Microbial infections among some fishes and crustacean species during blooming phenomenon in Qaroun Lake-Egypt

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Abstract: This study was designed to determine the blooming phenomenon effect on pathogenic organisms among fish (*Tilpia zilli* and *Gobius*) and crustacae species(Crab and Shrimp). The water discoloring yellow to red { Red-tides }formation. Fish and crustacean species was affected by increase in, Salinity, CO_2 and ammonia concentrations than the permissible limit and decrease in Dissolved Oxygen (DO_2) in Qarun Lake water. The water was infected with different organisms (refer to water pollution and fish pathogens.). Morbidity and mortality ***Tilpia zilli,*** Gobius, Crabs and Shrimp spp samples revealed some internal and external clinical signs.***V. anguillarum, P. fluroescens* and *St. pyogens*** infected Tilapia species experimentally and recorded higher **virulence** rates (100%) while ***Y. ruckeri*** recorded lower rates(40%). *V.* ***anguillarum,* recorded higher natural infection rate (32.81%) while *Aeromonas. Sobria*** and ***Y. ruckeri*** recorded lower infection rate (3.12%)) between morbidity and freshly dead Qaroun Lake fishes and crustacean species. Natural infection rates among fishes and crustacean were highly especially in Gobius fish which recorded (100%).while ***Tilpia zilli,*** Crab and Shrimp spp. recorded(75%),(66.6%)and(60%) respectively. Fish and crustacean species were affected by the blooming phenomenon which occurred its immune-suppression and mass mortalities with increase of aquatic microbial virulence during bad change of water quality.

[Abou El-Geit, E.N, Saad, T.T., Abdo, M. H. and Mona S. Zaki **Microbial infections among some fishes and crustacean species during blooming phenomenon in Qaroun Lake-Egypt.** *Life Sci J* 2013;10(2):1217-1224] (ISSN:1097-8135). http://www.lifesciencesite.com. 168

Keywords: Microbial pathogens, fish and crustacae, blooming (\Red- tide), Qaroun Lake.

1. Introduction

Qaroun Lake is one of most affected lakes in Egypt which exposed to continuous environmental changes. Many authors reported changes in water quality of Qaroun Lake (Bishai & Kirollus, 1980; Mansour *et al.,* 2000). Recurrent excessive blooms can cause dissolved oxygen depletion as a result of dead algae decay. In highly eutrophic (enriched) lakes, algal blooms were reported to lead to anoxia with consequent fish mortality during the summer (Oberemm, 1999) Qaroun Lake during study revealed that there are three factors causing massive mortalities of fishes, namely blooming phenomenon, poor water quality (trace metals & physico-chemical parameters) and microbial pathogens in the aquatic environment.(Abou El-Gheit *et al.,* 2012).Bacterial infection as more likely to occur at such polluted water as reason of stress factor. There are different types of bacterial species that generally used as indicators of pollution (Keough and Quinn, 1991).

Bacterial infections such as Vibrios, Aeromonads, Pseudomonads, Photobacteria, Streptococci and Staphylococci were recorded in several fingerlings, juveniles, adults and brood stocks of some marine fish species (Alicia *et al.,* 2005). crustacean suffered from shell displayed characteristic black spots lesions in exoskeleton due to bacterial induced degradation. Claire Vogan *et al*.(2002). The diseases of fresh water prawns and crabs are caused by a combination of pathogenic, nutritional, physiological, and environmental factors (Yang and Huang, 2003).

Delves and Poupard (2000) isolated chitinolytic vibrio Spp., but also Aeromonas and Pseudomonas are isolated from brown spot disease lesions, the lesions were brown to black spots occurred on all body surface and appendages (periopods and pleopods) with ulceration.

The study aimed to evaluate microbial infections status on *Tilpia zilli*, Gobius sp. and crustacae species (Crab and shrimp), which occurred due to the effect of water quality changes during blooming phenomenon at the end of February 2012 in Qaroun Lake.

2. Material and methods

Study area:

Lake Qaroun is the only enclosed saline lake in Egypt. It is located in the western desert in the deepest part of Fayoum depression and lies at 83 km of south west of Cairo. The lake receives the agricultural drainage water from the surrounding cultivated land. The drainage water reaches the lake by two greatest drains, El-Batts drain (at the northeast corner) and El-Wadi drain (near mid-point of the southern shore)

Water sampling:

Three water samples for physical and chemical parameters were collected during blooming phenomenon at the end of February 2012 from each station. First samples were collected from station 1, Lake northeast (between Shakshok research station and EL-Bats drain). Second samples were collected from station 2, (Lake Middle East in front of Shakshok research station, NIOF). Third samples were collected from station 3, (Lake south). Water analysis (physical and chemical) was measured according to APHA, (2002). Three water samples were collected from the same stations for bacteriological analysis. Sterile glass tubes were used for subsurface water samples.

Fish and crustacean sampling:

Eighty five of moribund and freshly dead aquatic animals (*Tilpia zilli* Gobius, shrimp and Crab species) were collected from Qaroun Lake (Table1). Fish and crustacean samples, placed in strong aseptic bags, put in an ice box and transported to the laboratory of shakshouk Research station (NIOF), at El-Faiyum governorate.

All samples processed directly for clinical, microbiological and postmortem examinations according to (Buller, 2004). All signs and lesions were recorded.

Culture media:

The following media (general and specific) were used for bacterial growth according to Popoff (1984). Nutrient agar, nutrient broth (oxoid), MacConky agar, Brain heart infusion agar (Oxoid), Tryptic soy agar and tryptic soy broth (Difco), blood agar,Triple sugar iron agar (Oxoid), thiosufate-citrate bile salt agar (TCBS), Rimler-Shots (R-S) media, (Oxoid and Difco), Salmonella-Shigella agar (SSA), Pseudomonas agar and Sabouraud dextrose agar. All media supplemented with 1.5 % NaCl and incubated at 25 ± 1 ºC for 48 hours.

Bacterial isolation and identification :

Aseptic swabs from skin lesions, gills, spleen, liver, kidney and water were inoculated onto general and specific media.The inoculated plates and broth were incubated aerobically at 25±1 ºC for 48 hours and 35±2ºC at aerobic and anaerobic conditions.The growth colonies were subjected to culture and morphological characters, motility and gram reaction. Also microbial isolates were identified using biochemical characterization according to Murray *et al.* (2003) and Austin and Austin (2007). Pure bacterial isolates were confirmed using the analytical profile index of API20E system (Buller, 2004).

Fungal isolation and identification:

Under aseptic condition tissue samples measuring approximately 5-10 mm were taken from dorsal fin, caudal peduncle, skin lesions and gills and also, Tissue and water samples were performed by dilution plate method described by (Hanlin and Ulloa,1979) and inoculated onto sabouraud dextrose agar plates.The plates were inoculated at 25±1 ºC for 2- 7 days. Pure cultures were identified using single spore isolation method (Booth, 1971). It was based on culture characteristics, namely colony color, type of mycelium, shape and septation of conidia.The spores were stained with Lacto phenol cotton blue microscopically examined.

Challenge experiments

Tilapia species were distributed randomly into 7 groups each with 30 fish (10 per replicate). Each group was injected intraperitoneal with 0.1 ml x 107 C.F.U. /ml of bacterial suspension. Mortalities were recorded for 14 days, where upon the survivors were examined pathologically after Austin & Austin (1989)

Statistical analysis:

Data were analyzed by one-way analysis of variance (ANOVA). When differences were found among treatments, Tukey's test was used to compare means by Minitab statistical software (Minitab). Differences were considered significant at $P < 0.05$

3.Results and discussion

The environmental changes is one of the major problems we face in the twenty –first century which is of vital importance to human existence and development, Qaroun Lake exposed continuously to environmental changes. Recurrent blooming phenomenon recorded in the last years (2008 to 2012) at autumn or winter seasons with remark water discoloration to red (Fig.1.). These phenomenon may be return to suddenly environmental changes and its location in the Western desert in the deepest part of Fayoum depression. This case may be lead to, increase algal toxin secretions in Lake water which caused immune suppression for fish and crustaceans, opportunistic organisms become more virulence and caused diseases and mass mortalities These findings supported by (Mansour & Sidky, 2003) who mentioned that Lake Qaroun is the largest reservoir of agricultural and sewage drainage of Fayoum province as well as the drainage from fish farms established around the lake. Clement *et al.* (2001)and Cambella *et al.* (2005) who supported our remark and in accordance recorded that Red-tides usually occur between February and May, These phenomenon also supported by Ibrahim (2007)who mentioned that, the most common type of harmful algal blooms (HABs) is referred to as a "Red Tide" because the bloom discolors the water, making it appear red. However, HABs may also be yellow, orange, brown, green, white, or pink, depending on which one of the three primary types of phytoplankton are responsible for the problem; dinoflagellate, diatoms, or blue green algae.

Poor water quality was recorded in all our study stations (depletion of oxygen level (3.8-5mg/1) below the permissible limits while concentrations of ammonia was increasing (860-1430μg/l) and high salinity (13.6- 28.5ppt.) table.(1).These results agree with who mentioned that, harmful algal blooms can block sunlight from phytoplankton under the waters surface leading to decreased food and oxygen levels for higher order consumers e.g. fish, a change that can be lethal to other aquatic organisms and cause kills.

As well as, Van Tassell 1998 mentioned that, the gobies die-offs observed in Lake Haroun may be the result of botulism type E poisoning. Toxins from the bacterium *Clostridium botulinum* and specifically Type E botulism, The botulism toxin is produced in the absence of oxygen. It remains unclear which factors trigger the bacterium to produce the neurotoxin and the ensuing fish and wildlife die-offs

Qaroun Lake water polluted by receiving the agricultural drainage water from El-Batts and El-Wadi drains, drainage of, fish farms established around the lake, Fisheris and domestic animal fecal pollutions.Microbial isolates from our study stations water were recorded as. *V. anguillarum*, *Streptococcus pyogenes*, Ps. Fluoroescens. Aeromonas Sp, *Enterococcus faecalis*. *Staph aureus* Y. ruckeri,. Lactococcus Sp., *Asperigilles niger*., *Candida albicans* (Table 2.).These results agreed with Keough and Quinn (1991)who mentioned that bacterial infection as more likely to occur at such polluted water as reason of stress factor. There are different types of bacterial species that generally used as indicators of pollution and Karabose *et al.*(2003)whose mentioned that, one of the most important factors of water pollution is the microbial contamination; especially with pathogenic microorganisms. Enteric pathogens are typically responsible for waterborne sickness.

Concerning to morbidity and mortality, *T.zilli*, Gobius fishes, Shrimp and Crabs samples revealed some clinical signs (these signs were different from case to case according to microbial virulence). These signs as, lethargic or lost balance, curving tail eroded and erected fins, loss of scales and, irregular ulcer with hemorrhagic lesions on the skin at the base of fins, distended abdomen with exophthalmia, eye cataract and vent inflammation. In crab and shrimp,soft and sloughed exoskeleton, black or brown spots on the shell and gills, necrosis and inflammation of lymphoid organs and gills. (Figs.2-.a,b,c.)

Post-mortem (P.M) of examined fish showed congestion in the internal organs with enlarged kidneys, spleen and swim bladder distended while in other fish the liver was grayish or pale color and the abdominal cavity filled with yellow to bloody exudates (Fig.3.). All internal and external signs were similar and remarked by Claire Vogan *et al.*(2002), Abou El-gheit (2005), Austin and Austin(2007), Moustafa *et al.* (2010), Amal and Zamari (2011).

Clinical signs of *T.zilli*, Gobius, Shrimp and Crabs were confused and may be return to the degree of microbial virulence and disease stages. These results agree with Alicia *et al.* (2005) whose mentioned that, Clinical signs (external and internal) caused by each pathogen are dependent on the host species, fish age and stage of the disease (acute, chronic, subclinic carrier). In addition, in some cases, there is no correlation between external and internal signs. In fact, systemic diseases (i.e., pasteurellosis, piscirickettsiosis) with high mortality rates cause internal signs in the affected fish but they often present a healthy external appearance. On the contrary, other diseases with relatively lower mortality rates flexibacteriosis, winter ulcer syndrome, some streptococcosis cause significant external lesions, including ulcers, necrosis, exophthalmia

Table (1) Physico-chemical analysis of Qarun Lake water during blooming phenomenon, at the end of February 2012

Stations	I	II	III	Permissible limits WHO, (1993)
Water Temperature ºC	18.5	19	19	25 – 35
Salinity ppt	13.6	27	28.5	-
DO (mg/l)	5	3.8	4.3	6 – 14
BOD (mg/l)	4	4.2	3.6	Up to 6
pH	7	6.5	7.1	7-8
NH_3 (μg/l)	1430	860	980	50 – 500

The incidence of natural Infection rates among morbidity and freshly dead marine fishes and crustacean recorded (75.29%)Which represented in (100%) Gobius fishes and (77.5%) *T. zilli*, (66.6%) Crabs and 60% Shrimp and the infection with Gram-negative bacteria was higher than the infection with Gram-positive bacteria. Also the infection prevalence were identified to Gram-negative bacteria as (32.81 %) *V. anguillarum* (14.06 %) *Ps. fluorescens*, (9.37 %) *A. hydrophila*, (3.12 %), *A. sobria*, (3.12 %) *Y. ruckeri* and Gram-positive bacteria represented in (21.87 %) *S. pyogens*, (9.34 %) *E. faecalis*, (4.68 %) *Staph. Aureus* and (1.56 %) Lactococcus sp. (Table 2).

These results was higher than those reported by Moustafa *et al.* (2010) whose revealed that the prevalence of bacterial infections was (53.06%) in fishes collected from the Gulf of Suez and (46.93%) from Lake Qaroun. The infection with Gram-negative bacteria prevailed the infection with Gram-positive bacteria during study of Suez Gulf and Qaroun Lake and agreed with our results. Also, the natural infection rates were lower than our results which represented in (9.38-8.16%)*V.anguillarum*,(11.02-5.71%) *V.alginolyticus*, (5.3-5.71%) *A. hydrophila*, (4.08-2.04%), *A. sobria*, (8.57-7.34%), *Ps. fluorescens*, (7.75-7.75%) *P. piscicida*, (5.3-8.16%) *S. fecalis*, (1.63-2.04%) *Staph. aureus* respectively. while Abou El-Gheit *et al.* (2012) mentioned that, natural infection rates was highly and recorded (81.1%) among fish in Qaroun Lake at year (2010) and the main isolations from *S.vulgaris* and mullet fish were higher in Gram-negative bacteria as (67.1 %) *V. anguillarum*, (10.95%) *Ps. fluorescens*, (2.37%) *A. hydrophila*, (2.37%), *A. sobria*, (8.21 %) *Y. ruckeri* and the lower in Gram-positive bacteria represented in (8.21%) *S. pyogens*, (1.37 %) *E. faecalis*, (1.37%) *Staph. Aureus* and (1.37 %) *Asperagillus niger* also during blooming phenomenon.

As well as, Zorrilla *et al.* (2003) recorded a high percentage of gram negative bacteria (Vibrio spp, Pseudomonas spp, *P. piscicida*, *Flavobacteria maritimus*, Aeromonas spp.) in gilthead seabream and low percentage of gram positive bacteria.

V. anguillarum was the highest infection rates for our study samples of (*T. zilli* and Gobius fishes, Crabs and Shrimp) while *Y. ruckeri* and *Staph. aureus* were the lowest infection rates (Table 2). These results supported by Moustafa *et al.* (2010); Abou El-Gheit *et al.* (2012) as mentioned before and Nakayama *et al.* (2006) who reported that one of the major bacterial diseases in shrimp aquaculture is vibriosis. Vibrio strains are predominant bacteria in the marine environment and usually constitute the majority of normal micro flora of farmed and wild shrimp. Delves and Poupard (2000) isolated Chitinolytic vibrio Spp, but also Aeromonas and Pseudomonas are isolated from brown spot disease lesions. also(Yang and Huang, 2003) mentioned that, the initial cause of trembling disease in fresh water crab is a bacteria. vibiosis and *Aeromonas hydrophila* have been isolated from diseased Crabs.

In the present study, *Tilapia zilli* Was challenged intra-peritoneal with bacterial isolates (*Vibrio anguillarum*, *Pseudomonas fluorescens*,, *Streptococcus pyogenes*,*Aeromonas hydrophila*, *Aeromonas sobria*, *Yersinia ruckeri* and, *E. faecalis* for virulence test. Reisolation of the inoculated bacterial isolates confirmed the virulence of bacterial isolates.

Challenged fish revealed variable results might be attributed to bacterial isolates (which isolated from different species, age and size.), bacterial toxins and hemolytic enzymes.

Highly mortality rates among inoculated *Tilapia zilli* fish recorded as (100 %) for each of *Vibrio anguillarum*, *Pseudomonas fluorescens* and *Streptococcus pyogenes*, while it was (80 %) for *Aeromonas hydrophila* (60 %)for every *Aeromonas sobria* and *E. faecalis* and (40 %) for *Yersinia ruckeri* (Table 3).These results agreed with Moustafa *et al.*(2010) who mentioned that *O. niloticus* experimentally infected I/P with the different bacterial isolates from Suez Gulf and Qaroun Lake and revealed high mortality rates among inoculated fish as (100 %) for *Vibrio anguillarum* and *Pseudomonas fluorescens*, (80 %)for *Aeromonas hydrophila* and highly in *E. faecalis* which recorded(90 %)and Nakayama *et al.* (2006) reported that, prawns and crabs larvae can be completely wiped out by Vibiosis; it is only 15 days from initial observations of trembling disease to mass mortalities of 80-100 percent.

Also supported Haenen & Davidse (2001) who mentioned that the pathogenicity of *P. anguilliseptica* varies according to fishes, temperature, type of water, size and age of the same fish species. In addition, Nottage & Birkbeck, 1987 who reported that the pathogenicty of *V. anguillarum* for experimentally infected *O. niloticus* may be attributed to extracellular toxins and enzymes. Most infectious diseases of produced by the bacterium.

Also, El-Attar & Moustaf (1996) recorded that the pathogenicty of *Ps. fluorescence* for experimentally infected *Oeorchromis niloticus* may be attributed to the production of extracellular enzymes and lethal toxins.

As will as the pathogenicty of *A. hydrophila* for experimentally infected *Oeorchromis niloticus* fish may be attributed to toxins and extracellular enzymes produced by *A. hydrophila* (Saavedra *et al.*, 2002).

In concern to the experimental infection of *Tilapia zilli* with *S. fecalis*, the pathogenicty of streptococci may be attributed to the effect of exotoxins produced by the bacterium (Kimura & kusuda, 1979 and Woo, 1999).

In conclusion, the main factors that have been associated with massive mortalities and outbreaks of fish diseases were, blooming phenomenon (Red tide). Poor water quality and microbial virulence. Increase of data about the environmental changes and microbial diseases in Qaroun Lake may be helpful to implement developmental strategies for blooming phenomenon control and fish disease prevention.

Table (2): Natural infection among marine fish,crustaceans and water in Qaroun lake during blooming phenomena (at the end of February 2010).

Microbail isolates Fish & Water samples	Examined Samples			Aeromonas spp.				Streptococcus spp.				*Y. ruckeri*		*V. anguillarum*		*Ps. fluoroescens*		*Staph. aureus*		Other organisms	
	Examined No.	Infected		*A.hydrophila*		*A. sobria*		*S. pyogens*		*E. Faecalis*											
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
T. zilli	40	31	77.5	2+E	6.45	-	-	7+L.Sp.	22.58	2	6.45	2	6.45	11	35.48	5	16.12	1	3.22	1 (L)	3..22
Gobius spp.	10	10	100	1	10	1	10	2	20	1	10	-	-	3	30	2	20	-	-	-	-
Shrimp spp.	20	13	65	2	15.38	1	7.69	3	23.07	1	7.69	-	-	4	30.76	2	15.38	-	-	-	-
Crab spp.	15	10	66.6	1	10	-	-	2	20	2	20	-	-	3	30	-	-	2	20	-	-
Total	85	64	75.29	6	9.37	2	3.12	14	21.87	6	9.37	2	3.12	21	32.81	9	14.06	3	4.68	1	1. 56
Water North east	3	+		+ E				-		+A.s		-		+		+		+ C		A/ *A. niger*	
Water Middle	3	+		+				-		+		+		+		+		-		C/ *Candida albicans*	
Water South	3	+		-	+			+A. hydrophila		-		-		+ LSp.		-		+		LSp../ Lactococcus Spp.	

(+*) = Some fish have more than one microbial infection.
+: A=*Asperigilles niger*. C = *Candida albicans*. L = *Lactococcus lactis*. LSp. = *Lactococcus Sp.*
Staph. = Staph.aureus. .Ps = *Pseudomonas fluorescens*. E. *Enterococcus faecalis.*
A.s. = *Aeromonas sobria*.

Table (3): Tilapia species experimentally infected intra-peritoneal with different bacterial isolates from Qaroun Lake.

Bacterial isolates	Total No.	Mortality Number / Day														Mortality %
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
A. hydrophila	30	-	3	-	-	9	3	6	-	3	-	-	-	-	-	80
A. sobria	30	-	-	6	3	-	3	-	-	6	-	-	-	-	-	60
Y. ruckeri	30	-	3	-	-	3	-	-	-	-	-	6	-	-	-	40
V. anguillarum	30	3	6	6	3	6	3	3	-	-	-	-	-	-	-	100
P. fluroescens	30	-	6	3	6	6	3	6	-	-	-	-	-	-	-	100
St. pyogens	30	3	6	-	6	6	6	3	-	-	-	-	-	-	-	100
E. faecalis	30	-	-	-	3	3	-	-	6	-	-	3	-	-	3	60

* Challenge dose / fish = 0.2 ml of 1 x 10^7 C.F.U.

Fig. (1) showing recurrent blooming phenomenon ("Red Tide") recorded in the last years 2012)at autumn or winter seasons with remark discoloration water to red.

Fig. (2) a,b,c;
- *T.zilli* and Gobius fishes showing curving tail, eroded and erected fins hemorrhages lesion on the skin and base of fins, abdominal distension, exophthalmia, eye cataract and vent inflammation.
-crab and shrimp revealed soft and sloughed exoskeleton, black spots on the skin and gills,,necrosis and inflammation of lymphoid organs and gills

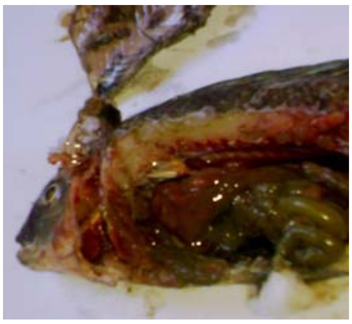

Fig.(3): *T. zilli* showing :
- Congestion of gills and internal organs,
-Spleen,liver and kidney enlargement and serous bloody exudates in the abdominal cavity.

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