Biomonitoring Studies of Environmental Pollution in Egypt Using Crayfish and Mosquito-fish with Emphasis on Bacteriological, Parasitological and Heavy Metal Assay

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Abstract

The present study was conducted to biomonitor the water quality in Abu-Rawash, Giza, Egypt by comparing the health status of two different aquatic organisms, red swamp crayfish (*Procambarusclarkii*) and mosquito-fish (*Gambusiaaffinis*).Bacteriological, parasitological and histo-pathological studies were performed.Physiochemical parameters (pH, Electrical conductivity, phosphate, ammonia and nitrate) and some heavy metals [copper (Cu), zinc (Zn), cadmium (Cd), nickel (Ni), and lead (Pb)] were analyzed in water samples. Heavy metal concentrations were also examined in different crayfish tissues (muscle, exoskeleton and hepatopancreas) and mosquito-fish.Some pathogenic and nonpathogenic bacterial species were isolated from both crayfish and mosquito-fish represented by *A. hydrophila*, *Ps. Fluorescens*, *Vibrio parahaemolyticus*, *E. coli*, Citrobacter spp., Enterobacter spp., staph and micrococcus. No parasites were found out in crayfish, while mosquito-fish showed high gill infection with encysted metacercariea. High levels of heavy metals were detected in water, they follow the order of Ni>Pb>Cd, while Zn and Cu were in normal values. Ni showed the highest bioaccumulation factor in both crayfish and mosquito-fishtissues, while Zn was in high concentration in the muscles of crayfish.Crayfish and mosquito-fish are two different biomonitoring aquatic species that give an early warning of the water pollution to take the responsible steps and avoid water pollution for saving our aquatic environment.

Keywords: Crayfish, Mosquito-fish, Biomonitoring, Bacteria, Parasites, Heavy metals.

Introduction

Willful introductions of non-indigenous species are sometimes intended to solve some troubles. The freshwater red swamp crayfish *Procambarusclarkii* was accidentally introduced to the Egyptian Nile water during the early 1980s. Mosquito-fish **(*Gambusia affinis*)** is an omnivorous, opportunistic cannibal, feeds on mosquito larvae, amphibian eggs and tadpoles, detritus, algae and aquatic plants. It lives in shallow slow flowing water bodies and can transmit parasites directly or indirectly to human; like *Haplorchistaichui* (**Chai *et al.*, 2005**). Crayfish and mosquito fishare two plentiful and different species in shallow aquatic environments. They reflect the water quality and thus considered as bioindicators of water pollution.

Some studies have revealed that crayfish was infected by bacteria, fungi, viruses, protozoa and metazoan parasites **(Saud *et al.*, 2013).** While others stated that the infection rate of crayfish by trematode metacercariae were declined since the 1970s (**Kim *et al.*, 2009**).

Heavy metal pollution in the water environment represents a serious problem. It finds its way to the water bodies through many sources; agricultural, industrial and household sources. These heavy metals enter the food chain and bioaccumulate in different tissues causing serious problems including man (**Naghshbandi*et al.*, 2007**). The present study was conducted to biomonitor water pollution in the River Nile through studying the bacteriological, parasitological and histopathological status of two different aquatic organisms; red swamp crayfish (*Procambarusclarkii*) and Mosquito-fish (*Gambusiaaffinis*) in addition to the heavy metal pollution.

Material and methods

Study site: The present study was conducted on a small branch of the River Nile,El-Mansoria canal, Abu-Rawash, Giza, Egypt. This branch receives high load of pollutants come from different anthropogenic sources.

Bacteriological examination:

Sampling and processing: 50 samples of *Procambarusclarkii* crayfish (45 - 65 gm body weight) and 50 samples of *Gambusiaaffinis* (2-4cm length and 4-5 gm body weight) were randomly collected from 5 different localities of the studied site. The specimens of crayfish were collected alive during the summer season of 2014and 2015 and transported in a plastic bags containing water and supplied with oxygen, while Gambusia fish samples were transportedwith the minimum time of delay in ice bags. Both were transported to the laboratory of the Hydrobiology Department, National Research Center Egypt. Swabs from gills, hepatopancreas with hemolymph samples were aseptically taken according to **(Lucía*et al.*, 2003),** while bacteriological samples in Gambusia fish were taken by cutting and separation of the kidney under the dorsal fin and cultured into Tryptic soya broth then smeared onto agar media (Brain heart infusion (BHI) agar media (Oxoid), Tryptic soya agar TSA (Oxoid), Aeromonas and Pseudomonas specific agar media for pure isolation of suspected isolates. The inoculated plates were incubated at 25 ^{o}C for 24 to 48 h. Representative numbers of the different colonial types detected on the media were

collected from plates and streaked on TSA for purification and identification.

Identification of isolates:

Identification of pure bacterial isolates was performed by following the criteria proposed by those described in **(Buller, 2004).**

Parasitological examination:

Wet smears from the branchial cavity and gills of each crayfish and mosquito fish were freshly examined, fixed with methanol, stained by 10% Gimsa stain and examined under the bright field microscope to identify the presence of any external protozoan parasites. Small pieces of gills, liver, hepatopancreas and muscles from both crayfish and mosquito fish were compressed between two glass slides (compressorium) and examined under the binocular dissecting microscope for the presence of metacercariae (**Pritchard and Kruse, 1982**).

Histopathological examination :

Small portions of gills, hepatopancreas and muscles were preapared according to **Bernet *et al.*, (1999),** examined microscopically and photographed by using a microscopic camera.

Water quality examination: Some water physicochemical parameters were analyzed according to **APHA (1995**).

Analysis of heavy metals:

Water samples: Water samples were collected at two consecutive summer seasons 2014 and 2015 from the study site. from the subsurface layer of different three points around the selected site (one meter apart from each other), acidified by concentrated nitric acid (5ml/L) and heavy metals (Cu, Zn, Cd, Ni, and Pb) were detected in one pooled sample by the atomic absorption spectrophotometer (Perkin-Elmer 3110, USA) (**APHA, 1995**).

Crayfish and mosquito fish: Crayfish was captured by hand net on summer months because of their absence in winter. The crayfish was dissected and hepatopancreas, muscles and exoskeleton were dried in an oven (120^{o}C), while mosquito fish was excavated and dried as a whole. Then these dried tissues were grounded in a ceramic mortar, and 0.5g of it were digested using concentrated nitric acid and the heavy metals concentrations were measured using the atomic absorption spectrophotometer (Perkin-Elmer 3110, USA) (**Riyahi, 2000).**

Accumulation factor (AF): It was calculated according to the following equation:

AF = Concentration of the heavy metal in the organ (mg/kg)/concentration of the heavy metal in water (mg/L) (**Authman *et al*., 2013**).

Results

Clinical and postmortem examination:

Clinical and postmortem examination of 50 crayfish revealed 45 samples were positive for bacterial isolation and 5 samples were negative. Affected crayfish were found to be anorexic, lethargic and had blistering on the end of the telson with necrosis and erosion were seen on the tail skin. Some of them were showing congestion and enlargement of hepatopancreas. On the other hand from 50 samples of mosquito fish 30 samples were positive for bacterial isolation.

Bacteriological examination: Bacteriological examination of Crayfish and mosquito was shown in table (1). **Parasitological examination:**

Parasitological examination of crayfish branchial cavity, gills and muscles showed no parasites. On the other hand, the examination of freshly compressed mosquito fish gills showed high infection with encysted metacercariae **(Fig. 1c).**

Water analysis: was shown in table 2&3

Table (1): percentages of bacterial spp. isolated from examined crayfish andmosquito fish.

Bacterial isolates	**Crayfish**		**mosquito fish**	
	No	% of isolation	No	% of isolation
Aeromonashydrophila	1	28.4	1	38.0
Pseudomonas fluorescens	7	20.0	9	26.0
Vibrio parahaemolyticus	1	6.7	1	16.0
E. coli	2	15.0	3	14.0
Citrobacter spp.	4	13.3	8	-
Enterobacter spp.	9	5.0	7	-
Staphylococcus spp.	8	3.3	-	-
Micrococcus spp.	3	8.3	-	6.0
	2		-	
	5		3	
Total	60	100	50	100

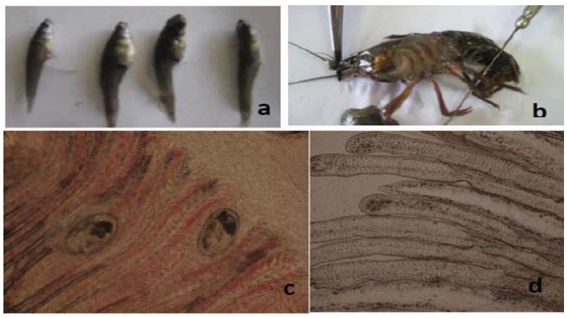

Fig. 1. Mosquito-fish, *Gambusia affinis* (a). Red Swamp Crayfish, *Procambarus clarkii* (b). Freshly compressed gills of *Gambusia affinis* showing infections with *Centrocestus sp*. (Heterophydae) encysted metacercariae (c). Freshly prepared gills of *Procambarus clarkii* showing absence of parasitic infestations (d).

Table (2): Physico-chemical characteristics and heavy metal analysis of water at the studied site in comparison to some reference permissible limits.

Parameter	Site result	Permissible limits	References
Physico-chemical characteristics			
pH	7.29	6-9	Egyptian Environmental Law No. 4 (1994)
EC (dS/m)	0.4	0.7-3	
PO_4 (ppm)	3.0	5	Egyptian Environmental Law No. 4 (1994)
NH_3 (ppm)	1.5	3	Egyptian Environmental Law No. 4 (1994)
		up to 12	WHO, 2003
NO_3 (ppm)	7.3	40	Egyptian Environmental Law No. 4 (1994)
Heavy metals			
Cu (ppm)	0.3	1	Egyptian law No. 48 (1982)
		1.5	Egyptian Environmental Law No. 4 (1994)
		0.009	USEPA (2009)
Zn (ppm)	0.31	1	Egyptian law No. 48 (1982)
		5	Egyptian Environmental Law No. 4 (1994)
		0.12	USEPA (2009)
Cd (ppm)	0.2	0.01	FAO (1983), Egyptian law No. 48 (1982)
		0.05	Egyptian Environmental Law No. 4 (1994)
		0.00025	USEPA (2009)
Ni (ppm)	0.71	0.01	Egyptian law No. 48 (1982)
		0.1	Egyptian Environmental Law No. 4 (1994)
		0.052	USEPA (2009)
Pb (ppm)	0.34	0.05	FAO (1983), Egyptian law No. 48 (1982)
		0.0025	USEPA (2006, 2009)

Table (3): Heavy metal concentrations (mg/Kgdry weight) in *Gambusiaaffinis* and *Procambarusclarkii*tissues at the studied site in comparison to some reference permissible limits.

	Cu	Zn	Cd	Ni	Pb
Gambusia (Whole fish)	6.9±1.1 (23)	101.1±5.9 (326.1)	9.3±0.9 (46.5)	142.9±8.2 (201.3)	11.3±1.3 (33.3)
Crayfish					
Hepatopancreas	42.8±3.4 (142.7)	51.2±3.2 (165.2)	4.8±0.2 (24)	165.6±7.2 (233.2)	3.9±0.09 (11.5)
Exoskeleton	33.3±1.7 (111)	35.1±1.2 (113.2)	1.9±0.03 (9.5)	150±3.6 (211.3)	2.7±0.08 (7.9)
Muscle	16.7±1.2 (55.7)	66.4±4.2 (214.2)	6.6±1.6 (33)	105±6.6 (147.9)	6.8±1.02 (20)
FAO (1983)	30	40	0.5		0.5
EC (2001)			0.5-1.0		0.2-0.4
[a]UKMAFF	20	50	Nd	Nd	1.0
[b]TPHR	30	40	5.5	Nd	Nd

Data are represented as mean value±standard deviation (accumulation factor).a and b: Seafood standards of heavy metal concentrations (µg/g wet weight) in various countries (UKMAFF: United Kingdom Ministry of Agriculture Fisheries and Food. TPHR: Tasmania Public Health Regulation) (**Huang, 2003**).

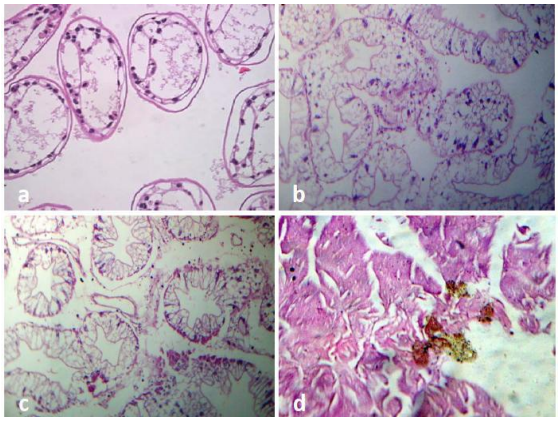

Fig. 2. Histopathological alterations noticed in *Procambarus clarkii*: Gill tissues showing slight degenerative changes and heamocytic cells infiltrations H&E, X400 (a). Hepatopancreatic sinuses showing vacuolar degeneration with heamocytic and eosinophilic granular cells infiltrations in the cell membrane lining H&E, X400 (b). Hepatopancreatic sinuses showing severe necrotic changes H&E X400 (c). Necrotic changes and melanomacrophages cells infiltrations in muscle tissue H&E, X400 (d).

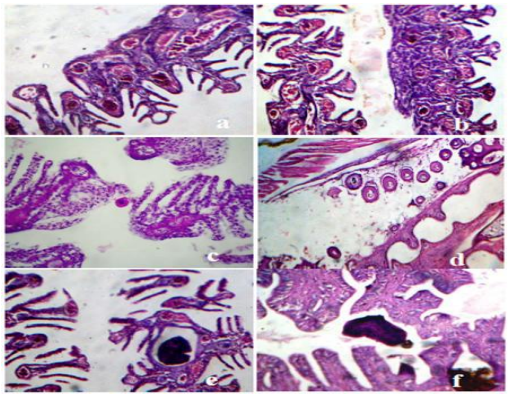

Fig. 3. Histopathological alterations noticed in *Gambusia affinis*: Gill tissue showing, congestion, degenerative, necrotic changes in the respiratory epithelium and hyperplasia H&E, X400 (a & b). Parasitic sections and cysts in between the gill tissues H&E, X400 (c, d, e & f).

Histopathological examination

The histopathological examination of red Swamp Crayfish, *Procambarus clarki*, revealed variable alterations. Gills showed vacuolar degeneration in the respiratory epithelium (Fig. 2a). Furthermore, swelling, vacuolation as well as necrotic changes were frequently denoted in the epithelial lining of the hepatopancreatic sinuses concomitantly with infiltrations of eosinophilic granular cells and heamocytic cells (Fig. 2b&c). Necrotic changes as well as melanomacrophages cells infiltrations occurred abundantly in between the muscle tissue (Fig. 2d).

On the other hand, regarding mosquito fish, *Gambusia affinis,* gills were congested (Fig. 3a & b)**.** Additionally, proliferative**,** degenerative and necrotic changes were commonly detected in the respiratory epithelium. Moreover, parasitic sections and cysts were noticed frequently in between gill tissues (Fig. 3c, d, e & f).

Discussion

An underappreciated aspect of crayfish biology is that the crayfish body is not simply a single organism, but rather a complex consortium of microbial and metazoan taxa. The crayfish exoskeleton can host a wide diversity of organisms. Many of these organisms may be only incidental associates, but others are obligate ectosymbionts **(Gelder 2010).**

Bacteria are often considered to be secondary or opportunistic pathogens of freshwater crayfish. However, a number of species or strains have been associated with serious mortality. They are frequently occur in the haemolymph of crayfish mostly belonging to the genera Vibrio, Aeromonas and Pseudomonas are most frequently associated with severe bacteraemia. Citrobacter, Pseudomonas, Acinetobacter and Enterobacter spp. are normal inhabitants of the gut of freshwater crayfish. In stressed crayfish, and/or those infected with a virulent strain, the bacteria may proliferate in the foregut, midgut and in the hepatopancreatic tubules causing necrosis in the epithelium **(Alderman,1996).**

With regard to our investigation of examined crayfish were found to be anorexic, lethargic and had blistering at the end of the telson with necrosis and erosion were seen on the tail skin. Some of them were demonstrating congestion and enlargement of hepatopancreas and this was in accordance with that demonstrated by **Lorraine and Peter (1994)** who found that affected crayfish were unable to walk and appeared to be stuck in the residue on the bottom of the ponds. Many had a white powdery coating over their bodies with blistering on the end of the

telson and on the uropod. Some of them exhibited erosion at the end of the telson and the tail integument exhibited necrosis and erosion with evidence of bacterial invasion.

Absence of parasites on crayfish examined in the current study will give spotlight on its role in decreasing the percentage of some epidemic diseases that were abundant in Egypt for a long time. Crayfish feeds on the snails which are the intermediate host of such diseases and consequently cut their life cycle controlling their incidence biologically (**Fishar, 2006)**. This is in accordance with **Kim *et al*., (2009)** who stated the decline of infection rate of digenetic trematodemetacercariaein crayfish since the 1970s.

On the other hand, the occurrence of mosquito fish in shallow and slow flowing water may make it more vulnerable to be infected with encysted metacercariaeand represents a dangerous in transmitting such parasites to human **(Chai *et al*., 2005).**

There are many reasons support the value of using crayfish in biomonitoring studies. The first one of them is its localization; it does not migrate, grow rapidly and reach its harvestable size in three to four months **(Banks and Brown, 2002).** Furthermore, it has a long life span of about 2 years and a continuous contact with both water and sediment since crayfish is typically epi- and sub-benthic in behavior, so itexposes to wide variety of contaminants that dissolved or suspended in the water column and also to those adsorbed to sediments. Moreover, it has a high enough position in the aquatic food web and the bioaccumulation and biomagnification are good represented in its tissuesthrough eating many smaller organisms **(Moss *et al*. 2010).**

The current study revealed high concentrations of heavy metals in the water that can enter the aquatic environment through discharge of waste water from sewage and/or industrial pollution. The highest concentration is nickel (0.7 ppm), which was higher than the permissible limits stated by **FAO (1983)** and **USEPA (2006)**.

Concentrations of Cu, Cd and Pb were ranged from 0.2-0.34 and were also higher than the compared permissible limits. This confirmed the presence of industrial and sewage waste water discharged in that branch of El-Mansouria canal.

Although Zinc was at a concentration of (0.31mg/l), which was not exceed the permissible levels, it has high concentrations in tissues especially the muscles and has also high accumulation factor. Zinc is an essential metal for the metabolic reactions in crayfish and its high concentration in the tissues independent on its concentration in the aquatic environment because the crayfish has the ability to manipulate its high levels for their own metabolic process (**Alcorlo*et al*., 2006**). This can be also emphasis with the fact that seafood are the greatest sources of zinc for humans.

Other analyzed metals during this study; Pb and Cd showed their high concentrations and bioaccumulations in the muscles of crayfish and this was confirmed by some previous studies which stated that the crayfish muscle (the human consumed part) is one of the preferential bioaccumulation tissue for heavy metals (**Higueras *et al*., 2006**). Besides that these metals are not involved in crayfish metabolism and increase in crayfish tissues with their increase in the surrounding aquatic environment and with longer exposure periods (**Alcorlo*et al*., 2006**).

The high concentration of Ni in the water and its consequent increase in the crayfish exoskeleton was in accordance with the opinion that the presence of some heavy metals in the exoskeleton may be due to their excretory role in elimination of heavy metals (**Macheviciene, 2002**).Also its high concentration in the hepatopancreas was emphasized because the hepatopancreas of the crayfish is concerned with more than one function; digestive juice secretion, food absorption, storage, and detoxification of pollutants, especially heavy metals (**Icely and Nott, 1992**). Moreover, the hepatopancreas concentrates the metals from the digestive tract and haemolymph and store them in its intracellular vacuoles (**Roldan and Shivers, 1987**).

Histopathological studies demonstrated variable degenerative, proliferative and infiltrative changes in histopathological sections. These alterations may be relevant to the damaging effects of the detected metals as well as may also be linked to the detected microbial infections. Similar alterations were noticed in previous studies **(Elgendy *et al*., 2015b).** The long exposure of aquatic animals to environmental pollutants, heavy metals in particular, influences their capability to defend against microbial infections by reducing the competence of their external and internal defense mechanisms including phagocytic activity of leukocytes and antibody synthesis **(Sinderman, 1995).** Moreover, majority of metals cause coagulation and precipitation changes of fish mucus, primary defense mechanism of aquatic animals, as well as cytological damages to the gills **(Burton *et al*., 1972)**. These injuries diminish gas exchange with tissue hypoxia and in some cases cause high mortalities. Moreover, gill lesions reduce the resistance against infectious agents and open portals of entry for establishing pathogenic bacteria especially when combined with unfavorable quality measures typical to that noticed in Elmansoria canal. On the other hand, we also argue that the elevated metals levels potentially created unsuitable conditions for existence of some ectoparasitic infestations in the investigated specimens in concordance with **(Kuperman, 1992)**.

Data extracted from our study clearly support the value of crayfish and mosquito fish in biomonitoring aquatic pollution. Results also demonstrated that these aquatic animals may acts as a significant reservoir of many microbial infections threatening fish as well as human beings. Hence keen monitoring via bioindicators can provide valuable data for assessment of environmental status which will support maintaining favorable water quality measures for aquatic animal health.

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