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Gill Lesions in the Perch, *Anabas testudineus*, Subjected to Sewage Toxicity

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Sewage is a major pollutant of the freshwater bodies of Gorakhpur region. The pollution of the large Ramgarh Lake (location: 26°42'-26°46' N, 83°23'-83°25' E; maximum area: 15 sq km), which is the chief fishery resource of this area, is particularly heavy since the untreated refuse of Gorakhpur city is discharged into the lake through a long and mostly open sewer (Narain and Srivastava 1979a). This sewage effluent has been found to cause considerable damage to the freshwater fish and shellfish of this region. The ill-effects of sewage on the blood cell counts and hemostasis of the crab, Paratelphusa spinigera, and on the blood and hepatic and renal tissues of the catfish, Heteropneustes fossilis, have already been studied (Narain and Nath 1982, 1986; Narain and Srivastava 1979a, 1979b, 1989, 1990; Srivastava and Narain 1982, 1985). The present study describes the histopathological changes produced in the gills of the perch, Anabas testudineus, maintained in sewage. Gills have been chosen for inspection in view of the fact that these organs are particularly vulnerable to environmental toxicants because of their external location and close contact with water and because of their permeability which makes them the principal sites of the uptake of toxicants from the medium (Roberts 1978). Damage to gills has, for this reason, been studied in a large variety of fish exposed to various kinds of environmental pollutants both at the light microscopic and electron microscopic levels (Eller 1975; Jagoe and Haines 1983).

MATERIALS AND METHODS

Anabas testudineus, collected from unpolluted habitats, were held in porcelain tanks and were acclimatized to

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the laboratory conditions for 4 wk. Each storage tank contained 1000 L of dechlorinated tap water which was changed daily. The tap water used for storing the fish had the following chemical composition: pH, 7.3 ± 0.001 SE; dissolved oxygen, 7.2 ppm ± 0.36 SE; free carbon dioxide, 11.07 ppm ± 0.41 SE; total alkalinity, 280 ppm ± 2 SE; total nitrogen, 0.002 ppm ± 0.0001 SE; sulphate, 3 ppm ± 0.6 SE; phosphate, 0.01 ppm ± 0.0008 SE; calcium 60 ppm ± 2 SE. Total nitrogen was estimated by Cope's modification of macro-Kjeldahl method (Hesse 1971). Other estimations were done according to the methods recommended by Indian Standards Institution (1972).

Sewage samples were collected just before the sewer entered the lake. Microscopic examination of sewage samples revealed the presence of ciliates, flagellates, other protozoans and roundworm ova. Bacterial cultures showed the presence of Pseudomonas sp., Klebsilla sp. and Bacillus subtilis, while fungal cultures revealed the presence of Candida sp. Chemical analysis of sewage revealed as follows: pH, 8.05 ± 0.15 SE; dissolved oxygen, nil; free carbon dioxide, 24 ppm ± 2.33 SE; total alkalinity, 1040 ppm ± 80 SE; total nitrogen, 0.36 ppm ± 0.02 SE; ammonia nitrogen, 1.85 ppm ± 0.01 SE; sulphate, 140 ppm ± 50 SE; phosphate, 48.05 ppm ± 3.46 SE; calcium, 120 ppm ± 50 SE.

Healthy fish (14-25 g body-wt, 12-19 cm fork-length) were transferred to a set of 15 cylindrical glass aquaria (diameter, 48 cm). Each aquarium contained 12 fish in 20 L of sewage which was diluted to 25% with tap water and was changed twice daily. Gills of the fish were examined before the start of the experiment, to provide the control picture, and thereafter every 24 hr. Ten fish were sampled for each observation. The tissue was fixed in Bouin's fluid and 10% formalin and paraffin sections were dyed with hematoxylin-eosin and Mallory's triple stains.

Exposure of the fish to sewage was continued for 10 d at room temperatures of 25-30°C because earlier studies with catfish (Narain and Srivastava 1979a; Srivastava and Narain 1982, 1985) had revealed that the stressed individuals started displaying hematological signs of tissue damage within 10 d of exposure to 25% of the sewage polluting Ramgarh Lake.

RESULTS AND DISCUSSION

The gill of Anabas testudineus is of the typical teleostean type (Fig. 1). It showed marked histological changes when the fish were subjected to the stress of sewage pollution (Figs. 2-6).

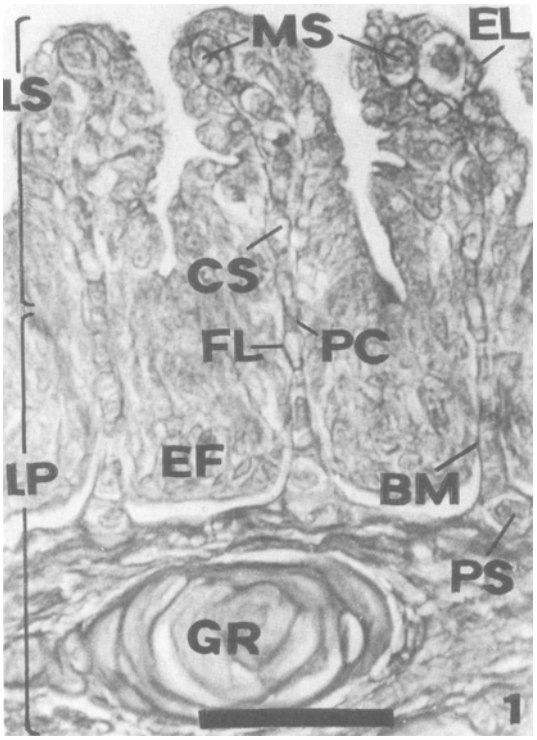
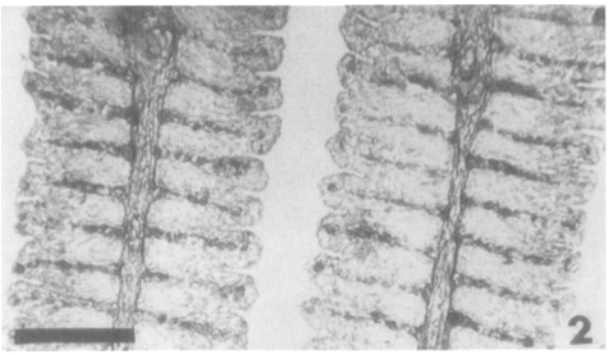
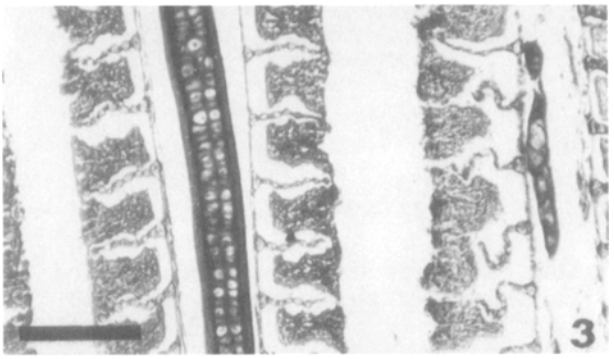
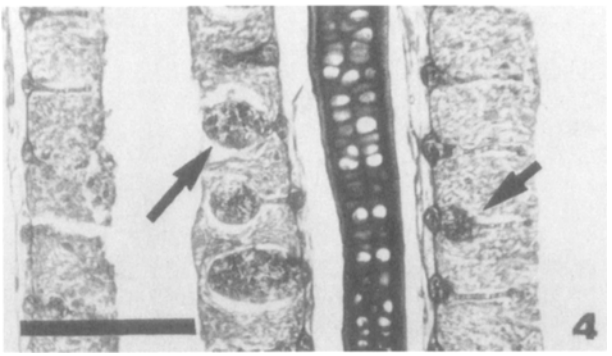


Figure 1. Photomicrograph of cross section of control gill of Anabas testudineus passing through a primary lamella or gill filament (LP) and three secondary lamellae (LS) and showing: lamellar epithelium (EL); filamental epithelium (EF); basement membrane collagen (BM); gill ray (GR); pillar cells (PC); pillar cell flanges (FL); proximal blood spaces (PS); central blood spaces (CS); marginal blood spaces (MS). Scale bar, 10 microns.

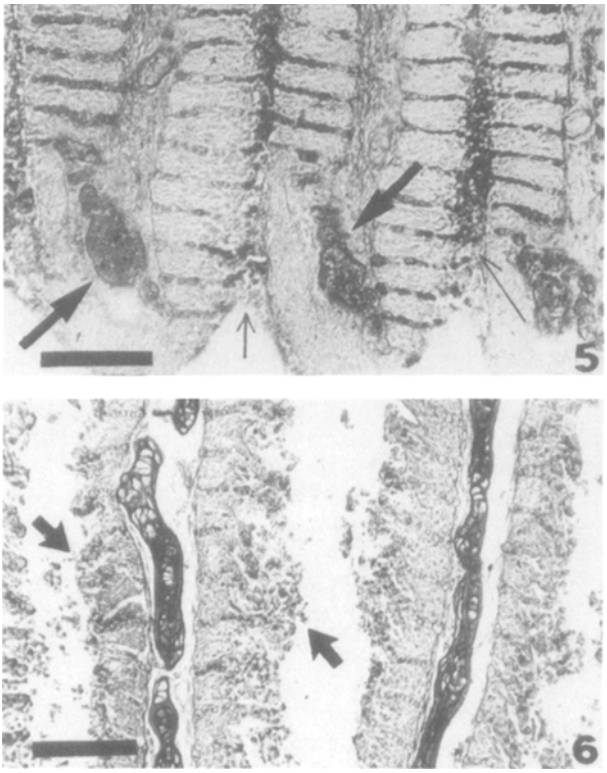
Hyperplasia of branchial epithelium was found to be the most pronounced damage caused to Anabas testudineus on account of sewage pollution. This effect appeared by the second day of exposure and advanced progressively so that the adjacent secondary lamellae became fused extensively with each other by the tenth day of exposure (Figs. 2-6). Osburn (1910) has concluded that proliferation of gill epithelium protects the gill filaments from constant irritation in silver salmon fingerlings which lack adequate gill covers. But, since proliferative thickening of gill epithelium is produced by most kinds of environmental toxicity (Skidmore and Tovell 1972; Eller 1975; Roberts 1978), this response may be considered as a







Photomicrographs of cross sections of gill of Anabas testudineus exposed to 25% sewage for 10 d.
Scale bar, 30 microns.
Figure 2. Showing lamellar fusion.
Figure 3. Showing separation of gill epithelium from its supporting elements, and curling of some pilaster stalks.
Figure 4. Showing stasis of lamellar blood circulation. Note the large stagnant masses of blood (arrows) within the lamellar blood spaces.



Photomicrographs of cross sections of gill of Anabas testudineus exposed to 25% sewage for 10 d. Scale bar, 30 microns.

Figure 5. Showing pooled blood (thick arrows) within the lamellae and hemorrhagic exudates in the branchial cavity (thin arrows).

Figure 6. Showing rupture of gill epithelium and release of hemorrhagic exudates into the branchial cavity (arrows).

general safety measure against irritation by environmental toxicants. Hyperplasia of branchial epithelium has been observed commonly in fish facing low tension of oxygen and high concentrations of ammonical wastes in the environment (Eller 1975; Roberts 1978). In view of this, the increased level of ammonia nitrogen and the near absence of dissolved oxygen in the sewage could be responsible for the hyperplasia observed in stressed Anabas testudineus. It may be recalled that ammonia

nitrogen has also been found to be among the more harmful chemical constituent factors of sewage with regard to the production of hematological disorders in the catfish, Heteropneustes fossilis (Narain and Srivastava 1979a, 1989).

The proliferated gill epithelium of Anabas testudineus exposed to sewage for 6-10 d was also seen to separate from the pilaster stalk and the gill ray in many gill filaments (Fig. 3). Such a lifting of lamellar epithelium usually occurs when the lymphoid space between the epithelium and its supporting elements gets enlarged by accumulation of fluid on account of factors like increased capillary permeability (Roberts 1978) or lowered efficiency of the epithelial cells in maintaining normal water balance (Skidmore and Tovell 1972).

In a number of secondary gill lamellae of Anabas testudineus damaged by exposure to sewage for 7-10 d, the pillar cell system also appeared to collapse. The pilaster columns were seen to get curled up (Fig. 3) and their blood spaces got engorged with stagnant masses of blood (Fig. 4). Pools of congested blood were also visible within the subepithelial space (Fig. 5). Such a collapse of the pillar cell system is believed to occur when a fall in the hydrostatic pressure causes this system to fail as a vascular endoskeleton (Skidmore and Tovell 1972).

Occasionally, the respiratory epithelium of Anabas testudineus maintained in sewage for 10 d got ruptured at different points so that the capillaries were exposed to water and hemorrhagic exudates could be seen at many places over the lamellar surface and in the branchial cavity (Figs. 5, 6).

In fish, the respiratory epithelium is the barrier between the blood and the surrounding water through which respiratory gases and other materials needed for sustenance are exchanged. So, any damage to this epithelium impairs not only the ventillatory process but also other vital processes, like ion-exchange, during the secretory and excretory functions of the gills.

Fusion of gill lamellae and lifting of gill epithelium from the supporting elements are such effects which reduce the surface area available for gaseous and other exchange and lengthen the distance over which the exchange diffusion occurs (Skidmore and Tovell 1972; Jagoe and Haines 1983); ultrastructural features like loss of surface macroridges of secondary lamellar epithelial cells also produce similar reduction in the exchange surface area (Jagoe and Haines 1983). When this

hampers the exchange of respiratory gases across the gill epithelium then respiratory insufficiency becomes a natural consequence. Respiratory distress also results when stress-induced damage in the gill epithelium leads to events like increased influx of hydrogen ions which reduces the pH of the blood and thus decreases the oxygen carrying capacity of hemoglobin (Haines and Schofield 1980). On the other hand, the ionoregulatory and excretory functions of the gill are hampered when epithelial damage disturbs the exchange of ammonium and bicarbonate ions of the blood with sodium and chloride ions of the medium which normally occurs across the gill epithelium of fish (Love 1980).

Effects like collapse of pillar cell system and rupture of gill epithelium tend to stagnate or even stop the lamellar blood flow (Skidmore and Tovell 1972). This consequence is also likely to limit the respiratory capacity of the gills.

Undoubtedly, therefore, as stipulated by Eller (1975), gill alterations, such as those observed presently, would represent basic physiological problems which the stressed fish may not ultimately be able to cope with. Respiratory distress would be one such problem specially under high temperature conditions, like those prevailing in this region (where the water temperature rises as much as 30°C in the month of June), which reduce the solubility of oxygen in water (Roberts 1978).

The present observations strengthen our recommendations (Narain and Srivastava 1979a) that sewage deserves more attention than it enjoys presently in comparison to other pollutants like heavy metals and pesticides.

REFERENCES

Eller LL (1975) Gill lesions in freshwater teleosts. In: Ribelin E and Migaki G (eds) The pathology of fishes. University of Wisconsin Press, Madison, pp 305-330

Haines TA, Schofield CL (1980) Responses of fishes to acidification of streams and lakes in Eastern North America. In: Restoration of lakes and inland waters, International symposium on inland waters and lake restoration, Portland, Maine, U.S. Environmental Protection Agency, Washington DC, EPA 440/5-81-010: 467-473

Hesse PR (1971) Text book of soil chemical analysis. John Murray Ltd, London

Indian Standards Institution (1972) Methods of sampling and test (physical and chemical) for water used in industry, edn 2. New Delhi

Jagoe CH, Haines TA (1983) Alterations in gill epithelium morphology of yearling sunapee trout exposed to acute acid stress. Trans Amer Fish Soc 112:689-695
Love RM (1980) The chemical biology of fishes, vol 2. Academic Press, New York
Narain AS, Nath P (1982) Ultrastructural cytoplasmic damage in the erythrocytes of the freshwater teleost, Heteropneustes fossilis, exposed to sewage pollution. Nat Acad Sci Letters 5:103-104
Narain AS, Nath P (1986) Submicroscopic hepatic and renal pathology of a teleost maintained in sewage. Bull Environ Contam Toxicol 37:266-273
Narain AS, Srivastava PN (1979a) Haematohistological responses of the Indian freshwater catfish, Heteropneustes fossilis, to environmental pollution by sewage, fertilisers and insecticides. Arch Biol Bruxelles 90:141-151
Narain AS, Srivastava PN (1979b) Pollution related changes in blood cell counts and blood cell clumping of the freshwater crab, Paratelphusa spinigera. Indian J Exp Biol 17:971-974
Narain AS, Srivastava PN (1989) Anemia in the freshwater teleost, Heteropneustes fossilis, under the stress of environmental pollution. Bull Environ Contam Toxicol 43:627-634
Narain AS, Srivastava AK (1990) Liver and kidney damage in freshwater teleosts (Heteropneustes fossilis and Anabas testudineus) exposed to sewage pollution. Acta hydrochim et hydrobiol (in press)
Osburn RC (1910) The effects of exposure on gill filaments of fishes. Trans Amer Fish Soc 40:371-376
Roberts RJ (1978) The pathophysiology and systematic pathology of teleosts. In: Roberts RJ (ed) Fish pathology. Bailliere Tindall, London pp 55-91
Srivastava PN, Narain AS (1982) Leucocytic and haemostatic reactions of the Indian catfish, Heteropneustes fossilis, subjected to environmental pollution by sewage, fertilisers and insecticides. Acta Pharmacol Toxicol 50:13-21
Srivastava PN, Narain AS (1985) Catfish blood chemistry under environmental stress. Experientia 41:955-957

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