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Histological Analysis of the Impact of Lagoon Pollution on *Chrysichthys nigrodigitatus* from Cote d'Ivoire

Abiba Sanogo Tidou · Armel-cyrille D. Yapi · Victor K. Kouamé · Serge Aoussi · David E. Williams

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Abstract Immunohistological and histopathological methods were used to highlight the importance of cell damages and some biomarkers for health risk assessment. A comparative study between 3 sites of the lagoons showed that the most polluted areas (Adiake and Ebrah), influenced by human activities, presented more damaged cells and stained cells in gills and livers of *Chrysichthys nigrodigitatus* than the less polluted area (Layo): gill cell hyperplasia and liver cells vacuolation were more evident in fish from Adiake than in fish from Ebrah and Layo. The percentage of proliferating cell nuclear antigen (PCNA)-positive cells in gill were 45.8 % ± 23.7 % for Adiake, 18.2 % ± 4.6 % for Ebrah and 11.4 % ± 6.51 % for Layo; The percentage of PCNA-positive in liver cells were 3.8 % ± 3.6 % for Adiake, 4.9 % ± 4.7 % for Ebrah and 2.6 % ± 2.5 % for Layo. Gills were more affected than livers. The Adiake site was the most contaminated area of the lagoon complex of Cote d'Ivoire.

Keywords PCNA · Immunohistochemistry · Fish tissue damages · Lagoon Cote d'Ivoire

Although the availability commercial food, most of the African people living around rivers and lagoons consume water and animals directly from these areas. The consumption of aquatic organisms living in contaminated waters can pose serious health hazards (Blas-Machado et al. 2000). In Cote d'Ivoire, lagoons present interesting problems of water pollution that impact on aquatic fauna and human health. Several studies have been performed in these lagoons and most concerned water quality in terms of phytoplankton and macrophytes invasion, chemical analysis, bacteriological analysis and stocks of fish and crustaceans. Many species of macrophytes dominate microplankton in the lagoon, especially during the beginning of the wet season**.** Bacterial pollution is characterized by the presence of fecal coliforms, *Clostridium perfringens* and *Escherichia coli*. With respect to chemical pollution sediment, fish, gastropod and shrimp are contaminated by chlorinated hydrocarbons and by heavy metal. Tilapia and catfish are a major component in the diet of West African. Tilapia is primarily an herbivorous Cichlidae while *Chrysichthys* is a benthic species, a predator of insects and bivalves. Juveniles eat plankton but adult diet comprises also detritus (Ikusemiju and Olaniyan 1975). *Chrysichthys* in the lagoon Ebrie is highly exposed to the pollutants in the sediment like in Lac Taabo (Roche and Tidou 2009).

The lagoon sediments continue to be contaminated by heavy metals. Coulibaly et al. (2012) obtained higher values of cadmium, copper, lead, mercury and zinc in sediments and water from Bietry Bay (Ebrie lagoon). That could cause biological, physiological, biochemical or carcinogenic effects on these brackish animals. Cell proliferations play an important role in these toxic mechanisms, especially carcinogenesis. The immunohistochemical (IHC) assay for the endogenous marker, proliferating cell nuclear antigen (PCNA) is one approach to evaluate carcinogenic risk (Ortego et al. 1995; Berntssen et al. 2004). PCNA is an auxiliary protein in the S-phase of cell division

A. S. Tidou (✉) · A. D. Yapi · V. K. Kouamé
UFR of Management and Environmental Sciences, University of Abobo Adjame, 02 bp 801, Abijan 02, Cote d'Ivoire
e-mail: atidou@hotmail.com

S. Aoussi
UFR of Natural Sciences, University of Abobo Adjame, 02 bp 801, Abijan 02, Cote d'Ivoire

D. E. Williams
Environmental and Molecular Toxicology, Oregon State University, ALS 1007, Corvallis 97331-7301, OR, USA

(Bravo et al. 1987). In the present paper, a catfish *Chrysichthys nigrodigitatus*, was used to compare the potential impact of water pollution on fish quality at three sites of the lagoon complex of Cote d'Ivoire. Histological pathology was also observed in the fish tissues at the studied sites.

Materials and Methods

The Lagoon Ebrie is a long (120 km) and narrow (1–9 km) lagoon complex which receives the input of several rivers. It is also connected to the Atlantic Ocean by the Vridi canal. The mean surface temperatures are 25–31°C. The pH is variable (6.5–10). Water is turbid, especially near Abidjan (Table 1).

The Layo site is located in the western part of the Ebrie lagoon (5°N18; 4°W), 40 km from Abidjan. Layo receives water runoff from agricultural sites and industrial lands. The Layo water quality seems to be good for aquaculture (site of the Research Center of Aquaculture) and was considered as the less polluted site in the present study. Adiake and Ebrah sites are located at the eastern part of the lagoon complex.

Table 1 Some parameters of the sites in the lagoons

	Period: September–December 2009		
	Adiaké	Layo	Ebrah
pH	9.19 ± 0.02	7.91 ± 0.04	6.85 ± 0.01
Temperature (°C)	31.90 ± 0.01	30.05 ± 1.01	29.10 ± 1.03
Salinity	1.50 ± 0.02	2.1 ± 1.01	0.10 ± 0.01
Dissolved O_2 (mg/L)	3.04 ± 1.30	4.01 ± 1.3	6.57 ± 0.30
Transparency (cm)	50 ± 0.10	0.8 ± 0.01	45 ± 1.60
Depth (m)	5 ± 0.02	3 ± 0.01	45 ± 0.05

Ebrah (5°N22; 3°W82) is located between Grand Bassam and Bingerville 30 km from Abidjan, the biggest city. Ebrah is more influenced by urban activities and small farms. No river tributary feds the Ebrah lagoon; only a seasonal opening in the sea outlet.

Adiake (5°N60; 3°W30) is located in the Aby lagoon where the input of freshwater is more important. The Aby lagoon is shared with the Republic of Ghana for traffic navigation and fishing.

Adiake and Ebrah are considered the most polluted areas in this study. Adiake is a boundary lagoon where the use of banned pesticides by farmers and fishermen is frequently reported (endosulfan, paraquat, diquat, etc.).

The temperature was mostly stable. The pH of the water was alkaline at the three sites. Layo seemed to have the highest salinity and was also the most turbid.

Chrysichthys nigrodigitatus were caught with gillnets at two sites of the lagoon Ebrie (Layo, Ebrah) and at one site of the Lagoon Aby (Adiake) (Fig. 1).

Fifteen catfish were collected at each site during the flood season (September and December 2009) and measured and weighed (Table 2).

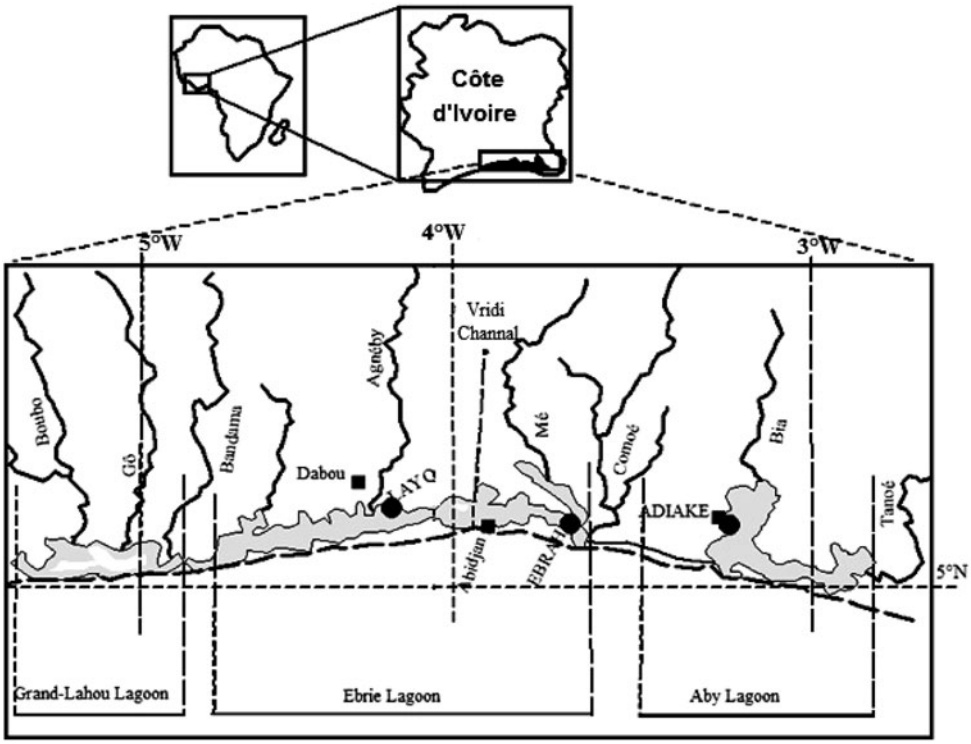

Fig. 1 Localization of sampling stations

Table 2 Size and body weights of fish under study

Sites	Size (cm)	Weight (g)
Layo	31.2 ± 4	212.2 ± 43.8
Adiake	22.2 ± 2.4	73.8 ± 2
Ebrah	19.4 ± 1.9	53.3 ± 16.1

The fish from Adiake and Ebrah were similar in size and were significantly smaller than Layo's fish ($p = 0.004$).

Livers and gills were carefully isolated and fixed in AFA (alcohol, formalin and acetic acids) and embedded in paraffin. A total of 10 bocks from fish at each site were examined by IHC and histopathology.

After deparaffinization and rehydration, sliced sections of 5 µm were made from fish tissues for IHC analysis according to the protocol performed in the Veterinary Diagnostic Laboratory of Oregon State University (OSU) using a Dakoautostainer. Primary antibody (mouse anti-PCNA, PC10 (Dako)) was applied 30 min at room temperature at a dilution of 1:1,000 in a diluent containing background reducing compounds (Dakocytomation). The negative control was Universal Negative control for Rabbit antibodies (N1699 Dakocytomation). High temperature antigen retrieval was carried out in a microwave pressure cooker using Dako target retrieval solution for 10 min of pressure, followed by 20 min at room temperature. Endogenous peroxidases were blocked with 3 % H_2O_2 for 10 min. Following a wash in Tris-buffered saline with Tween, slides were blocked in Dako serum–free protein block for 10 min and the primary antibody or negative control was applied. Envision + horseradish peroxidase Rabbit polymer (Dakocytomation) was applied for 45 min followed by the chromagen Nova Red (Vector Laboratories, Burlingame, CA) for 5 min. Slides were counterstained in Dakohematoxylin, cleared in xylene, and coverslipped. For histopathology, 3 slides of 3 sections were done and stained with hematoxylin and eosin (H&E).

For PNCA quantification procedure, 6 slides of 5 sections of each tissue were examined under higher magnification at 40× and 10–15 photographs were done per section. All stained cells and unstained cells of gills and livers were counted for each photograph. Stereological procedures for counting cells were not used because of non-homogeneous liver sections. The percentage of stained cells was determinate for each site (Table 3).

Table 3 Percentages of stained cells in *C. nigrodigitatus* tissues

	Layo	Adiake	Ebrah
% of gill stained cells	11.4 ± 6.6	45.8 ± 23.7	18.2 ± 4.6
% of liver stained cells	2.6 ± 2.5	3.8 ± 3.6	4.9 ± 4.7

Lesions and others abnormalities were identified and described under high magnification at 40× to 400× for histopathological analyses.

The sizes of fish from the three sites were compared by using ANOVA followed by Scheffe's and Bonferroni–Dunnett post hoc tests. Inter-site variations of %PCNA-positive cells were done using a 2 × 2 contingence table with determination of Chi-square with Yates correction. A p value <0.05 is considered to be significant.

Results and Discussion

In the gills, PCNA-positive cells with brown stained nuclei were more intensive in the filament epithelium from all three studied sites than the lamellar epithelium (Figs. 2, 3, 4). But only lamella cells were counted. The percentage of immunoreactive cells was 11.4 % ± 6.6 % in Layo; 45.8 % ± 23.7 % in Adiake; 18.2 % ± 4.6 % in Ebrah (Table 3). Adiake showed the highest significant rate of proliferating cells (Fig. 5). Highly significant differences in PCNA labeling were found between the Adiake site and the two others sites. The p value was <0.0001 for Layo-Adiake and for Adiake-Ebrah. The Layo site, where there are many aquaculture activities, showed the lowest PCNA staining in gill. Adiake and Ebrah are influenced by urban and rural activities (navigation traffics, tourism, etc.). Ebrah is located between two important cities, Bingerville and Grand Bassam, where the dynamic of the outlet changes the structure of the fish populations. Whereas, *C. nigrodigitatus* was found permanently at this site. The high percentage of stained cells in gill at Ebrah site could be caused by increased pollutants due to seasonal closure of the sea outlet by sand barriers. Also, concerning the Adiake site, located in the Aby Lagoon, banned pesticides and their use for fishing were usually reported. And there, many river runoffs in Aby lagoon increase the water pollution. All these cocktails of pollutants as revealed by previous and recent studies could be responsible for the highest rate of *C. nigrodigitatus* gills PCNA-positive cells at that eastern part of the lagoon system. Monteiro et al. (2009) also showed in tilapia (*Oreochromis niloticus*) a significant increase in proliferating filament cells and relative cell volume at the highest copper concentration. In addition, higher concentrations of mercury, cadmium and lead were found in the tissues of *Sarotherodon melanotheron* in Ebrie lagoon by Coulibaly et al. (2012). These authors showed that higher levels of heavy metal in fish were related to high contamination of sediments and water.

Proliferating cell nuclear antigen (PCNA)-positive nuclei were also observed in several liver cells from all the sites (Figs. 6, 7, 8). Ebrah site showed the highest level of liver proliferating cells with 4.9 % ± 4.7 % (Fig. 9). So, Liver PCNA-positive cells were most highly expressed in

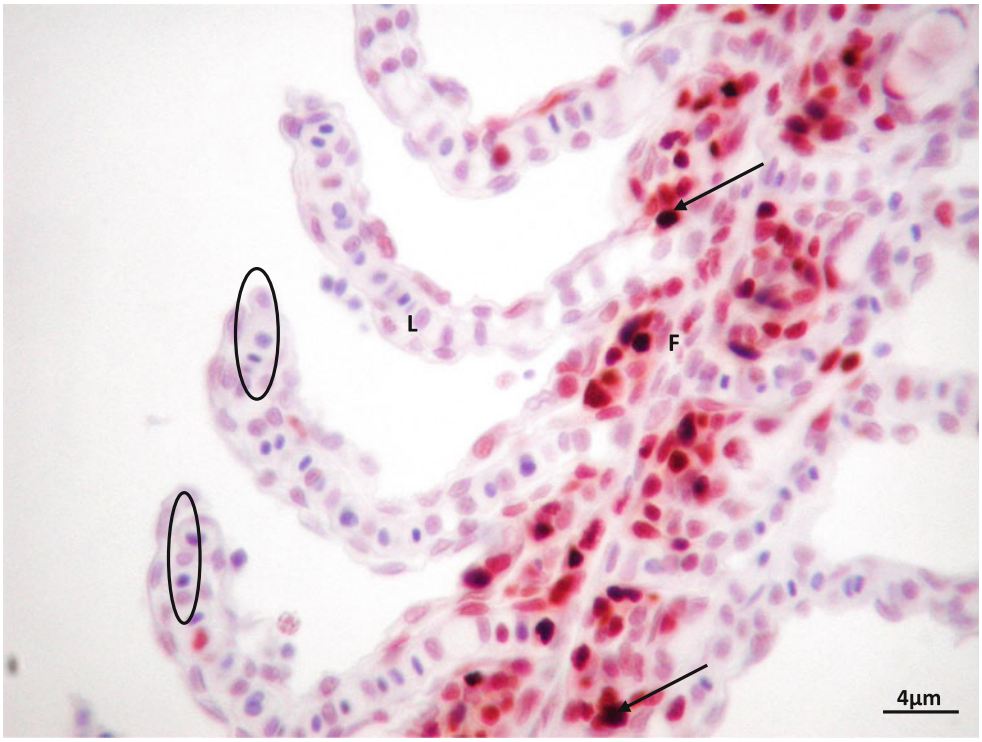

Fig. 2 Sagittal sections of *C. nigrodigitatus* gills from Layo: strongly stained cells (*arrows*) were only abundant in filament epithelium (*F*)

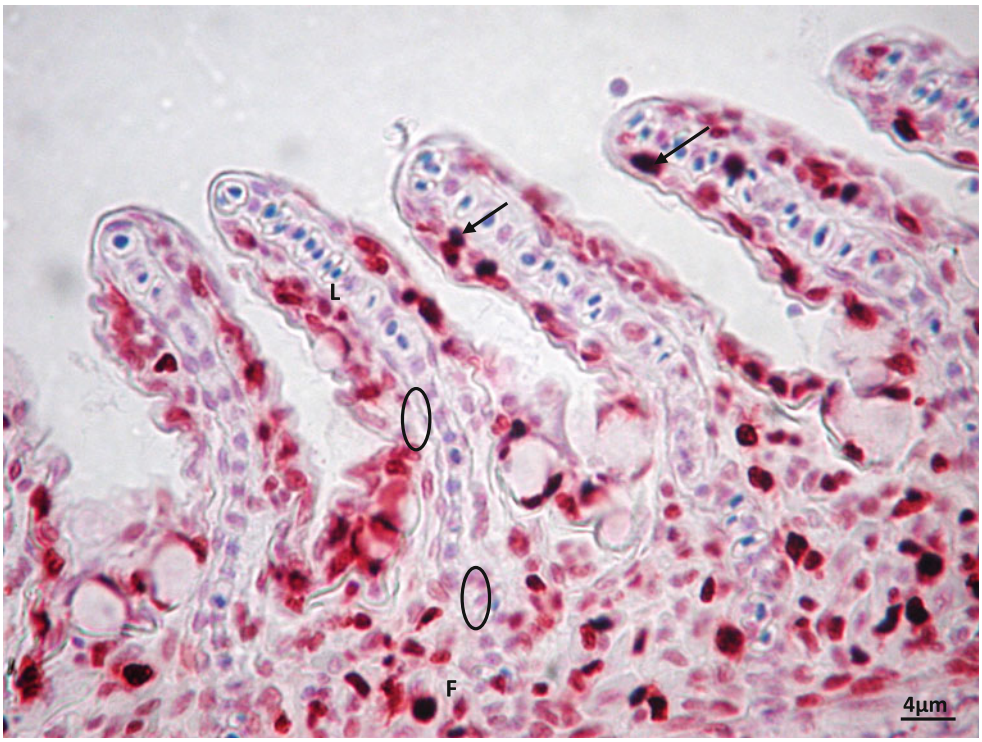

Fig. 3 Sagittal sections of *C. nigrodigitatus* gills from Adiake: strongly stained cells were clearly seen in both lamellar (*L*) and filament epithelium

more polluted areas (Table 3) than the Layo site. The p value was <0.0001 for Layo-Adiake and for Layo-Ebrah; the p value was 0.0263 for Adiake-Ebrah.

Otherwise, cell proliferations were more intensive in gills than in livers ($p < 0.0001$; Table 3). The structure and the respiratory function of fish gills expose them directly to water pollutants (Wilson and Laurent 2002).

Fish gills from Adiake showed more proliferating cells than Layo and Ebrah; but because of the greater size of the fish from Layo, it would be difficult to predict if Layo fish

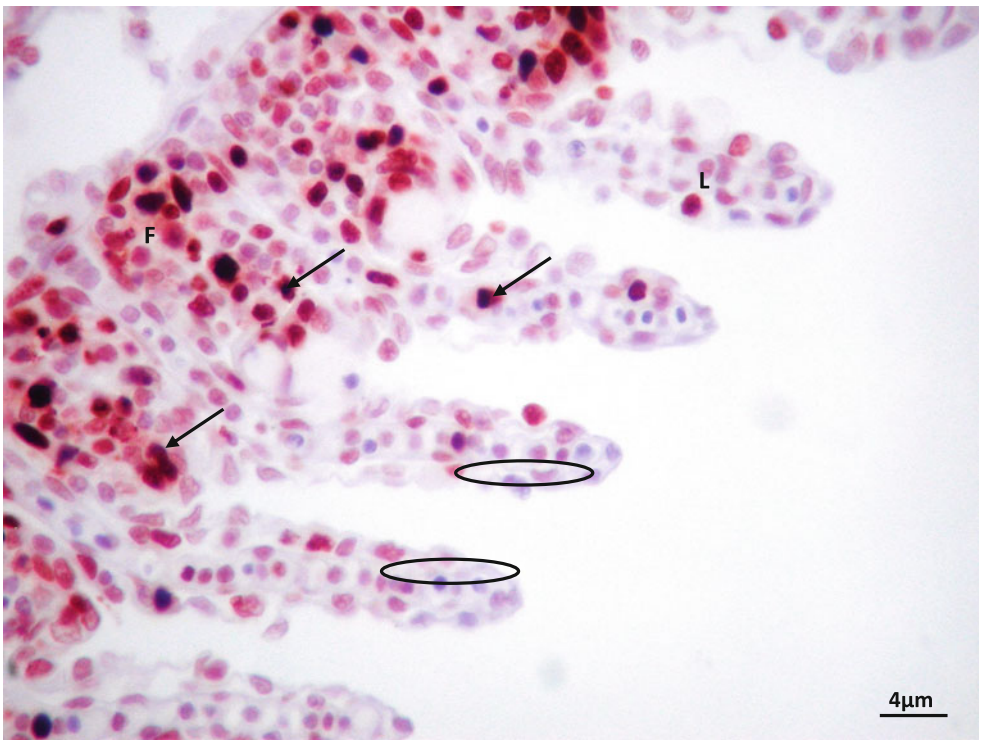

Fig. 4 Sagittal sections of *C. nigrodigitatus* gills from Ebrah: Lamellar cells were weakly stained (*oval*) and less abundant than those of Adiake

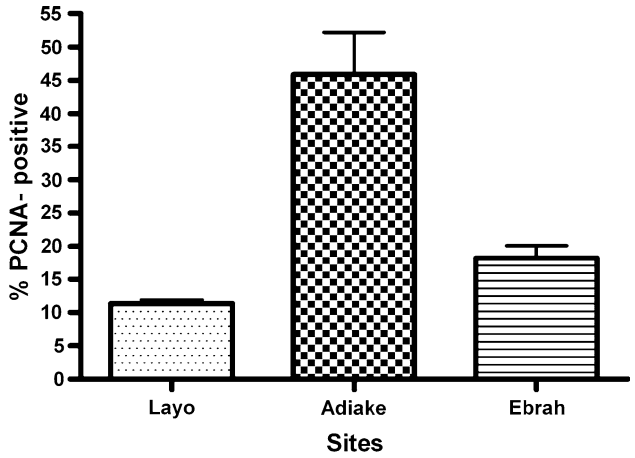

Fig. 5 Percentages of stained cells in *C. nigrodigitatus* gills from the three sites

were less affected by the pollutants than those of Adiake and Ebrah. It is known that proliferating cells are more active in juvenile gill than adult. Whereas, with a similar size of fish, PCNA-positive gill was significantly higher in Adiake than in Ebrah. The increase of the PCNA expression in Adiake fish gills could be the potential direct or chronic effects of pollutants in this site compared to Ebrah site and Layo site at this time of the year. That leads to the conclusion that the pollution of water in Adiake affected more the fish gills in the complex of lagoons in Cote d'Ivoire.

Liver, as a detoxification organ, accumulates toxic substances and is also used in histopathology and IHC studies. Kohler and Cornelis (1998) correlated enzymic parameters were with PCNA expression and histopathology in the flatfish liver: in the altered hepatocytes, all stages of carcinogenesis, from early foci to well-organized trabecular basophilic tumours and anaplastic carcinomas, showed significantly higher initial velocities of glucose 6-phosphate dehydrogenase (G6PDH) but not of Phosphogluconate dehydrogenase (PGDH) and a higher PCNA labelling index (43 %–65 %).

Concerning the present study, histopathological analysis showed that cell changes at the Adiake site were more remarkable than in Layo and Ebrah with moderate hyperplasia in gill cells and moderate vacuolation in the livers of the catfish. Gills from Layo displayed minimal hyperplasia while Ebrah fish had few protozoan and metazoan parasites. In Adiake, most fish had occasional protozoan and trematodes (Table 4). Hepatic cells displayed vacuolation and minimal hepatitis (Table 5).

At the Layo site, livers had patchy mild centrilobular hepatocellular lipidosis and there were few nematodes in pancreatic tissues. At Adiake site, most of the fish examined showed moderate hepatocellular vacuolation, largely due to glycogen storage (as per periodic acid-schiff (PAS) stain) and a smaller degree of lipid storage.

Some livers from fish caught at the Ebrah site had minimal to mild pericholangitis and/or hepatitis; vacuolation of hepatocytes was not observed at the Ebrah site.

These changes in fish from the Adiake site confirmed that high PCNA-positive cells found in that area of the

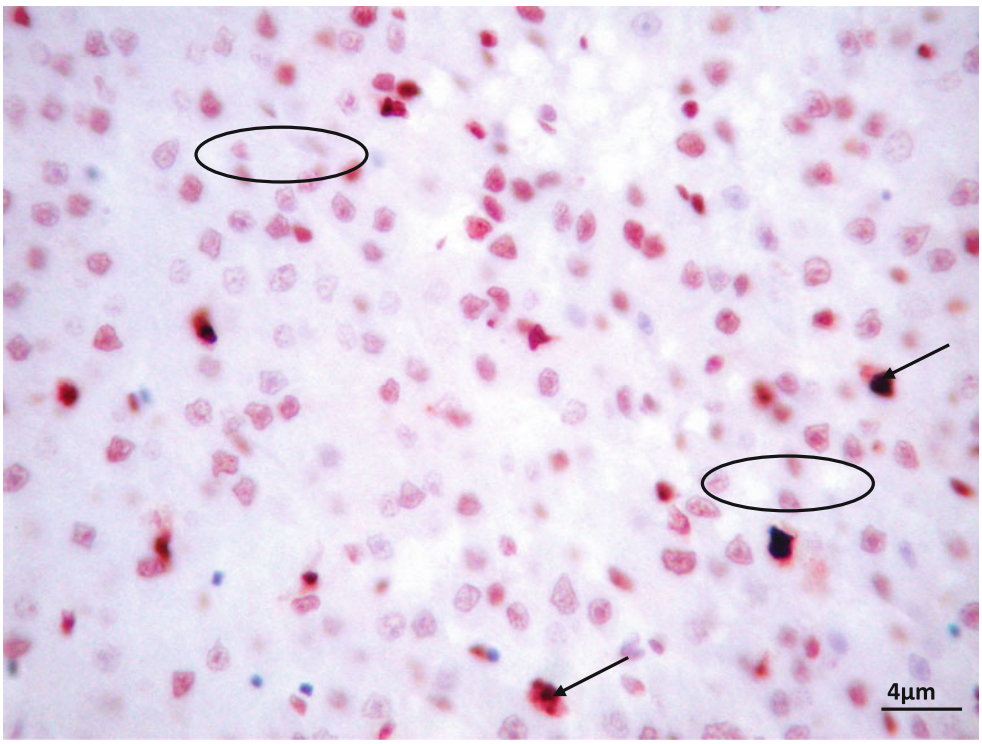

Fig. 6 Sagittal sections of *C. nigrodigitatus* livers from Layo: with less strongly stained cells

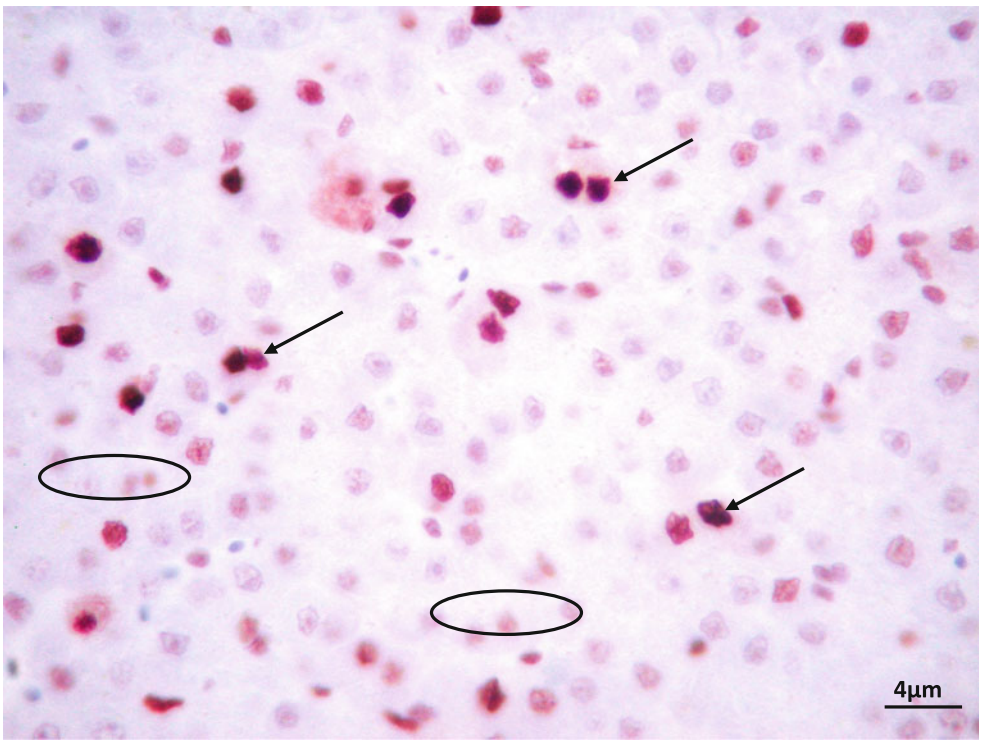

Fig. 7 Sagittal sections of *C. nigrodigitatus* livers from Adiake: with abundant stained cells

lagoon, reflected the poor water quality compared to Ebrah and Layo. Olarinmoye et al. (2009) observed that *C. nigrodigitatus* livers displayed vacuolar hepatocellular degenerations and necrosis and architectural disruption and dissociation of the Bilroth cords with lesions in Lagos lagoon specimens. Other changes detected in gills cells were lamellar fusions as also noted by Zilbergo and Munday (2000) with salmon infection by amoebae. Structural changes in gill tissues seem to be non-specific (Mallatt 1985) and reflect exposure to a wide spectrum of toxic chemicals and environmental stress. Roy and Munshi (1991) noted in fish exposed to insecticides inflammatory

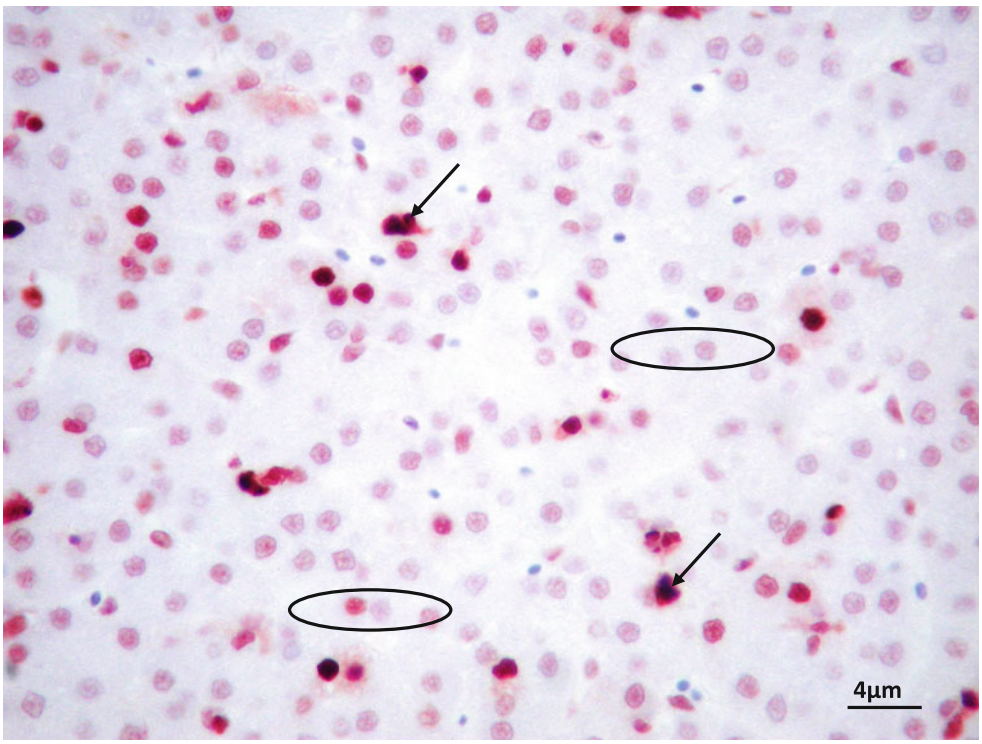

Fig. 8 Sagittal sections of *C. nigrodigitatus* livers from Ebrah: with abundant stained cells

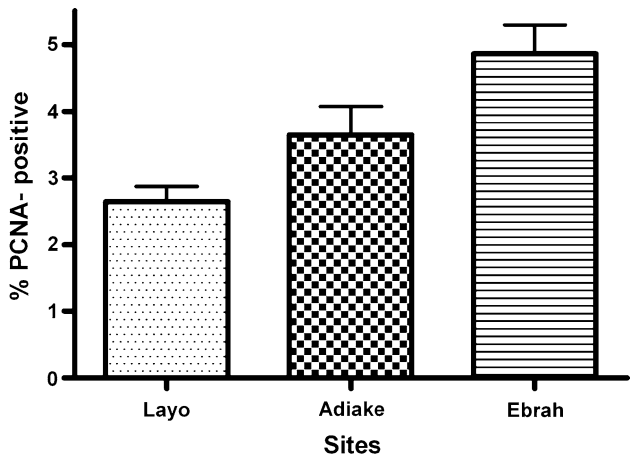

Fig. 9 Percentages of stained cells in *C. nigrodigitatus* livers from the three sites

changes such as swelling, lifting of lamellar epithelium and hyperplasia in the gill lamellae. Dang et al. (2000) observed an increase of apoptotic gills cells in tilapia (*O. mossambicus*) exposed to copper. All these changes could be adaptive mechanisms or physiological responses to face the sublethal pollution.

Despite the lower level of pollutants in organism reported by previous studies, damages in *C. nigrodigitatus* tissues could reduce of fish stock through biological and physiological mechanisms. Weis and Weis (1989)

Table 4 *C. nigrodigitatus* gills pathologies in the three sites of the lagoon

	Hyperplasia	Parasites	Others
Gills Layo			
1	–	–	–
2	–	–	–
3	Minimal	–	–
4	–	–	–
5	–	–	–
Gills Adiake			
1	+++	Large protozoan cysts	Hemorage multifocal
2	–	–	Very little material
3	++	Scattered metazoan (digenean)	–
4	++	–	–
5	–	–	–
Gills Ebrah			
1	+		–
2	+	Rare protozoan	–
3	+		–
4	+		–
5	+	Scattered metazoan	–

Table 5 *C. nigrodigitatus* hepatic pathologies in the three sites of the lagoon

	Vacuolation	Necrosis	Fibrosis	Hepatitis	Others	Pancreas
Livers Layo						
1	–	–	–	Minimal multifocal	Rare central veins with prominent smooth muscle	Rare nematodes
2	Patchy mild fatty centrolobular +	–	–	–	Many central veins with prominent smooth muscle	–
3	Patchy mild fatty centrolobular +	–	–	–	–	–
4	Patchy mild fatty centrolobular +	–	–	Minimal focal pericholangitis +bile storage at pancreatic/ hepatic interface	–	–
5	Patchy mild fatty centrolobular +	–	–	–	–	–
Livers Adiake						
1	Diffuse fatty ++	–	–	–	Bile laben macrophage	–
2	Peripheral lobular multifocal diffuse vacuolation (glycogen) ++	–	–	–	Bile laben macrophage	–
3	–	–	–	–	Granules fine grey	–
Livers Ebrah						
1	–	–	–	–	–	–
2	–	–	–		Multifocal pericholangitis +	
3	–	Scattered fibrinoid necrosis central veins	–	–	Minimal multifocal hepatitis	Bile macrophages +
4	–	Rare scattered fibrinoid necrosis central veins	–	–	Pericholangitis +	–
5	–	–	–	–	–	Rare bile macrophages

indicated that essential and non-essential metal could alter embryonic development of fish embryos causing a normal development, disability of organs or mortality. Otherwise, the increase of rodlet cells near the surface of primary lamellae of gills were observed in several parasite infected fishes (Dezfuli et al. 2003). The rodlet cells secretion has an antibiotic function (defensive role) against parasites (Leino 1996).

In conclusion, immunohistochemistry and histopathology in fish tissues are a useful tool for eco toxicological investigations in aquatic ecosystems and highlight the effects of pollutants. An advantage is that it allows one the visualization of damage to the tissues that could be important for people in developing countries, consuming these fish at a relatively high percentage of their diet.

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