response observed in fish exposed to a variety of contaminants. The severity of damage to the gills depends on the concentration of the toxicant and the period of exposure [Oliveira et al. (1996); Karlson-Norrgen et al. (1985); Mallat (1985); Franchini et al. (1994)]. This response is manifested by tissue alterations observed in this study that could be attributed to contamination of the bay of Poudre d’Or. The contaminant(s) may exist as organic nitrogenous compounds, nonylphenol ethoxylate and heavy metals like chromium and copper (Burke, 1999).

Toxic environmental conditions can result in two types of structural changes in tissues of the organism. One is the result of the direct toxic effect of the pollutant leading to degeneration and necrosis. The second is a result of compensatory mechanisms that deal with the environmental stressor, as in cellular hyperplasia (Hughes & Perry, 1976). In the present case, it seems that both types of structural responses are operational. Furthermore, white blood cell infiltration, displacement of epithelial cells and oedema could contribute to an increase in the diffusion distance from surrounding water to capillaries and simultaneously increase the amount of tissue in the secondary lamellae. This hypertrophy could result in a decrease of the respiratory dead volume between the lamellae and impair the diffusion of oxygen through the swollen epithelium.

The pathological changes observed in the gills could be attributed to metal toxicity (Bhagwant, unpubl. manuscript). An increase in the lamellar dense cells in the secondary lamellae of brook trout exposed for long to acid and aluminium, was previously reported by Tietge et al. (1988). The fusion of the secondary lamellae could cause a decrease in free gas exchange, thus affecting the general health of the fish (Skidmore & Tovell, 1972; Gardner & Yevich, 1970). The compensatory changes may, nonetheless, become maladaptive if the duration of the stress factor(s) exceeds the biological tolerance limits (Wedemeyer et al., 1990).

To our knowledge, the present study provides the first description of pathological lesions in the gills of *M. flavolineatus* and *Mug. cephalus* from the Bay of Poudre d’Or in Mauritius. The teleangiectasia observed in both fish species may affect blood circulation leading to respiratory impairment. Moreover, this type of structural damage shows close similarity to lesions brought about by elevated levels of other environmental pollutants such as zinc (Skidmore & Tovell, 1972), ammonia (Smart, 1976) and sediment-borne contaminants (Hargis Jr. & Zwerner, 1988). Chevalier & Gauthier (1985), using histological and electron microscopic studies of gill tissues of brook trout from acidified lakes, observed the sloughing off of chloride cells, and degeneration of branchial epithelial cell type altogether, leading to cell death.

The presence of mucous in the ballooning dilatation observed in the gill filaments may be considered as an ion trap to concentrate trace
PLATE II. Fig.9. Shifting of some chloride cells (CC) along the secondary lamellae in *Mugil cephalus*. Cellular hyperplasia accompanied by degeneration of chloride cells and oedema are common features [H & E x1000]. Fig. 10. Ballooning dilatation in *Mug. cephalus* consists of epithelial cells and red blood corpuscles (orange) [PAP x200]. Fig. 11. Dilatation of the venous sinus and haemorrhage (arrow) observed in the secondary lamellae of *Mug. cephalus* [PAP x400]. Fig. 12. Cellular hyperplasia, necrosis of the secondary lamellae in *Mug. cephalus* [PAS x200]. Fig 13. Ballooning dilatation clustered on both arms of the primary lamellae of *Mug. cephalus*. Part of the filament is unaffected [H & E x400]. Fig. 14. Ballooning dilatations of the secondary lamellae in *Mulloidichthys flavolineatus* [H&E x400]. Fig. 15. Magnified view of the ballooning dilatation in *Mug. cephalus* showing leukocyte infiltration and mucin filled spaces [PAS x1000]. Fig. 16. Thickening of the epithelial lining of the gill raker (GR) on the gill arch of *Mug. cephalus* depicting an acute inflammatory reaction [H&E x400]
PLATE III. Fig. 17. *Mugil cephalus* gill filament characterised by disorganised secondary lamella, ballooning dilatation (bud-like) structure and interlamellar hyperplastic lesion (x64). Fig. 18. Reduction in height and fusion of the secondary lamellae accompanied by abnormal collection of blood in the lamellae of *Mul. flavolineatus* gill result in teleangiectasia (x180). Fig. 19. Obliteration of space between secondary lamellae with some swollen (oedematous) type secondary lamellae in *Mug. cephalus* (x254). Fig. 20. Cytoplasmic protrusion from secondary lamellae of *Mul. flavolineatus* gill (x1150). Fig. 21. Necrosis of secondary lamella and ballooning dilatation in *Mul. flavolineatus* x251. Fig. 22. Extensive swelling of the basal part of the secondary lamellae and hyperplastic lesion in *Mul. flavolineatus* (x1070)
elements from water and favour cell adhesion between the neighbouring secondary lamellae. It may as well serve to protect the epithelia against both mechanical abrasion and infection as suggested by Olson & Fromm (1973). Such pathological lesions may be attributed to the sediment-borne contaminants since both fish species are omnivorous. However, the presence of white blood cells, mainly macrophage, lymphocytes and neutrophils, clearly indicates an inflammatory reaction and cellular infiltration thus reflecting an immunological response of the fish to environmental contaminants (Zeeman & Brindley, 1981; Tao et al., 2000).

The present study has shown that *Mullloidichthys flavolineatus* and *Mugil cephalus* from the Bay of Poudre d’Or exhibit pronounced gill lesions, which might be attributed to the presence of toxicants in the Bay that are detrimental to fish health.

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