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Histological Changes In Gills, Skin And Intestine Of Fresh Water Fish, *Channa Marulius*, Exposed To Bacterial Pathogens In Wainganga River District Chandrapur (M.S.)

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Abstract
The present study reveals the histological effects of pathogenic bacteria on the gills, skin and intestine of fresh water fish Channa marulius collected from Wainganga river of Chandrapur district. Several histological changes were observed in fish organs would serve useful purpose in evaluating the toxic effects of bacterial pathogens. The results revealed that the gills, skin and intestine of control infected Channa marulius showed pronounced difference in their structure and completely damaged. Histological changes in gills, skin and intestine observed microscopically showed increasing degrees of damage in the tissues in correlation with the concentration of pathogens. Bacterial pathogens associated with fish can be transmitted to human beings from fish used as food or by handling the fish causing human diseases. The study concludes that observation of fish gills provides an opportunity to assess fish health status as well as information on possible health hazards coming from their environment.
Key-Words: Histology, Gills, skin, intestine, Channa marulius, human health.

Introduction

India is primarily an agro-based country with more than 60–70% of its population dependent on agriculture. However, 30% of its agricultural production is lost owing to pest infestation. In the absence of a better alternative deployment of pesticides becomes inevitable despite their known hazardous effects. Application of pesticides in India contributed 3% of the total world's consumption and is increasing at the rate of 2–5% per annum (Bhadbhade *et al.*, 2002). One of the most economically exploited fishery resources worldwide has experienced an intense global expansion since 1955. Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs such as the gills, muscle, liver and kidney (Dutta, 1996). Histological investigation may therefore prove to be a cost effective tool to determine the health of organisms hence reflecting the health of an entire aquatic ecosystem. Histology of fish liver could therefore serve as a model for studying the interactions between stress factors which include bio-toxins, parasites, infectious germs, physicochemical parameters and pollutants (Brusle and Anadon, 1996). Pathogens produce pathological changes in fishsuch as necrosis in the liver, tubular damage of kidney and gill lamellar abnormalities (Ramalingam, 1985).

Histological investigations have long been recognized to be reliable biomarkers of stress in fish (Van der Oost, Beyer, & Vermeulen, 2003). Histological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies. One of the great advantages of using histological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer, Pawet, Schramm, Müller, and Triebskorn, 2001).

The non-indigenous contaminate the fish or the habitat one way or the other and examples include *Escherichia coli, Clostridium botulinum, Shigella dynteriae, Staphylococcus aureus, Listeria monocytogens* and *Salmonella*. The indigenous bacterial pathogens are found naturally living in the fish's habitat for example *Vibrio* species and *Aeromonas* species. The bacteria from fish only become pathogens when fish are physiologically unbalanced, nutritionally deficient, or there are other stress conditions, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to prevail. Pathogenic and potentially pathogenic bacteria associated with fish and shellfish include *Mycobacteium, Streptococcus spp., Vibrio spp., Aeromonas spp., Salmonella spp.* and others (Lipp and Rose, 1997). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta, Rios, Romao, Vianna, & Freiberger, 2003), and serve as warning signs of damage to animal health (Hinton & Laurén, 1990). Since gill and gastrointestinal tract in fishes considered the main passage for entrance of pollutants to the internal body organs

like liver and kidney through the blood (Takashima & Hibiya, 1995). Gills are the first target of waterborne pollutants due to the constant contact with the external environment (Perry & Laurent, 1993).The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply (Van der Oost *et al.,* 2003) it is also one of the organs most affected by contaminants in the water (Rodrigues & Fanta, 1998). Hence, this study was undertaken to examine the effect of different sublethal bacterial pathogens concentrations on histological aspects of gill, skin and intestine of fresh water fish, *Channa marulius*.

II. MATERIALS AND METHODS

Study Area

This study was conducted on fish species collected from Wainganga River flowing through Gadchiroli and Chandrapur district. In Gadchiroli district the river flows nearby Armori tehsil and in Chandrapur district it is near Bramhapuri tehsil. So the fish samples i.e. *Channa marulius*, collected from the both tehsil areas.

Experimental Design

A total of 20 adult fish of both sexes were used. The average weight of the fish was 101.4±2·9 g. The experiments were conducted in aerated glass aquariums (120 x 40 x 30 cm) each containing 5 fish in 100 L of contaminated test solution and tap water for the control and allowing one hour for acclimation to laboratory conditions.

Laboratory Analysis

Forty fish samples were collected from Wainganga River between the periods of March to July, 2013. Twenty samples of *Channa marulius* were collected aseptically and immediately from two district areas separately and transported in a thermal bag to the laboratory and processed within 3hrs of acquisition, and samples were kept in the refrigerator (4–8^{0}C).

Sampling

The bacterial counts on the external surfaces, intestines and tissue were estimated as follows:

Skin Surfaces

Sample from different locations of the skin of 40 raw fish was taken by rubbing the sterilized cotton swab over the skin and then inoculated into 9 ml of Nutrient broth, MacConkey broth and Selenite F broth which are dispensed in separate tubes. 10 fold serial dilution of the bacterial suspension inoculated in peptone water was prepared induplicate and viable aerobic bacterial counts were enumerated using 0.1ml and 1ml inoculums in standard plate count agar as described by (Slaby *et. al.,* 1981)**,** and then incubated at 37^{o}Cfor 48 hrs.

Intestines, Gills & Tissues

1g of the fish sample was dissected out, blended and mixed properly in a mortar. It was aseptically transferred to a sample bottle containing 9 mls of 0.1% sterile peptone water. The bottle was closed and shaken thoroughly for 10 minutes and allowed to stand for 20 minutes, after which a 10 fold serial dilution was carried out in duplicates and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37^{o}C for 48 hrs as described by (Slaby *et. al.,* 1981)**.** Coliform organisms and gram negative enteric bacteria counts were determined using pour plate method with MacConkey agar, EMB Agar respectively. Pseudomonas isolation agar for *Pseudomonas spp*. *Salmonella spp*. and *Shigella spp*., were enumerated using Salmonella Shigella Agar (SSA) and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar for pathogenic *Vibrio spp*. The plates were incubated at 37^{o}C for 24hrs. The observed colony growth were counted using Coulter™ Colony counter according to plate count method. Identification of the organisms was done using the phenotypic and biochemical characteristics as described by (Cheesbrough, M. 1984) and (Slaby *et. al.,* 1981)**.**

Histological Observation

For histological studies different tissues such as gill, skin and intestine were dissected from both control and infected fingerlings of *Channa marulias*. The isolated tissue samples were fixed in Bouin's fixative for 24 hrs and washed with distilled water. The samples were dehydrated in different grades of alcohol series and processed further. Sections of 5-6 µm thickness were taken using a microtome and stained using haematoxylin and eosin. Respectively mounted using DPX and observed under a compound microscope.

Microscopy Examination

At the end of exposure period, 5 fish were taken from each replicate tank. According to Humason (1967), the specimens were processed as usual in the recognized method of dehydration, cleared in xylene and finally embedded in paraffin wax before being sectioned at 5 µm using a Senior Rotary Microtome (Model No. MT - 1090). The specimens were stained with hematoxylin and eosin. Finally, the prepared sections were examined and photographically enlarged using light microscopy (Hamilton compound photomicroscope).

Results and Discussion

In this study, for all the fish samples ranged between 6.60 x 10^6 and 25.60 x 10^6cfu/ml as shown in table 1. Out of the 40 fish samples analysed, for the skin had the highest number of bacteria with 23.6 x 10^6cfu/ml in *Channa marulias*. The gills had the lowest isolation with 8.60 x 10^6cfu/ml in *C. marulias*. The *Pseudomonas spp.* was highest in *Channa marulias* 19.88 x 10^6cfu/ml on skin.

Table 1: Count of bacteria present at different parts of examined sample fishes

Fish	Parts	*Coliforms*	*E. coli*	*S. aureus*	*P. aeruginosa*	*V. cholerae*	*S. typhi*	*S. dysenteriae*
		(cfu/ml)	(cfu/ml)	(cfu/ml)	(cfu/ml)	(cfu/ml)	(cfu/ml)	(cfu/ml)
		10^6	10^6	10^6	10^6	10^6	10^6	10^6
Channa marulias	Intestine	8.56	14.5	6.18	17.2	8.19	5.17	1.06
	Gill	10.5	12.04	3.84	19.49	-	4.18	3.64
	Skin	13.6	9.08	5.46	19.88	-	1.2	1.2
	Mouth	16.68	15.2	2.48	16.8	2.48	4.1	3.1

Table 1 revealed the isolation of *Pseudomonas spp.* with the skin having the highest number in *C. marulias*. The *Vibrio spp.* isolated had the lowest count of 2.48x 10^6cfu/ml from the mouth of *C. marulias* as compared with the skin of fish samples.

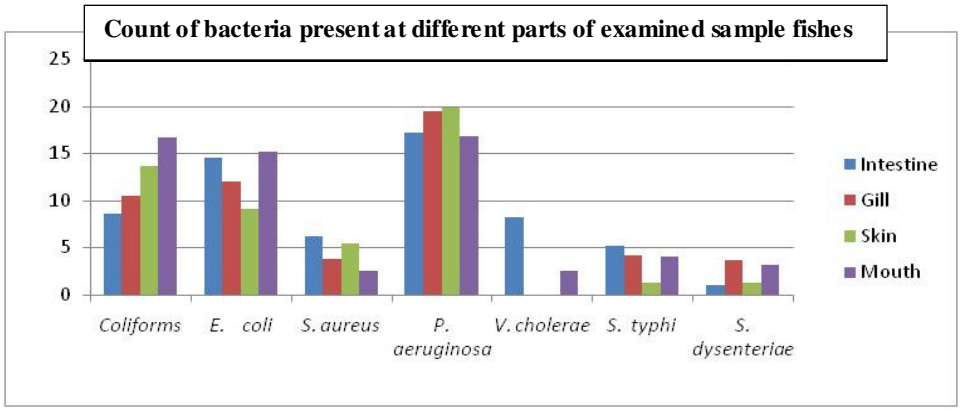

Figure . 1. *Graphical representation of bacterial count of different parts of Channa marulius.*

The intestine is the most colonized part of the examined areas in the fish with *C. marulias* having the highest count of 17.2 x 10^6cfu/ml, while the lowest count was exhibited in the *C. marulias* (1.06 x 10^6cfu/ml). The gills likewise showed possible colonization but in the lowest count as compared to other parts. No isolation of *Vibrio spp.* on the gills and skin of fishes. *E. coli* isolation showed the highest count in *C. marulias* (15.2 x 10^6cfu/ml). The intestine and gills were also heavily populated by *E. coli* with the highest exhibited in the gills of *C. marulias* (12.04 x 10^6cfu/ml). *Staphylococcus aureus* had a low isolation rate in all samples analysed as generally compared with other isolated organisms that had the lowest counts. The human bacterial pathogens that were isolated and identified include *S. aureus, Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae and Salmonella typhi* as indicated in the table.

Histopathologicl Lesions of Gills

Histological study of the gills shows a typical structural organization of the lamellae in the untreated fish. The treatments with bacterial strains (Fig. 2, 3, 4, 5 and 6) resulted in several forms of Histological changes such as cellular hypertrophy or hyperplasia in the epithelial layer of primary filaments and fusion of secondary lamellae. Other observations during the experiment include epithelial lifting, interstitial edema and blood congestion in the vascular axis of primary filaments.

In addition, a few bacterial infections were also observed at gill lamellae. The examination showed that bacterial caused cellular degeneration which result in necrosis of gill epithelial tissues. The gills are important organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion (Heath, 1987). They are directly exposed to poisons occurring in the external environment which often cause pathology in fish (Mallatt, 1985). The gills are among the most vulnerable structures of the fish because of their external location and intimate contact with the water. So, they are liable to damage by any irritant materials whether dissolved or suspended in the water (Roperts, 1978). Karlsson, Runn, Haux, and

Forlin (1985) mentioned that, the increase of cellular layers of lamellar epithelium may be due to an increase in the number of mitotic divisions of the lamellar epithelium. Kantham and Richards (1995) suggested that the gill hyperplasia may increase the epithelial thickness so as to retard into the blood stream. Cell proliferation with thickening of gill filament epithelium may lead to the lamellar fusion (Figueiredo-Fernandes, *et.al.* 2007).

Results of histological examination of infected intestine, gills and skin of *Channa marulius* showed various types of destructions in tissues. Loss of epidermal layer with complete necrotization of dermis and hypodermis were observed. The continuous exposure of these respiratory surfaces to the toxicant within the reconstituted fish culture water necessitated the observed spontaneous reactions (Fig. 2, 3, 4, 5 and 6).

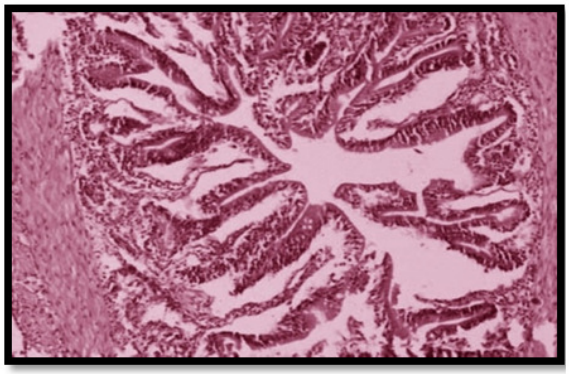

Figure 2: Intestine of healthy *Channa marulias* showing normal elongated lumen in villi. Circular muscles, longitudinal muscles and serosa are normally seen.

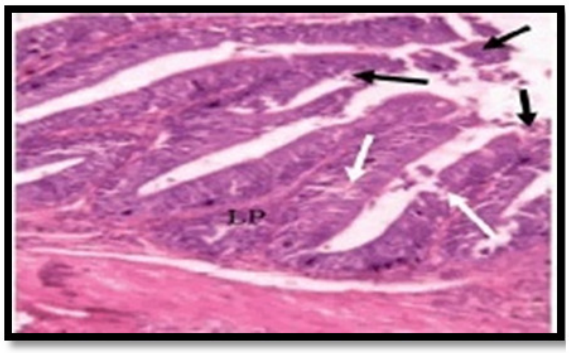

Figure 3: Intestine of infected *Channa marulias* showing damage lamina propria (LP), damage columinar epithelial cells (CEC) (white arrow) and distortion of villi (black arrow).

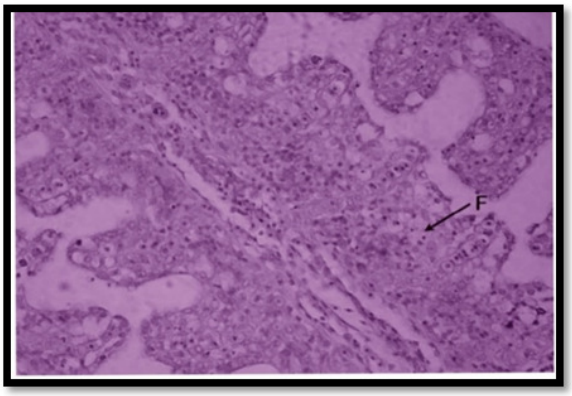

Figure 5: Photography of the gills of infected Channa marulius, noted the lamellar oedema (arrow) and lamellar fusion (F).

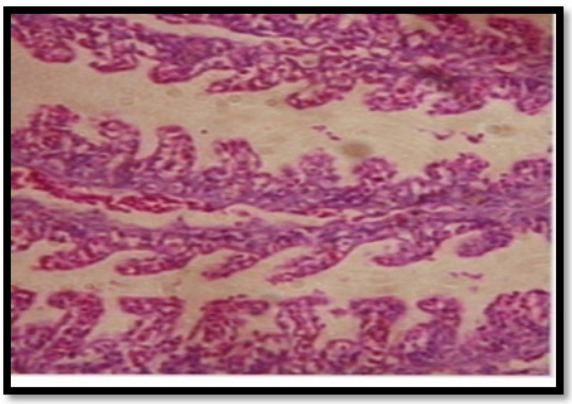

Figure 4: Showing (a) gills of normal *Channa marulius* and (b) gills of infected *Channa marulius*.

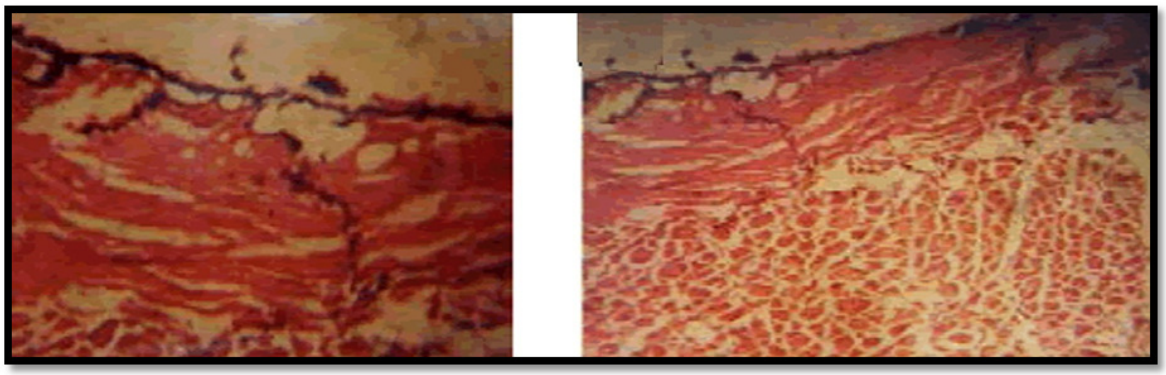

Fig. 6: Histology of skin: (a) Necrotic changes in the skin of affected *Channa marulius* (at 100 x) and (b) Necrotic changes in the skin of affected *Channa marulius* (at 40x)

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