Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

September 2010, 31(5) 715-720 (2010)
For personal use only
Commercial distribution of this copy is illegal

Ultrastructure changes in hepatocytes of catfish *Clarias gariepinus* from Lake Mariut, Egypt

Ashraf M. Abdel-Moneim*[1] and Hala A. Abdel-Mohsen[2]

[1]Department of Zoology, Faculty of Science, Alexandria University, Alexandria - 215 11, Egypt
[2]National Institute of Oceanography and Fisheries, Kayet-Bay, Al-Anfoushy, Alexandria, Egypt

(Received: February 26, 2009; Revised received: July 18, 2009; Accepted: August 25, 2009)

Abstract: *In the present study, specimens of catfish (Clariidae) were collected from a polluted location (Main Basin) and a relatively clean area (East Basin) in Lake Mariut, one of the Nile Delta Lakes in Egypt. Fifteen fish were taken from each site. Liver preparations of fish from the two sources were comparatively examined for cellular changes using transmission electron microscopy. Fish hepatocytes from the polluted area showed accumulation of the heterochromatin, enlarged nucleoli, and an extremely folded nuclear envelope. Perichromatin granules were increased and progressively formed small clusters closely associated with patches of heterochromatin. In the cytoplasm, fractionation, dilation, and vesiculation of rough endoplasmic reticulum (RER), and elevated amounts of smooth endoplasmic reticulum (SER) tubules were noted. The most frequent pathological modifications were the swelling of mitochondria, cristae regression and changes in the electron-transparency of the matrix. Lysosomes showing myelin-like stacks of membraneous material (phospholipidosis), glycogenosomes (i.e., glycogen rosettes enclosed by membranes) and cytoplasmic myelinated bodies were strongly developed. Furthermore, increasing numbers of secondary lysosomes with degraded cell organelles were found. With reference to the storage vesicles, there appeared to be an increase in the lipid droplets (lipidosis) within many hepatocytes. This study reinforces the need to select representative sentinel species from different habitats for biomonitoring purposes and it provides further support for the use of biomarkers in assessing the health of aquatic ecosystems.*

Key words: *Clarias gariepinus, Liver, Ultrastructure, Pollution, Lake Mariut*
PDF of full length paper is available online

Introduction

Inland water, including seas, rivers and lakes receive massive flux loaded with industrial and anthropogenic wastes that exert huge impact on aquatic life (Barton and Iwama 1991; Adham, 2002; Padmanabha and Belagali, 2008; Toroglu and Toroglu, 2009; Srivastava *et al.*, 2009). In Egypt, lakes of the Nile Delta that used to be of special economic and social importance are now under potential risk due to uninterrupted runoff of many types of polluted discharges (Adham, 2002). Lake Mariut is the smallest of these lakes and the most polluted one. It has suffered over the years from the untreated sewage, agricultural and industrial wastes dumped into it (Hamza, 1999; Amr *et al.,* 2005). Fish quality and quantity were dramatically affected and eventually becoming unfit for human consumption due to the poor water quality of the lake (Adham *et al.*, 1997; Amr *et al.,* 2005). Many fish species like *Mugil cephalus*, *Mugil capito*, *Anguilla vulgaris*, *Cyprinus carpio* and *Morone labrax* have disappeared from the lake due to the increased pollution problem (Saleh *et al.*, 1983a,b; Saleh and Hamza, 1986). Only few fish species survived including the Nile sharptooth catfish or *Clarias gariepinus*. A synonym of *Clarias gariepinus* is *Clarias lazera* (Teugels, 1986).

Clarias gariepinus is the most important species for aquaculture among 32 valid species of the genus *Clarias* recognized by Teugels (1984). It is probably the most widely distributed fish in Africa (Adham, 2002). This fish species grows up to 60-170 cm total length and is of considerable importance as food fish (Admek and Sukop, 1995). It is omnivorous feeder and a general scavenger with a marked tendency to feed on benthic organisms and detritus (Bok and Jongbloed, 1984). As other clariids, this species is highly resistant to muddy waters and can survive extremes of aquatic hypoxia and even desiccation (Bok and Jongbloed, 1984).

Polluted water habitats exert extensive stress impacts upon aquatic animals (Barton and Iwama, 1991). Histopathological biomarkers of xenobiotic exposure and histopathology of fish liver have been increasingly recognized as valuable tools for the detecting adverse chronic effects of contaminant exposure and uptake on aquatic organisms (Myers *et al.,* 1998; Pietrapiana *et al.,* 2002). Hepatic lesions, including neoplasms, focal lesions, degenerative or necrotic lesions are commonly detected in fish from contaminated environments (Myers *et al.,* 1998). Such lesions are similar to those experimentally induced by toxicant or a carcinogen exposure in fish under chronic exposure to contaminated sediments and diets. Therefore, they have been related to environmental contaminant exposure in several field studies (Moore and Myers, 1994).

The use of fish as bio-indicators of pollution in Lake Mariut has received the attention of many workers. Much has been documented about the accumulation of heavy metals and pesticide residues in fish collected from polluted sites in the lagoon (Saad *et al*., 1982; El-Rayis and Saad, 1990; Amr *et al*., 2005). In addition, the connections between changes in physiological and molecular parameters such as acetylcholinesterase, alkaline phosphatase, glutathione S-transferase, DNA damage and free amino acid

* **Corresponding author:** ashraf_abdelmoneim@yahoo.com

composition, in fish blood or target organs, and pollution stress in the lake have been studied (Adham *et al.*, 1997,1999,2001; El-Demerdash and Elagamy, 1999; Matta *et al.*, 2007). Furthermore, sperm deformations in fish have been used as a model for predicting environmental hazards in Lake Mariut (Abdelmeguid *et al*., 2007). However, the effects of pollutants on liver ultrastructure of benthic fish (*e.g.*, catfish) collected from the lake have not been examined so far. Accordingly, the aim of the present study was to mark the possible cytological changes in hepatocytes of *Clarias gariepinus* as affected by chemical pollution in Lake Mariut.

Materials and Methods

Study area: Lake Mariut is an important fishing lake at the southern area of Alexandria City, Egypt (Fig. 1). It has no direct connection with the Mediterranean Sea. Lake Mariut is mainly divided into five principal basins; these are (main, MB; east, E; northwest, NW; southeast, SE; southwest, SW) (Adham *et al.,* 2001). The East Basin (also known as Fish Farm) extends 6 km beside the desert road with an average depth of 130 cm. It receives most of its water from the New Mariut Hydraulic Pumps and Umoum Drain at the southern margin. The Fish Farm connects with Qualaa Drain by a movable gate at its northern extremity. However, this gate is usually closed (Smaan and Abdel-Moneim, 1986). The Main Basin or Lake Proper is of about 600 acres, however, its area has reduced greatly due the recent establishment of many investment projects on the lake. It is boarded by highways from three sides and by the Nubarriya Canal and Umoum Drain at the west (Smaan and Abdel-Moneim, 1986). It receives flow from three main sources of pollution; the first is Qualaa Drain which carries agricultural wastewater, sewage discharge and industrial effluents to the basin. The second source is the West Treatment Plant (WTP), while the third one is Umoum Drain (El-Rayis, 2005). According to El-Rayis and El-Sabrouti (1998), the surplus water from the lake proper is allowed to flow into the lower reach of Umoum Drain before pumping the mixed waters (6.8 hm^3 d^{-1}) to the Mediterranean Sea at El-Max (Fig. 1). This pumping process maintains surface water level in the lake at about 2.5 m below sea level, to work properly as a recipient of different effluents.

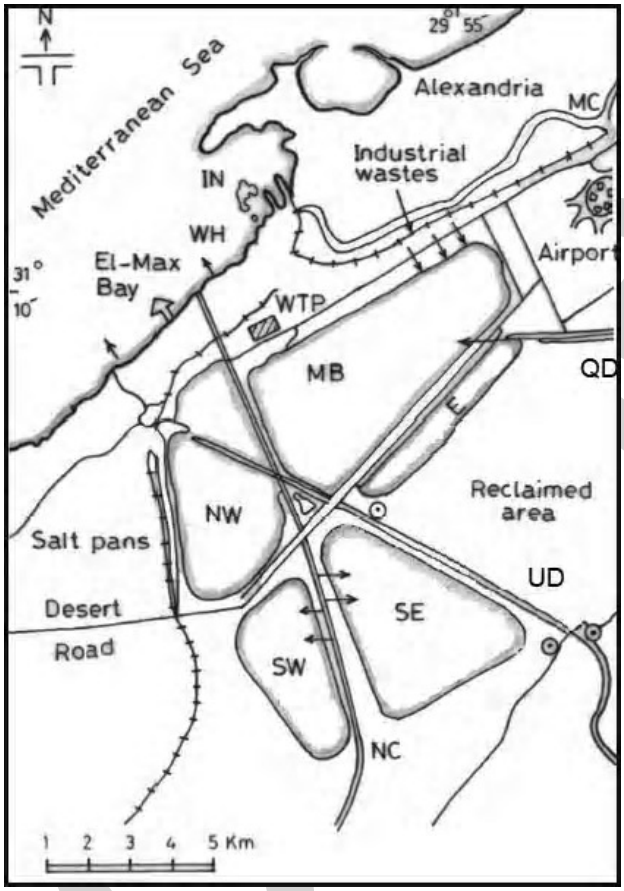

Fig. 1: Map of lake Mariut showing its position in relation to the Mediterranean sea as well as the supporting canal system (Adham *et al.,* 2001). MB, main basin (polluted area); E, east basin (relatively clean area); NW, northwest basin (cleaned area); SE, southeast basin; SW, southwest basin; IN, inner harbor; WH, west harbor; MC, Mahmoudiya Canal; NC, Nubarriya Canal; QD, Qualaa Drain; UD, Umoum Drain; WTP, west treatment plant

Fish sampling: During the summer months (June - August) of 2007, about 15 male specimens of *Clarias gariepinus* were collected alive from various fishermen from each of the two sampling sites of Lake Mariut and were then brought to the laboratory in plastic containers. Total lengths of specimens ranged between 27.3 and 40.7 cm and their weights fluctuated between 125 and 515 g. Maturation stages of the collected specimens were visually determined from the general morphology of gonads, according to Sehriban and Yilmaz (2007). All examined specimens were mature ones. As soon as the fish samples reached the laboratory, they were dissected and liver tissues were taken for transmission electron microscopy (TEM).

Ultrastructure study: Liver samples were fixed by immersion in 2.5% glutaraldehyde in phosphate buffer solution (pH 7.2) at 4°C. The specimens were post-fixed in 2% osmium tetroxide for 2h at 4°C. They were rinsed again in buffer, dehydrated in a graded ethanol series, and embedded in epon-araldite mixture. Ultrathin sections were cut and stained with uranyl acetate and lead citrate according to Reynolds (1963). The specimens were viewed in Jeol 100 CX TEM. To quantify hepatocellular modifications as a consequence of pollution impacts, a semi-quantitative evaluation was carried out by classifying the extent of the changes into six categories: - = absent; + = very weak; + + = weak; + + + = strong; + + + + = very strong.

Results and Discussion

Catfish hepatocytes from the East Basin (relatively clean area) were distinctly subdivided into an organelle-rich zone around the centrally located nucleus and a peripheral area with glycogen rosettes interspersed with lipid deposits (Fig. 2a). Nuclei showed heterochromatin randomly distributed in the nucleoplasm with small concentrations underneath the nuclear envelope. The organelle-rich portion of the cytoplasm was found to be comprised of mitochondria, peroxisomes, tubules and cisternae of smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER) stacks with non-fenestrated cisternae arranged in parallel array. In addition, golgi fields consisting of cisternae and occasional

lipid droplets were also observed. Thus, the ultrastructure of reference hepatocytes closely resembled that described for control fish hepatocytes in other experiments (*e.g.*, Braunbeck *et al.,* 1989, 1990).

Cytological changes in fish hepatocytes from the polluted area (Main Basin) are summarized and quantified in Table 1. Part of the hepatocytes showed an increasing tendency of the heterochromatin to condense in the nuclear periphery and around the nucleolus (Fig. 2b). Perichromatin granules (Fig. 2c) were increased and progressively formed small clusters closely associated with patches of heterochromatin. Significantly enlarged nucleoli indicating enhanced hepatocellular activity were clearly seen. An extremely folded nuclear envelope could also be observed (Fig. 2d). Moreover, cytoplasmic effects comprised a less regular compartmentalization of different components. RER underwent a progressive loss of structural integrity. Although part of the RER cisternae still displayed typical parallel arrays, increasing numbers of cisternae appeared fractionated, dilated and vesiculated. Furthermore, RER cisternae were occasionally transformed into circular arrays (Fig. 2e). In contrast to the RER, elevated amounts of SER tubules were noted. The most frequent pathological modifications were the swelling of mitochondria, cristae regression and changes in the electron-transparency of the matrix (Fig. 2b). On the other hand, increasing heterogeneity in the lysosomal matrix was commonly observed. Lysosomes showing myelin-like stacks of membraneous material (phospholipidosis), glycogenosomes (*i.e.* glycogen rosettes enclosed by membranes) and cytoplasmic myelinated bodies were strongly developed (Fig. 2b). Furthermore, increasing numbers of secondary lysosomes with degraded cell organelles were found (Fig. 2f). In peroxisomes, no structural changes were evident. However, small clusters of peroxisomes were observed. With reference to the storage vesicles, there appeared to be an increase in the lipid droplets (lipidosis) within many hepatocytes (Fig. 2f).

Table - 1: Semiquantitative analysis of ultrastructural alterations in the liver of catfish (*Clarias gariepinus*) collected from two locations in lake Mariut

	Relatively clean area (East Basin)	Polluted area (Main Basin)
Nucleus		
Irregular outline	-	+ +
Dilation of nuclear membrane	+	+ + +
Amount of heterochromatin	+ +	+ + +
Perichromatin granules	+	++
Mitochondria		
Cristae regression and electron lucent matrix	-	+ + +
Swelling	-	+ + +
Peroxisomes		
Structural integrity	+ + +	+ + +
Formation of clusters	-	+
Lysosomes		
Heterogeneity of lysosomal matrix	+	+ +
Secondary lysosomes	+	+ + +
Phospholipidosis (myelin formation)	-	+ +
Glycogenosomes	-	+ +
Myelinated bodies in cytoplasm	-	+ +
Vacuoles	+	+ + +
RER		
Parallel stacks of RER cisternae	+ + +	+
Fragmentation of cisternae	-	+ +
Dilation of membranes	+	+ +
Vesiculation of membranes	+	+ + +
Transition into concentric arrays	-	+ +
SER		
Quantity	+	+ + +
Golgi fields		
Activity	+ +	+ +
VLDL-particles	+ +	+ +
Storage materials		
Amount of lipid	+	+ + +
Amount of glycogen	+ + +	+

Data are given as means from 15 fish

Abbreviations: - = Not present, + = Little developed, + + = Moderately developed, + + + = Strongly developed, RER = Rough Endoplasmic Reticulum, SER = Smooth Endoplasmic Reticulum, VLDL = Very low density lipoprotein

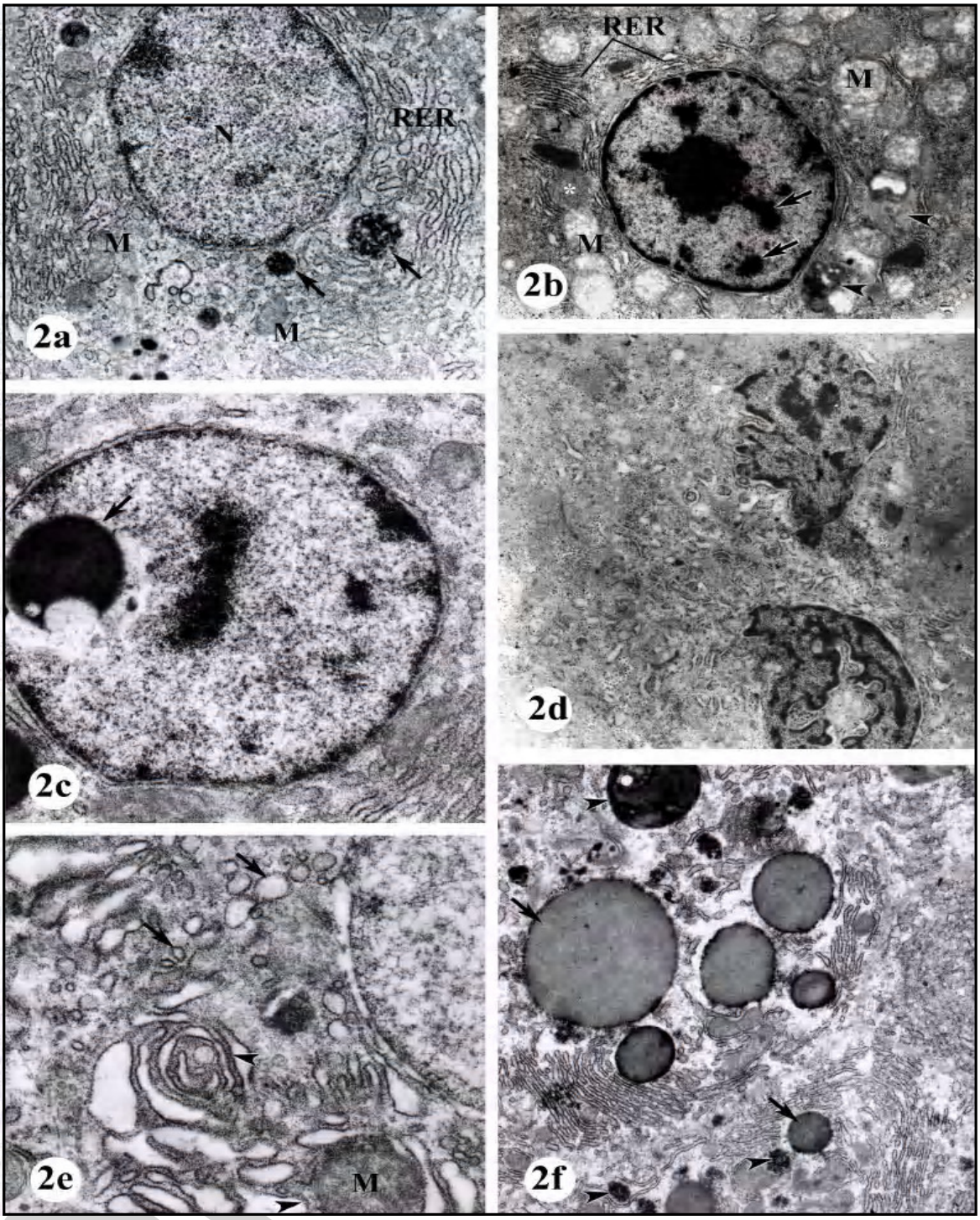

Fig. 2: Ultrastructure of hepatocytes of *Clarias gariepinus* collected from the East Basin (relatively clean area) (a) and the Main Basin (polluted area) (b-f). (a) The nucleus (N) is large and centrally located. There are low numbers of secondary lysosomes (arrows). The cytoplasm contains mitochondria (M), and RER cisternae. The remainder of the cytoplasm represents glycogen storage, X 7500. (b) Accumulation of heterochromatin (arrows) is evident. Note fractionation of the RER cisternae, degenerated mitochondria (M) and the presence of numerous and large myelinoid bodies (arrowheads) within hepatocyte. Peroxisomes (asterisk) are mostly concentrated in fields of dilated RER cisternae, X 7500. (c) Note large intranuclear inclusion body (arrow), perichromatin granules can also be noted, X10000. (d) The nuclei exhibit severe irregularities in shape, along with the development of SER, X 7500. (e) Note dilation, fragmentation and vesiculation of RER cisternae (arrows) are strongly developed. Degranulation and transition of RER cisternae into circular arrays (arrowheads) enclosing glycogen or, mitochondria (M) are also observed, X 10000. (f) Augmentation of lipid droplets (arrows) with decrease of cellular organelles and proliferation of lysosomal elements (arrowheads) can be seen, X 5000

Because of the deleterious impact of pollutants in aquatic ecosystems (mainly in continental and coastal areas), the histo-cytological responses of fishes to various classes of xenobiotic compounds need to be determined and characterized (Hinton *et al.,* 1978; Couch, 1988; Braunbeck *et al.,* 1989, 1990). Fish liver structure and ultrastructure proved to be valuable as sensitive indicators of toxicant-induced injury (Hinton *et al.,* 1988; Braunbeck and Volkl, 1991). In the present study, liver cells of catfish from polluted area in Lake Mariut showed toxicant-related alterations in ultrastructure. Most ultrastructural changes such as progressive dilation of RER cisternae, irregular nuclear outline, and induction of glycogenosomes and phospholipidosis are of unspecific nature, since they were also reported in fish after exposure to several toxicants such as lindane (Sylvie *et al.,* 1996), polychlorinated biphenyls (Hugla and Thome 1999), and mercury (Giari *et al.,* 2008). On the other hand, myelin-like figures in the cytoplasm of cells may be used as an index of autophagy of certain damaged membranous components as well as a high degree of turnover of cell organelles (Ghadially, 1997). Myelin-like figures in hepatocytes of catfish collected from the polluted Main Basin were noticed several times.

One of the main alterations in liver cells of catfish was the increase in size and number of lipid droplets. Increase in lipid droplets in hepatic cells of fish exposed to toxicants was reported by Biagianti-Risbourg and Bastide (1995), Biagianti-Risbourg *et al.* (1996), Braunbeck (1998), Strmac and Braunbeck (2002), and Thophon *et al.* (2004). A large increase in the number of lipid droplets in the cell cytoplasm reflected the decline of protein synthesis that accompanies hepatocyte injury which blocks the utilization of lipid-protein conjugation (Cheville, 1994). On the other hand, glycogen inclusions declined in hepatocytes of catfish caught from the Main Basin. Similar observation was noted in other fish species such as *Channa punctata*, *Oncorhynchus mykiss*, *Danio rerio* and *Liza ramada*, following exposure to several toxicants (Murthy and Devi, 1982; Biagianti-Risbourg and Bastide, 1995; Braunbeck, 1998) as well as in fish from natural polluted water (Teh *et al.,* 1997). This could be due to either increased glycolytic activity to meet the energy demands imposed by enhanced metabolic activity, hormone-mediated stress phenomena (Hanke *et al.,* 1983; Gluth and Hanke, 1985) or reduced intestinal absorption of carbohydrates (Braunbeck and Appelbaum, 1999).

The mitochondrial degeneration may account for the impaired oxidative capability of hepatocytes in fish from the polluted location. Indeed, marked ultrastructural changes including the presence of swollen mitochondria with loss of functional cristae have already been reported in the liver tissue of freshwater catfish exposed to methyl parathion (Tripathi and Shukla, 1990). Furthermore, SER proliferation is generally regarded to be indicative of the induction of biotransformation processes (Hinton *et al.,* 1978; Braunbeck *et al.,* 1989, 1990). In addition to SER proliferation, RER proliferation and fenestration (Braunbeck *et al.,* 1989, 1990; Lester *et al.,* 1993) as well as circular ER arrays were all suggested to be indicative of mixed-function oxidases (MFO) induction (Hawkes, 1980). It is worth mentioning that RER changes in catfish from contaminated area may well be interpreted as the morphological counterpart of ethoxycoumarin-O-deethylase (ECOD) and ethoxyresorufin-O-deethylase (EROD) induction. Similar findings were reported in rainbow trout after exposure to endosulfan and disulfoton (Arnold *et al.,* 1995), and in the demersal fish following intraperitoneal injection of benzo(a)pyrene (Au *et al.,* 1999). Most likely, the increase in perichromatin granules (observed in the present study) also represents aberrations in protein synthesis (Ghadially, 1997). In agreement, Adham *et al.* (2001) reported improper growth, protein inadequacy, and functional impairment in *Oreochromis niloticus* inhabiting polluted sites of Lake Mariut, in particular the Main Basin. These were reflected by data of specific formulae as RNA/DNA and the relative RNA content in cells of the liver and gill arches as well as by the relative mobilization of serum protein fractions.

In conclusion, our results suggest that water pollution in Lake Mariut is capable of inducing morphological alterations in liver, which may cause physiometabolic dysfunction in clariid species. This study reinforces the need to select representative sentinel species from different habitats for biomonitoring purposes and it provides further support for the use of biomarkers in assessing the health of aquatic ecosystems.

Acknowledgments

The authors wish to express their gratitude to Prof. Dr. Nabila E. Abdelmeguid, Professor of Cell Biology, Department of Zoology, Faculty of Science, Alexandria University, Egypt and to Prof. Dr. Hamed H. Saleh, Professor of Aquatic Pollution, National Institute of Oceanography and Fisheries, Alexandria, Egypt.

References

Abdelmeguid, N.E., A.M. Kheriallah, C.A. Matta and A.M. Abdel-Moneim: Environmental contaminant-induced spermatozoa anomalies in fish inhabiting Lake Mariut, Alexandria, Egypt. *Int. J. Appl. Environ. Sci.*, **2**, 1-18 (2007).

Adham, K.G.: Sublethal effects of aquatic pollution in Lake Mariut on the African sharptooth catfish *Clarias gariepinus* (Burchell, 1822). *J. Appl. Ichthyol.*, **18**, 87-94 (2002).

Adham, K., A. Kheirallah, Abou-Shabana, N. Abdelmeguid and A. Abdel-Moneim: Environmental stress in Lake Maryût and physiological response of *Tilapia zillii* Gerv. *J. Environ. Sci. Hlth., A*, **32**, 2585-2598 (1997).

Adham, K.G., I.F. Hassan, N. Taha and T.H. Amin: Impact of hazardous exposure to metals in the Nile and Delta Lakes on the catfish, *Clarias lazera*. *Environ. Monit. Assess.*, **54**, 107-124 (1999).

Adham, K.G., S.S. Hamed, H.M. Ibrahim and R.A. Saleh: Impaired functions in Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1757), from polluted waters. *Acta Hydrochim. Hydrobiol.*, **29**, 278-288 (2001).

Admek, Z. and I. Sukop: Summer outdoor culture of African catfish (*Clarias gariepinus*) and tilapias (*Oreochromis niloticus* and *Oreochromis aureus*). *Aquat. Living Resour.*, **8**, 445-448 (1995).

Amr, H.M., M.M. El-Tawila and M.H. Ramadan: Assessment of pollution levels in fish and water of main basin, Lake Mariut. *J. Egypt. Pub. Hlth. Association*, **80**, 51-76 (2005).

Arnold, H., H.J. Plutab and T. Braunbeck: Simultaneous exposure of fish to endosulfan and disulfoton *in-vivo*: ultrastructural, stereological and biochemical reactions in hepatocytes of male rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.*, **33**,17-43 (1995).

Au, D.W.T., R.S.S. Wu, B.S. Zhou and P.K.S. Lam: Relationship between ultrastructural changes and EROD activities in liver of fish exposed to Benzo[a]pyrene. *Environ. Pollut.*, **104**, 235-247 (1999).

Barton, B.A. and G.K. Iwama: Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Ann. Rev. Fish Dis.*, **1**, 3-26 (1991).

Biagianti-Risbourg, S. and J. Bastide: Hepatic perturbations induced by a herbicide (atrazine) in juvenile grey mullet *Liza ramada* (Mugilidae, Teleostei): An ultrastructural study. *Aquat. Toxicol.*, **31**, 217-229 (1995).

Biagianti-Risbourg, S., C. Pairault, G. Vernet and H. Boulekbache: Effect of lindane on the ultrastructure of the liver of the rainbow trout, *Oncorhynchus mykiss*, sac-fry. *Chemosphere*, **33**, 2065-2079 (1996).

Bok, A.H. and H. Jongbloed: Growth and production of sharptooth catfish, *Clarias gariepinus* (Pisces: Clariidae), in organically fertilized ponds in the Cape Province, South Africa. *Aquaculture*, **36**, 141-155 (1984).

Braunbeck, T.: Cytological alterations in fish hepatocytes following *in vivo* and *in vitro* sublethal exposure to xenobiotics - structural biomarkers of environmental contamination. *In*: Fish ecotoxicology (*Eds*.: T. Braunbeck, D.E. Hinton and B. Streit). Birkhauser Verlag, Basel, Switzerland. pp. 61-140 (1998).

Braunbeck, T. and A. Volkl: Induction of biotransformation in the liver of eel (*Anguilla anguilla* L.) by sublethal exposure to dinitro-o-cresol: An ultrastructural and biochemical study. *Ecotoxicol. Environ. Saf.*, **21**, 109-127 (1991).

Braunbeck, T. and S. Appelbaum: Ultrastructural alterations in the liver and intestine of carp *Cyprinus carpio* induced orally by ultra-low doses of endosulfan. *Dis. Aquat. Organ.*, **36**, 183-200 (1999).

Braunbeck, T., V. Starch and R. Nagel: Sex-specific reaction of liver ultrastructure in zebrafish *(Brochydunio rerio)* after prolonged sublethal exposure to 4-nitrophenol. *Aquat. Toxicol.*, **14**, 185-202 (1989).

Braunbeck, T., P. Burkhardt-Helm and V. Starch: Liver pathology in eels *(Anguilla anguilla* L.) from the Rhine river exposed to the chemical spill at Basel in November 1986. *Limnologie Aktuell*, **1**, 371-392 (1990).

Cheville, N.F.: Ultrastructural Pathology. Iowa State University Press, Ames (1994).

Couch, J.A.: Role of pathobiology in experimental marine biology and ecology. *J. Exp. Mar. Biol. Ecol.*, **118**, I-6 (1988).

El-Demerdash, F.M. and E.I. Elagamy: Biological effects in *Tilapia nilotica* fish as indicators of pollution by cadmium and mercury. *Int. J. Environ. Health Res.*, **9**, 173-186 (1999).

El-Rayis, O.: Impact of man's activities on a closed fishing-lake, Lake Maryout in Egypt, as a case study. ***M****itigation Adapt. Strat. Global Change*, **10**, 145-157 (2005).

El-Rayis, O.A. and M.A.H. Saad: Heavy metal pollution in lagoon Mariut on the southern coast of the eastern Mediterranean Sea. *J.K.A.U. Sci.*, **1**, 17-26 (1990).

El-Rayis, O.A. and M.A. El-Sabrouti: Pollution problems and proposals for restoration. *J. Arab Acad. Sci. Technol.*, **23**, 16-28 (1998).

Ghadially, F.N.: Ultrastructural pathology of the cell and mMatrix. Butterworths, London (1997).

Giari, L., E. Simoni, M. Manera and B.S. Dezfuli: Histo-cytological responses of *Dicentrarchus labrax* (L.) following mercury exposure. *Ecotoxicol. Environ. Saf.*, **70**, 400-410 (2008).

Gluth, G. and W. Hanke: A comparison of physiological changes in carp, *Cyprinus carpio,* induced by several pollutants at sublethal concentrations. *Ecotoxicol. Environ. Saf.*, **9**, 179-188 (1985).

Hamza, W.: Differentiation in phytoplankton communities of Lake Mariut: A consequence of human impact. *Bull. Fac. Sci. Alex. Univ.*, **39**, 159-168 (1999).

Hanke, W., G. Gluth and R. Mtiller: Physiological changes in carps induced by pollution. *Ecotoxicol. Environ. Saf.*, **7**, 229-241 (1983).

Hawkes, J.W.: The effects of xenobiotics on fish tissues: Morphological studies. *Fed. Proceed.*, **39**, 3230-3236 (1980).

Hinton, D.E., J.E. Klaunig and M.M. Lipsky: PCB-induced alterations in teleost liver: A model for environmental disease in fish. *Mar. Fish Rev.*, **40**, 47-50 (1978).

Hinton, D.E., J.A. Couch, S.J. Teh and L.A. Courtney: Cytological changes during progression of neoplasia in selected fish species. *Aquat. Toxicol.*, **11**, 77-I 12 (1988).

Hugla, J.L. and J.P. Thome: Effects of polychlorinated biphenyls on liver ultrastructure, hepatic monooxygenases, and reproductive success in the barbel. *Ecotoxicol. Environ. Saf.*, **42**, 265-273 (1999).

Lester, S.M., T.A. Braunbeck, S.J. Teh, J.J. Stegeman, M.R. Miller and D.E. Hinton: Hepatic cellular distribution of cytochrome P-450 IA1 in rainbow trout (*Oncorhynclius mykiss):* an immunohisto- and cytochemical study. *Cancer Res.*, **53**, 3700-3706 (1993).

Matta, C.A., A.M. Kheirallah, N.E. Abdelmeguid and A.M. Abdel-Moneim: Effects of water pollution in Lake Mariut on gonadal free amino acid compositions in *Oreochromis niloticus* fish. *Pak. J. Biol. Sci.*, **10**, 1257-1263 (2007).

Moore, M.J. and M.S. Myers: Pathobiology of chemical-associated neoplasia in fish. *In*: Aquatic toxicology: Molecular, biochemical and cellular perspectives (*Eds*.: D.C. Malins and G.K. Ostrander). CRC press, Boca Raton, FL. pp. 327-386 (1994).

Murthy, A.S. and P. Devi: The effect of endosulfan and its isomers on tissue protein, glycogen and lipids in the fish *Channa punctata. Pest. Biochem. Physiol.*, **17**, 280-286 (1982).

Myers, M.S., L.L. Johnson, O.P. Olson, C.M. Stehr, B.H. Horness, T.K. Collier and B.B. McCain: Toxicopathic hepatic lesions as biomarkers of chemical contaminant exposure and effects in marine bottom fish species from the Northeast and pacific coasts, USA. *Mar. Pollut. Bull.*, **37**, 92-113 (1998).

Padmanabha, B. and S.L. Belagali: Ostracods as indicators of pollution in the lakes of Mysore. *J. Environ. Biol.*, **29**, 415-418 (2008).

Pietrapiana, D., M. Modena, P. Guidetti, C. Falugi and M. Vacchi: Evaluating the genotoxic damage and hepatic tissue alterations in demersal fish species: A case study in the Ligurian Sea (NW-Mediterranean). *Mar. Pollut. Bull.*, **44**, 238-243 (2002).

Reynolds, E.S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.*, **17**, 208-212 (1963).

Saad, M.A.H., M.M. Abu-Elamayem, A.H. El-Sebae and I.F. Sharaf: Occurrence and distribution of chemical pollutants in lake Mariut, Egypt. I. Residues of organchlorine pesticides. *Water Air Soil Pollut.*, **17**, 245-252 (1982).

Saleh, H.H. and A. Hamza: Study on the health condition of *Tilapia zillii* (Gerv.) living in the polluted water of Merghim zone, lake Mariut, Alexandria (Egypt). *FAO-UNEP*, **334**, 132-141 (1986).

Saleh, H.H., A. Hamza and B.S. El-Baghadadi: Effect of water pollution in Lake Mariut on mortality and survival rates of *Tilapia zillii* (Gerv.). *Bull. High Inst. Pub. Hlth.*, **13**, 59-76 (1983a).

Saleh, H.H., A. Hamza and B.S. El-Baghadadi: Effect of water pollution on fish population of Lake Mariut. *Bull. High Inst. Pub. Hlth.*, **13**, 233-247 (1983b).

Sehriban, C. and E. Yilmaz: Gonad development and sex ratio of sharptooth catfish (*Clarias gariepinus* Burchell, 1822) cultured under laboratory conditions. *Turk. J. Zool.*, **31**, 35-46 (2007).

Smaan, A. and M.A. Abdel-Moneim: Some physical features of the polluted basin and Fish Farm in Lake Mariut, Egypt. *Bull. Inst. Oceanogr. Fish.*, **12**, 149-163 (1986).

Srivastava, N., G. Harit and R. Srivastava: A study of physico-chemical characteristics of lakes around Jaipur, India. *J. Environ. Biol.,* **30**, 889-894 (2009).

Strmac, M. and T. Braunbeck: Cytological and biochemical effects of a mixture of 20 pollutants on isolated rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Ecotoxicol. Environ. Saf.*, **53**, 293-304 (2002).

Sylvie, B.R., C. Pairault, G. Vernet and H. Boulekbache: Effect of lindane on the ultrastructure of the liver of the rainbow trout, *Oncorhynchus mykiss*, sac-fry. *Chemosphere*, **33**, 2065-2079 (1996).

Teh, S.J., S.M. Adams and D.E. Hinton: Histopathologic biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. *Aquat. Toxicol.*, **37**, 51-70 (1997).

Teugels, G.G.: The nomenclature of African *Clarias* species used in aquaculture. *Aquaculture*, **38**, 373-374 (1984).

Teugels, G.G.: A systematic revision of the African species of the genus *Clarias* (Pisces; Clariidae). *Ann. Mus. R. Afr. Centr.*, **247**, 1-199 (1986).

Thophon, S., P. Pokethitiyook, K. Chalermwat, E.S. Upatham and S. Sahaphong: Ultrastructural alterations in the liver and kidney of white sea bass, *Lates calcarifer*, in acute and subchronic cadmium exposure. *Environ. Toxicol.*, **19**, 11-19 (2004).

Toroglu, E. and S. Toroglu: Microbial pollution of water in Golbasi lake in Adiyaman, Turkey. *J. Environ. Biol.,* **30**, 33-38 (2009).

Tripathi, C.L. and S.P. Shukla: Enzymatic and ultrastructural studies in a freshwater catfish: Impact of methyl parathion. *Biomed. Environ. Sci.*, **3**, 166-182 (1990).