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Disease Causing Organisms in *Procambarus clarkii* and *Gambusia affinis* with Emphasis on their Role in Biomonitoring of Aquatic Pollution

W.S. Soliman, Wafaa T. Abbas*, Taghreed, B. Ibrahim, Amany, M. Kenawy and M.Y. Elgendy
Department of Hydrobiology, Veterinary Research Division, National Research Centre, Cairo, Egypt.

THE HEALTH status of red swamp crayfish, *Procambarus clarkii* and mosquito fish, *Gambusia affinis* collected from Elmansoria canal, Giza, Egypt was investigated. The canal is known to receive lofty loads of pollutants from diverse anthropogenic sources. 113 bacterial isolates were obtained from the investigated fish specimens. Isolates were phenotypically identified as, *Aeromonas hydrophila* 26.54%, *Vibrio parahaemolyticus* 21.23%, *Pseudomonas fluorescens* 14.15%, *E. coli* 10.61%, *Citrobacter* sp. 7.96%, *Enterobacter* sp. 8.84%, *Staphylococcus* sp. 4.42% and *Micrococcus* sp. 6.19%. High gill infestations with *Centrocestus* sp. encysted metacercariae were noticed in mosquito fish. No parasitic infestations were recorded in crayfish. Challenge experiment confirmed the pathogenicity of *Aeromonas hydrophila* isolates. The water analysis revealed high heavy metals levels with values, Ni 0.71, Pb 0.34 and Cd 0.2 ppm while Zn and Cu were in normal values. Metals analysis in crayfish and mosquito fish tissues denoted bioaccumulation. Crayfish muscles showed, Ni >Zn >Cu >Pb>Cd while their levels in mosquito fish demonstrated, Ni >Zn>Pb>Cd>Cu. Proliferative, degenerative and necrotic alterations were evident in histological sections. Results suggest that both crayfish and mosquito fish can serve as carriers for some fish disease pathogenic agents and a convenient tool for biomonitoring aquatic pollution.

Keywords: Bacteria, Parasites, Pollution, Crayfish, Mosquito fish.

Pathogenic microorganisms distribute wildly in the aquatic environment especially in polluted habitats. There is close relationship between emergence of aquatic animal diseases and coexistence of diverse pollutants in the aquatic environment (Elgendy *et al.*, 2015a). Polluted water deteriorates fish host defenses allowing increased opportunities for microbial agents to affect fish populations (Arkoosh *et al.*, 1998). Moreover, some pollutants and wastes are nutritious and cause eutrophication increasing bacterial load and algae in water as well as induce critical oxygen deficiencies (Ansari *et al.*, 2011). Accordingly,

*Corresponding author: WafaaTawfik Abbas, Assistant Professor, 00201001125171.
Email: wtabbas2005@yahoo.com

the early detection of these pollutants strongly assists to restrict the spread of many detrimental microbes to aquatic animals and/or human beings (Kuklina *et al.*, 2014).

Procambarus clarkii and *Gambusia affinis* are two models used largely in biomonitoring studies. They are extremely adaptable aquatic organisms endure wide range of critical environmental conditions (Abdelghany, 2002). Both are obstinately introduced into many non-indigenous aquatic habitats in the aim to solve some troubles. They feed on variety of food resources including algae, detritus, gastropods as well as numerous invertebrates (Whitledge and Rabeni 1997, Rincon *et al.* 2002). Meanwhile they are also preyed upon by various fish species, aquatic birds and mammals (Holdich, 2002) and can play prominent roles in water ecosystem, the burrowing activities of crayfish help to create appropriate habitats for many other small aquatic organisms (Pintor and Soluk, 2006**).** Furthermore, crayfish can biologically control many snails which represent important vectors as well as intermediate host of numerous pathogenic agents (Fishar, 2006).Confirmatory previous reports highlighted their significant role in the limitation of some parasitic infestations endemic to aquatic habitats (Haddaway *et al.*, 2012 and Du Preez, 2013).

Interestingly, crayfish does not migrate and usually localize in their habitats (Banks and Brown, 2002). Furthermore, it has a long life span extending up to 2 years with continuous contact with water and sediment as well as tolerate polluted environments consequently it can act as good biomonitors for aquatic pollution since it accumulates respective elements in their tissues (Moss *et al.*, 2010).

On the other hand, mosquito fish, *Gambusia affinis*, are willfully introduced in many countries as a bio-control for mosquito larvae especially in African countries. Its aggressive feeding habits on the eggs and larvae of fish have been accused for the decline of a number of fish species (Rincon *et al.*, 2002). Additionally, these fish constantly cause finnipping for other cohabitant fish subsequently stress them and potentiate their infection by lots of opportunistic bacterial and fungal agents (Lloyd, 1990).

Among pollutants, heavy metals are of particular concern due to their toxicity and competence to bio-accumulates in aquatic ecosystems (Miller *et al.*,2003) subsequently, metals affect human causing chronic toxicity and possibly cancer (Mohamed *et al.*, 2016 and Zhao *et al.*, 2014).

The present study aimed to assess the health status of two important aquatic organisms, *Procambarus clarkii* and *Gambusia affinis* collected from Elmansoria canal, Abo-Rawash, Giza, Egypt. This was performed in bio-monitoring studies to investigate the effect of water pollution on the existence of bacterial and parasitic agents as well as to demonstrate the accompanied histopathological alterations.

Material and Methods

Area of study and sampling

The present study was conducted in Elmansoria canal, a small branch from the River Nile, Abo-Rawash area, Giza, Egypt, during 2015 summer season. This branch receives high load of pollutants come from different anthropogenic sources. 50 samples of each red swamp crayfish, *Procambarus clarkii* (45-65 g) and mosquito fish, *Gambusia affinis* (4-5 g) were collected by fishermen. The specimens were transported alive in plastic bags containing water and supplied with oxygen, within the minimum time of delay to Hydrobiology Department, National Research Centre, Egypt. Water samples were collected early in the morning in sterile bottles from the subsurface layer of different three points around the selected site.

Bacteriological examination

Swabs from gills, hepatopancreas and hemolymph were aseptically obtained from crayfish specimens according to (Lucía*et al.*, 2003).On the other hand, regarding mosquito fish, loopfuls were retrieved from gills and kidneys. Inoculi were further enriched in tryptic soy broth then smeared onto agar media, Brain heart infusion agar (BHI) (Oxoid), Tryptic soy agar (TSA) (Oxoid), Aeromonas and Pseudomonas specific agar media (Oxoid). The inoculated plates were incubated at 25 ^{o}C for 24 to 48 h. Water samples obtained from Elmansoria canal were also analyzed microbiologically. Representative numbers of the different colonial types detected on the media were collected from plates and streaked on TSA for purification and identification according to Buller (2004).

Identification of isolates

Identification of pure bacterial isolates was performed by studying their morphological and biochemical characteristics according to schemes demonstrated by Bergey's Manual of Systemic Bacteriology (1982) using traditional as well as commercial API 20 E and API NE systems following the criteria described in (Buller, 2004).

Parasitological examination

All crayfish and mosquito fish samples were thoroughly investigated for external parasites by visual inspection via naked eye. Furthermore, wet smears from the cephalothoracic cavity and gills of crayfish and skin and gills of mosquito fish were freshly examined, fixed with methanol, stained by 10% Giemsa 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 encysted metacercariae (Pritchard and Kruse, 1982).

Experimental infection

Randomly selected *A. hydrophila* isolate was used for challenge experiment.1.24 × 10^7 CFU/ml inoculi were prepared according to (Elsherry, 2004) then injected into three different separated species, crayfish, mosquito fish and *Oreochromis niloticus*. Experimental animals were initially adapted to the wet lab conditions, kept in glass aquaria supplemented with sufficient water for an entire period of two weeks before the onset of challenge experiment. Each group contained 10 fish of the same species. Three other control groups, one for each species, were injected with phosphate buffer saline. Crayfish were injected into the haemolymph while mosquito fish and tilapia fish were challenged using the intra peritoneal route. All fishes were observed for two weeks after challenge with *A. hydrophila*. Dead fishes were processed microbiologically to re-isolate *A. hydrophila* in the aim to confirm the specificity of pathogenicity.

Water quality examination

Conductivity and pH of water samples were measured on spot while collecting the samples using digital portable apparatus, (pH meter model HI 8314 and digital conductivity meter HI2300 Hanna Ins. Romania). After acidification of water samples, phosphate, ammonia and nitrate were determined by colorimetric methods in the lab (APHA, 1995).

Heavy metals analysis

Water samples

Water samples were 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

The same metals were detected in the tissue samples. Crayfish samples were dissected, hepatopancreas, muscles and exoskeleton were dried in an oven (120^{o}C). On the other hand, mosquito fish was excavated and dried as a whole. Dried tissues were grounded in a ceramic mortar, 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)

Accumulation factor (AF) 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).

Histopathological examination

Small portions of gills, hepatopancreas and muscles were fixed in Davidson’s fixative for 48 hrs, then dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned at 5 microns. Sections were stained with Hematoxylin and Eosin method (Bernet *et al.*, 1999), examined microscopically and photographed by using a microscopic camera.

Results

Clinical examination

Investigated crayfish were lethargic, had some blistering on the end of the telson with necrosis and erosion on the tail region. Some specimens also demonstrated congestion and enlargement of hepatopancreas. On the other hand, mosquito fish specimens showed no specific signs except for erosions of skin and fins. Some specimens demonstrated petechial hemorrhages on the external body surfaces (Fig. 1a&b).

Bacteriological examination

Total number of 113 bacterial isolates was retrieved from investigated fish specimens, 72 isolates from crayfish and 41 isolates from mosquito fish. Retrieved isolates were further identified as, *Aeromonas hydrophila*26.54%, *Vibrio parahaemolyticus* 21.23%, *Pseudomonas fluorescens* 14.15%, *E. coli* 10.61%, *Citrobacter* sp. 7.96%, *Enterobacter* sp. 8.84%, *Staphylococcus* sp. 4.42% and *Micrococcus* sp. (6.19%). Concerning water samples, the total bacterial count was $2.5X10^6$, additionally, similar bacterial isolates were also detected in water samples collected from the canal.

Parasitological examination

Parasitological examination of mosquito fish demonstrated high frequency of *Centrocestus* sp. encysted metacercariae in gills with 75% infection rate, 2-5 cysts per gill filament (Fig. 1c). On the other hand, crayfish showed no parasitic infestations (Fig. 1d).

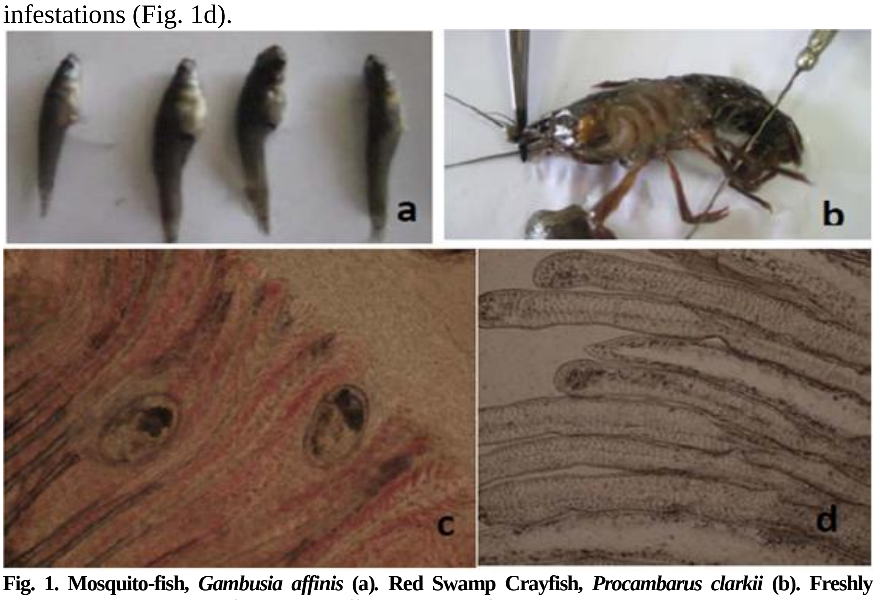

Fig. 1. Mosquito-fish, *Gambusia affinis* (a). Red Swamp Crayfish, *Procambarus clarkii* (b). Freshly compressed gills of *Gambusia affinis* showing infections with *Centrocestus sp.* (Heterophydae) encysted metacercariae (c). Freshly prepared gills of *Procambarus clarkii* showing absence of parasitic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

Experimental infection

Challenge experiment indicated the pathogenicity of *A. hydrophila* isolates. 60%, 100% and 80% of, crayfish, mosquito fish and tilapia fish respectively were died during the 2 weeks experimental period. Succumbed fish showed petechial hemorrhages on the external body surfaces and different degrees of erosion. Marked congestion of kidney was noticed in tilapia and mosquito fish. Some fish died without exhibiting any signs. *A. hydrophila* was re-isolated from all succumbed fishes.

Water analysis

Physicochemical water quality measures

The average recorded values for pH, EC, nitrate, ammonia and phosphate exhibited normal values when compared with the some international permissible limits (Table 1).

Heavy metals in water

Analysis of heavy metals in water samples demonstrated higher concentrations of some metals over the permissible limits, the uppermost concentration was detected for Ni (0.71ppm) and the lowest one was Cd (0.2ppm). On the other hand, Zn and Cu values were within normal standards (0.3ppm) (Table 1).

TABLE 1. Physicochemical and heavy metals analysis of water samples collected From Elmansoria canal in comparison to some reference permissible limits.

Parameter	Site	Permissible limits	References
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)
Cu (ppm)	0.3	1	Egyptian law No. 48 (1982)
		1.5	Egyptian Environmental Law No. 4 (1994)
		0.009	USEPA (2006)
Zn (ppm)	0.31	1	Egyptian law No. 48 (1982)
		5	Egyptian Environmental Law No. 4 (1994)
		0.12	USEPA (2006)
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 (2006)
Pb (ppm)	0.34	0.05	FAO (1983), Egyptian law No. 48 (1982)
		0.0025	USEPA (2006)

Heavy metal residues and bioaccumulation factor in tissue samples

Ni was the predominant metal detected in all tissues of mosquito fish as well as crayfish, followed by Zn. The metal concentrations and their bioaccumulation factors are illustrated in Table 2. The metals recorded in mosquito fish was Ni (201.3), Zn (326.1), Pb (33.3), Cd (46.5) and Cu (23) ppm. On the other hand, concerning crayfish, the uppermost level was recorded for Ni (233.2), (211.3) and (147.9) ppm in hepatopancreas, exoskeleton and muscles respectively. Concentrations of Zn, Cd and Pb in crayfish were higher in muscles (214.2), (33) (20) ppm respectively followed by hepatopancreas and exoskeleton, while Cu and Ni were higher in hepatopancreas, followed by exoskeleton and muscles.

TABLE 2. Heavy metal concentrations (mg/kg dry weight) in *Gambusia affinis* and *Procambarus clarkii* tissues in comparison to some reference permissible limits.

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

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).

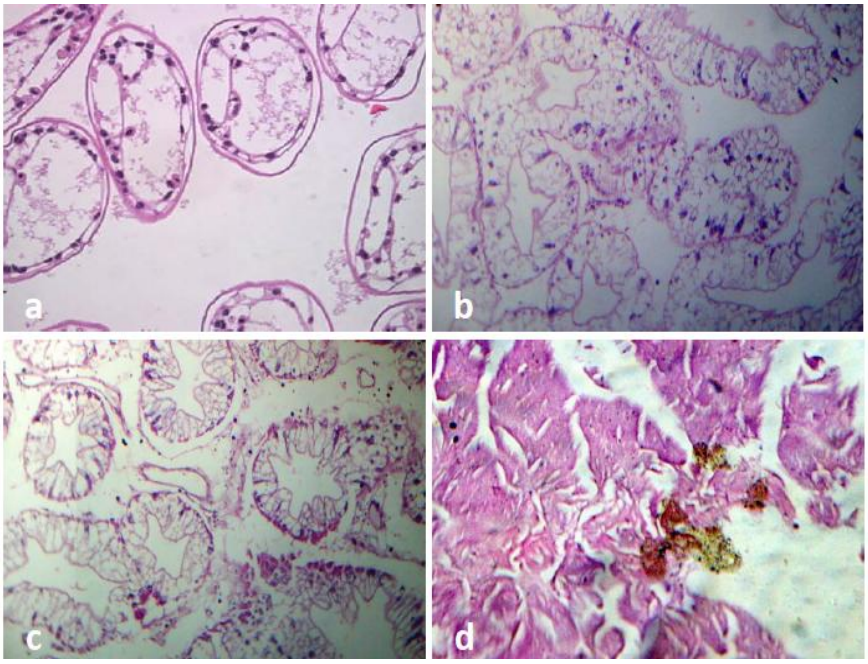

Fig. 2. Histopathological alterations noticed in *Procambarus clarkii*: Gill tissues showing slight degenerative changes and heamocytic cells infiltrations H&E, X400 (a). Hepatopancreatic sinuses showing vacuolar degeneration with heamocytic and eosinophilic granular cells infiltrations in the cell membrane 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).

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

Even though the hazards of water contamination on the health status and survivability of aquatic animals are well acknowledged, it remains a matter of interest due to its detrimental outcomes on both fish and human beings. Maintaining favorable water quality measures is critical for aquatic animal health. Hence keen monitoring via bioindicators can provide valuable data for assessment of environmental status (Burger, 2006).

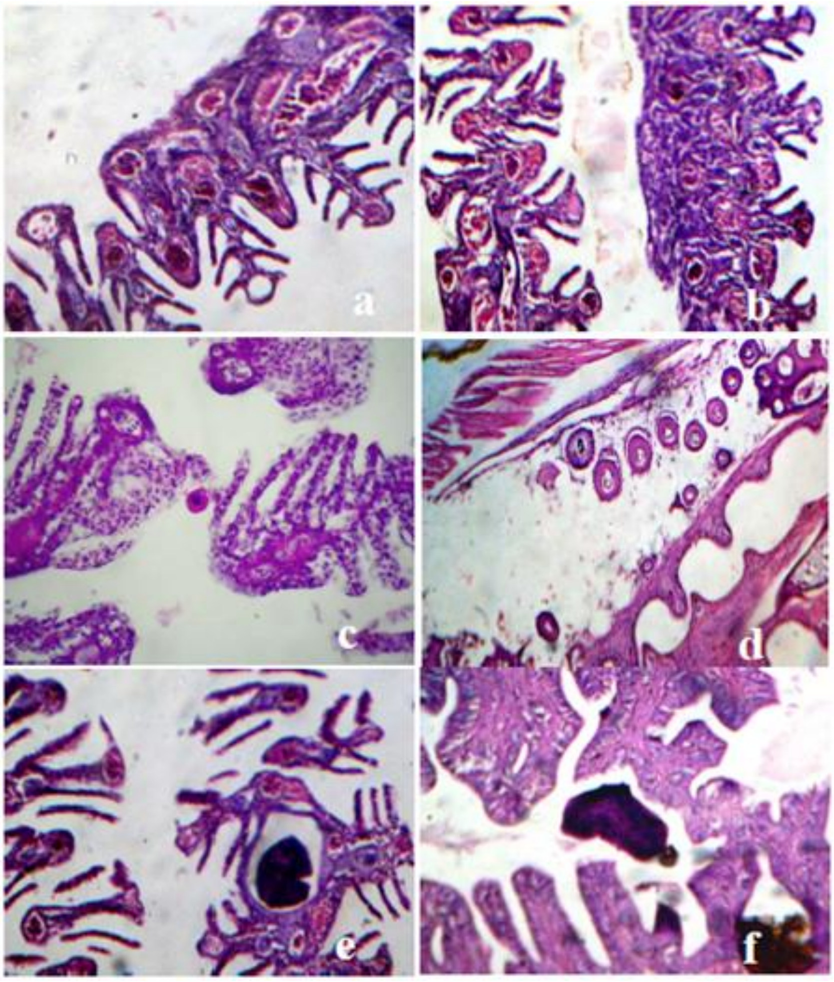

Fig. 3. Histopathological alterations noticed in *Gambusia affinis*: Gill tissue showing, congestion, degenerative, necrotic changes in the respiratory epithelium and hyperplasia H&E, X400 (a & b). Parasitic sections and cysts in between the gill tissues H&E, X400 (c, d, e & f).

A substantial body of scientific evidence has been published on the close relationship between pollution and microbial infections in aquatic animals. Infections are common in aquatic animals especially those exposed to unfavorable environmental conditions (Sinderman, 1995, Elgendy *et al.*, 2013 and Moustafa *et al.*, 2014).

Bacteriological examination demonstrated that *A. hydrophila* was the most predominant bacterial isolates 26.54%. The ubiquitous nature of this pathogen in aquatic environment may clarify this relatively high prevalence. Losses attributed to this microorganism in fish and shellfish have been recorded worldwide (Tulsidas *et al.*, 2008 and Elgendy, 2015b). *A. hydrophila* are frequently retrieved from both diseased and apparently healthy individuals, interestingly, even both types cause problems and mortalities in many farmed aquatic species (Quaglio *et al.*, 2006a).

Furthermore, *Vibrio parahaemolyticus* recorded 21.23%. Previous reports have showed that several *Vibrio* sp. Were frequently involved in infections as well as mortalities affecting crayfish including, *V. mimicus* and *V. cholera* (Thune *et al.*, 1991 and Wong *et al.*, 1995).Vibrios infections are commonly associated with no characteristic signs (Eaves and Ketterer,1994).Moreover, vibrios can do infections in many cultured as well as wild aquatic animals predisposed susceptible by underlying poor conditions (Edgerton *et al.*, 2002,Quaglio*et al.*, 2006b,Moustafa *et al.*, 2015 and Elgendy *et al.*, 2015a).

Results also demonstrated the recovery of other bacterial species including *Pseudomonas fluorescens*14.15%, *E. coli* 10.61%, *Citrobacter* sp. 7.96%, *Enterobacter* sp. 8.84 %, *Staphylococcus* sp. 4.42 % and *Micrococcus* sp. (6.19%). Results are in concordance with previous reports discussing microbial infections in crayfish denoting that many microorganisms are frequently detected including, *Coxiella cheraxi*, Acinetobacter, Nocardia, Bacillus, Spiroplasma, Citrobacter, Corynebacterium, Flavobacterium, Micrococcus, Pseudomonads, Staphylococcus and several *Vibrio* species (Edgerton *et al.*, 2002, Quaglio *et al.*, 2006b and Jiravanichpaisal *et al.*, 2009). Many bacterial infections can colonize the external exoskeleton and haemolymph of crayfish with the competence to impact this species in its natural habitat when their existence combined with unfavorable environmental conditions (David *et al.*, 2005).The lofty loads of discharges from sewage and agriculture drainages being thrown into the Elmansoryia canal are loaded with many bacterial species.

Pathogenicity studies confirmed the virulence of isolated bacteria not only for crayfish and mosquito fish but also for tilapia fish. Challenge experiment confirmed that crayfish can acts as a reservoir for many pathogenic microorganisms threatening cohabitant fish species. Wong *et al.* (1995) demonstrated that *Vibrio mimicus* retrieved from the hemolymph of crayfish suffering from asymptomatic bacteraemia were found to be primary pathogens, able to produce disease in unstressed, healthy aquatic animals. On the other hand, the finnipping habits of mosquito fish for other cohabitant fish species may potentiate transmission as well as establishment of many microbial infections (Lloyd, 1990).

Parasitological examination demonstrated absence of parasitic infestations in crayfish specimens which may be relevant to their potential resistance against some infections. On the other hand, mosquito fish were found to be vulnerable to *Centrocestus* infections. Similar infestations with *Centrocestus* sp. were reported in many fish species including *Gambusia affinis* (Nishigori, 1924 and Mitchell *et al.*, 2005). The detections of *Centrocestus*sp.in mosquito fish despite the existence of crayfish in their environment may be related to the limited competence of crayfish to consume some thick-shelled snails (Hofkin*et al.*, 1992) as well as may be also linked to the host specificity of parasites (Bauer *et al.*, 2000). Previous studies have reported that the snail intermediate hosts of *Centrocestus* sp. have high potentials to endure harsh environmental conditions as well as numerous insecticides (Mitchell *et al.*, 2005).

Earlier reports have confirmed the marked effect of invasive predator aquatic species, like crayfish, on the diversity and abundance of parasitic communities of cohabitant native species through interference with their pathway from intermediate to definitive hosts, (Holmes, 1979 and Poulin *et al.*, 2011).Several reports have signified the potential role of crayfish in the biological control of some epidemic diseases, in particular, infestations that include snails in their life cycle like, schistosomiasis, fascioliasis and heterophyid sp. (Mkoji *et al.*, 1995, 1999 and Khalil & Sleem, 2011) through attacking and consuming parasites hosts (Holmes 1979, Poulin*et al.*, 2011 and Pulkkinen *et al.*, 2013)

Results of water analysis revealed high concentrations of some heavy metals in the investigated water samples which may be claimed to the surplus discharges coming from sewage, agriculture and industrial activities near Elmansoria canal. The majority of evaluated metals were found in higher concentrations in water, Ni>Pb>Cd while Zn and Cu were within normal values. Nickel was the uppermost detected metals (0.7 ppm), extremely higher than the Egyptian environmental laws, 1982 & 1994 and the permissible limits USEPA (2009).Many industrial activities can contribute largely to amplify its levels including, stainless steel, ceramics, batteries and nickel plating (Al-Attar, 2007). This was consequently followed by the high concentration of nickel and its accumulation factor in both mosquito fish and crayfish tissues. Concentrations of Cd and Pb (0.2 and 0.34 ppm respectively) were also higher than the permissible limits, which probably be relevant to the presence of industrial and sewage waste water discharges in Elmansouria canal.

Results confirmed the ability of crayfish and mosquito fish to accumulate heavy metals since tissue concentrations were several times higher than the detected metal levels in water signifying the value of both fish species in biomonitoring studies. Although Zinc concentration in water (0.31mg/l) was not exceeding the permissible levels, it recorded high tissues concentrations and accumulation factor, especially in crayfish muscles. Zinc is an essential metal for metabolic reactions and its high tissues concentrations is independent on its water levels (Jakimska *et al.*, 2011).Additionally, previous reports have also confirmed that crayfish has considerable ability to manipulate elevated Zn levels through their metabolic processes (Alcorlo *et al.*, 2006).
The tendency of heavy metals to bioaccumulate in the investigated crayfish tissues varied. Some metals, Pb and Cd, bioaccumulated mainly in muscles in comparison to their levels in other organs in concordance with previous studies reporting muscles as the most organ for heavy metals bioaccumulation (Higueras *et al.*, 2006).These metals are not involved in crayfish metabolism hence it

amplifies in tissues with longer exposure to elevated metal levels in the surrounding aquatic environment (Alcorlo *et al.*, 2006).

In accordance with previous studies, other organs including, exoskeleton, hepatopancreas and gills have been also found to bioaccumulate metals from aquatic environments. In the current study, Ni was found to maximize more in crayfish exoskeleton that may be alleged as an attempt to eliminate out metals from the body through excretion (Macheviciene, 2002).The detected high concentration of Cu in crayfish hepatopancreas may also reflect the storage capacity of this organ for Cu (Bagatto and Alikhan, 1987). The hepatopancreas concentrates metals from the digestive tract and haemolymph, consequently store them in its intracellular vacuoles (Roldan and Shivers, 1987).

Generally, the indiscriminate disposal of industrial wastes, domestic sewage, agriculture drainages and other human activities into aquatic resources have long been considered a potential source for entrance of an assortment of heavy metals into aquatic ecosystems (Rajeshkumar & Munuswamy, 2011 and Maceda-Veiga*et al.*, 2012).

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 (Moustafa *et al.*, 2015, Elgendy *et al.*, 2013 and 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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الكائنات المسببة للأمراض في إستاكوزا المياة العذبة (*Procambarus clarkii*) و أسماك الجامبوزيا (*Gambusia affinis*) مع التأكيد على دورها في الرصد البيولوجي للتلوث المائى

وليد صلاح الدين سليمان، وفاء توفيق عباس، تغريد برهان الدين إبراهيم، أمانى محمد قناوى وممدوح يوسف الجندى
قسم الأحياء المائية - شعبة البحوث البيطرية - المركز القومى للبحوث - القاهرة - مصر.

تمت دراسة الحالة الصحية العامة لإستاكوزا المياة العذبة (*Procambarus clarkia*) وأسماك الجامبوزيا (*Gambusia affinis*) والتي تم تجميعها من ترعة المنصورية بالجيزة، مصر.حيث تتعرض هذة الترعة للعديد من الملوثات من مصادر متنوعة. تم الحصول على 113 عزلة بكتيرية من عينات الأسماك. وقد تم تحديد أنواع هذة العزلات ونسب تواجدها كالأتى: الإيروموناس هايدروفيلا (26.54٪)، الفيبريو باراهيموليتيكس (21.23٪)، السيدوموناس فلوروسنس(14.15٪)، الإيكولاى (10.61٪)، الستروباكتر (7.96٪)، الإنتيروباكتر (8.84٪)، الإستافيلوكوكس (4.42٪) والميكروكوكس (6.19٪). كما لوحظ وجود إصابات طفيلية عالية بالحويصلات اليرقية لطفيل السينتروسيستسفى خياشيم أسماك الجامبوزيا. بينما لم تسجل الدراسة أي إصابات طفيلية في إستاكوزا المياة العذبة. وقد أكدت تجربة إعادة العدوى بأنواع البكتيريا المعزولة (تجربة التحدى)على التأثير المرضي لعزلات الإيروموناس هايدروفيلا. و قد أظهرت نتائج تحليل عينات المياه من هذة المنطقة وجود نسب عالية من بعض المعادن الثقيلة حيث كانت كالأتى : النيكل (0.71) ، الرصاص (0.34) ، والكادميوم (0.2) جزء في المليون. بينما كانت نسب تواجد عنصرى الزنك والنحاس في القيم الطبيعية. و أكد تحليل المعادن الثقيلة فى أنسجة كلاً من إستاكوزا المياة العذبة و أسماك الجامبوزيا على التراكم البيولوجى لهذة المعادن. و كان ترتيب وجود المعادن الثقيلة فى عضلات إستاكوزا المياة العذبة كالأتى: النيكل> الزنك> النحاس> الرصاص> الكادميوم في حين كان ترتيبها فى أنسجة أسماك الجامبوزيا كالأتى: النيكل> الزنك> الرصاص> الكادميوم> النحاس. وأظهرت النتائج أيضاً وجود بعض التغيرات الهستوباثولوجية الواضحة فى أنسجة الأسماك موضع الدراسة. وتخلص هذة الدراسة إلى أن كلاً من إستاكوزا المياة العذبة و أسماك الجامبوزيا يمكن أن تكون ناقلة لبعض أمراض الأسماك كما أن لها دوراً هاماً فى الرصد البيولوجي للتلوث المائي.

الكلمات الدالة: البكتيريا - الطفيليات - التلوث - إستاكوزا المياة العذبة - الجامبوزيا.