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# A contribution of crustacean isopodoa, bacterial infections and physicochemical parameters in mass mortalities among fishes in Lake Qarun.

**Younes AM*[1], Noor Eldin A I[1], Abd Ellatif MA[2].**

[1]Hydrobiology dept., National Research Centre, Dokki, Cairo, Egypt.
[2]Fish diseases and management, Faculty of Veterinary Medicine, Moshtohar branch, Banha University.

**ABSTRACT**

*Tilapia zilli* and *Solea vulgaris* live fish were collected from Lake Qarun, El Fayoum governorate, during mass mortality at summer season. The main clinical sings of infected fish were slimy dark skin with signs of asphyxia, presence of hemorrhagic lesions on the skin, fins, and gills. Investigation of *T. zilli* fish revealed that either bilateral and/or unilateral infection of their gill chambers with one species of Cymothoidae identified as *Nerocila orbignyi.* Bacterial isolation identified that 100% of examined fishes were found to be infected with either one type or mixed bacteria. *V. alginolyticus* was the most common bacteria isolated, followed by *A. sobria*, then *Staph aureus.* The results of water analysis showed increase in concentration trace elements especially iron 2.7 mg/l. Therefore, the present investigation was conducted to shed light on multifactorial causes of mass mortalities among fishes in the Lake.

**Keywords:** Lake Qarun, *Tilapia zilli*, *Solea vulgaris*, Isopoda, bacteria, Iron.

***Corresponding author***

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## INTRODUCTION

In Lake Qarun, the fish production was supplied widely from high water salinities. The majority of research and studies on fish diseases focused on a single cause and neglect other causes, although the causes of almost fish diseases are multi-factorial. Previous studies confirmed the role of parasites in enhancing bacterial infections of fish. The invasion and movement of parasites from one location to another on fish cause mechanical injuries to epithelium tissues. These injuries may serve as portals of entry for bacterial invasion and act as stress factors rendering the fish more susceptible to other diseases and bacterial infection [1,2]. Isopods considered as a large ectoparasites crustacean group, found in marine, brackish and freshwater fish species worldwide. External crustaceans and digenetic trematodes of marine-water fish could be considered as the most prevalent causes of diseases affecting gills causing gill inflammation and distortion of normal anatomy which impairing their respiratory foundation [3,4].

Bacteria are indirectly profit from environmental change and decreased host resistance which result in increased virulence and replication rates. The reduction of resistance against *Vibrio alginolyticus* and other marine bacteria due to a decreased immune response were reported under changed salinity [5]. Vibrios are ubiquitous to marine environment. The clinical disease outbreaks only occur when fish exposed to infectious agent in existence of sharply stressed factors [6].

Therefore, the present investigation was conducted to shed light on concurrent infections among fish species from Qarun Lake with isopodosis and bacteria. Isolation and identification of the causative agents caused the outbreak. Also, to show the effect of increasing salinity, iron and nutrient concentrations on virulence of isopodosis and bacterial infections.

## MATERIALS AND METHODS

### *Fish samples*

A total of 200 live *Tilapia zilli* (n=100) and *Solea vulgaris* (n=100) fishes were collected live from Lake Qarun, El Fayoum governorate, Egypt during an outbreak in summer, 2015. Fishes were inspected for parasitic infestation. The fishes were transported alive to the laboratory of fish diseases at Hydrobiology department, National Research Centre. In large tanks filled with water of the same sources supplied with battery air pumps and examined as soon as possible. Fish skin, fins and gills were firstly examined by the naked eye for detection of any macroscopically visible lesions.

### *Bacterial isolation and phenotypic characterization*

Samples for bacterial isolation were taken from liver and kidney of moribund and freshly died fish, and cultured on general and selective media; Tryptic soy broth (TSB), Tryptic soy agar medium (TSA), Aeromonas agar media with ampicillin supplement, and Thiosulphate citrate bile salt sucrose agar (TCBS). The media were prepared with addition of 2% NaCl. The inoculated plates were incubated at 25°C for up to 24h. Identification of all isolates was done by cultural, morphological and biochemical characters through using commercial API20NE kit (BioMerieux, France) according to the manufacturer's instructions.

### ***Water analysis:***

Clean water sample flasks, one liter volume was equipped with a cork stopper. The flasks were rinsed several times with distilled water and sterilized in a hot air oven at 180$^{o}$C for one hour. The collected water samples bottles were labeled with the locality, date and time. Water physicochemical parameters were measured according to methods described in APHA [7].

## RESULTS AND DISCUSSION

Lake Qarun is a closed system acts as a reservoir for agricultural and sewage drainage water of El-Fayoum province, receiving its water from two main drains, EL-Bats and El-Wadi losing water by evaporation only. Salinity of the lake depends on the quantity of drainage water inflow and evaporation process. The mean salinity recorded in this study was 38%. The previous studies concerning salinity recorded that the mean

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salinity of the water increased from 11.06% in 1906 to 31.73% in 1970, and to 36.2% in 1978 [8]. In 2009, the lake salinity ranged from 29.2-35.4%, 27.1-36.2%, 29.3-38.0% and 29.7-35.4% in winter, spring, summer and autumn respectively [9]. Also, the salinity of the water samples in 2013 was varied from 34 to 43% with a mean value 37.67±2.92% [10]. All freshwater fish species that were originally living in the lake are extinct except *Tilapia zilli* due to increasing lake salinity. The agriculture drainage waters discharged into the lake are high in solids, nutrients, pesticides, heavy metals and organics. Moreover, a remarkable increase in the bacterial indicators of sewage pollution in the lake was recorded [10,11,12,13].

**Fig (1): Showing infested *T. zilli* and *S. vulgaris* with isopoda *Nerocila orbignyi*. (A) *T. zilli* infested with isopoda *Nerocila orbignyi* embedded in gill filament and arch (b). *S. vulgaris* (C): Light photomicrograph of *Nerocila orbignyi* dorsal view and (d): Ventral view.**

Investigation of *T. zilli* fish revealed that either bilateral and/or unilateral infection of their gill chambers with one species with morphological description of the parasitic isopod of Cymothoidae and identified as *Nerocila orbignyi* (Guerin-Meneville). The intensity of infestation was one or two per fish. Examination of the infested fish revealed the presence of parasite in the gill chamber (unilateral or bilateral) or attached on the skin. Slight protrusion of gill cover (operculum), atrophy and hemorrhage at site of attachment (Fig 1). These may be attributed to the low respired oxygen of destructed gill epithelium which caused by feeding activity, attachment, fixation and locomotion of crustaceans. These results are in agreement with those reported by Eissa *et al.* [14]. Also, these results agree with Kayış and Ceylan [15] who found a Female *Nerocila orbignyi* between the operculum and pectoral fin of *Solea solea*. Isopod parasites

have received considerable scientific attention because they cause serious damage to fishery resources. Isopods are a highly diverse group of crustaceans, with more than 10,300 species known to date, approximately 6,250 of these being marine or estuarine. Few families of Isopoda are parasitic such as; Bopyridae, Cryptoniscidae, Cymothoidae, Dajidae, Entoniscidae, Gnathiidae and Tridentellidae. The family Cymothoidae are obligate parasites of both marine and freshwater fishes and there are currently 40 recognized cymothoid genera worldwide. These isopods are large (>6 mm) parasites, and ectoparasites of marine and freshwater teleost fishes around the world [3,4]. Nerocila is a large genus of the family Cymothoidae including at least 65 species living attached on the skin or on the fins of fishes [16].

The parasite may act as a vector for bacterial entry, Cusack and Cone [17] found bacterial colonies on the surface of Gyrodactylus by scanning electron microscopy. The invasion and movement of parasite from one location to another on fish cause mechanical injuries to the epithelium making micro-lesions on the skin that might function as entry sites for the bacteria. These injuries making cultured Nile tilapia fish infested with *Gyrodactylus niloticus* more susceptible to *S. iniae* [2]. Busch *et al.* [1] suggested that an enhancement of *Flavobacterium psychrophilum* bacterial invasion by ectoparasite *Gyrodactylus derjavini*. Moreover, ectoparasite *Argulus coregoni* enhanced susceptibility to the pathogenic *Flavobacterium columnare* in rainbow trout [18]. Ravichandran *et al.* [3] found a greater number of Vibrio count in the infested fish with Cymothoids ectoparasite compared with normal one.

The bacterial incidence of natural infection rates among moribund and freshly dead fishes was 100% from *T. zilli* and *S. vulgaris*. The fish was found to be infected with either one type or mixed bacteria. Similar to this results, Abou El-Geit *et al.* [19] found that the incidence of natural infection rates among dead marine fishes and crustacean recorded from Qarun Lake during blooming phenomenon were (75.29%), (100%), (77.5%), and (66.6%) in Gobius fishes, *T. zilli*, Crabs, and Shrimp respectively. Additionally, Abou El-Gheit *et al.* [20] mentioned that, natural infection rates was high (81.1%) among *S. vulgaris* and mullet fish in Qarun Lake at year 2010. While these results was higher than those reported by Moustafa *et al.* [21] who found the total prevalence of bacterial infections was (46.93%) in infected fish spp. and distributed as follow; (41 %) among *S. vulgaris*, (40 %) among *T. zilli*, and (34 %) among *Mugil capito* collected from the Lake Qarun.

Species wise, in this study the most common Gram-negative oxidase positive bacteria were *V. alginolyticus* followed by *A. sobria* from representative samples from *T. zilli* and *S. vulgaris*. Gram-positive *Staph. aureus* was isolated from 15% of total fish. Other bacterial isolates found with few numbers were *Pseudomonas aeruginosa, Burkholderia cepacia*, Streptococcus spp., and Enterobacter spp. The previous bacterial prevalence from Qarun Lake were *V. anguillarum*, *Ps. fluorescens*, *A. hydrophila*, *A. sobria*, *Y. ruckeri*, *S. pyogens*, *E. faecalis*, *Staph. aureus* and Lactococcus sp. [19]. Whereas, Moustafa *et al.* [21] found *V. anguillarum*, *V. alginolyticus*, *A. hydrophila*, *A. sobria*, *Ps. fluorescens*, *P. piscicida*, *S. fecalis*, and *Staph. aureus* respectively from naturally infected fish from Qarun Lake. Moreover, Abou El-Gheit *et al.* [20] isolated *V. anguillarum*, *Ps. fluorescens*, *A. hydrophila*, *A. sobria*, *Y. ruckeri*, *S. pyogens*, *E. faecalis*, *Staph. aureus* and *Asperagillus niger* from *S. vulgaris* and mullet fish during blooming phenomenon in the lake at year 2010. This variation in bacterial spp. may be attributed to the differences in salinity level, the sample site/time, climate change, and immune status of fish.

Concerning prevalence of *V. alginolyticus* in naturally examined fishes, vibriosis is one of the most serious bacterial diseases in cultured marine fish worldwide. Vibrio species are the most dominant heterotrophic bacteria in the marine environment and are widely distributed in the coastal seawaters and /or brackish waters and usually constitute the majority of normal micro-flora of farmed fish [6,22]. These results agreed with Balebona *et al*. [23] who recorded that the main isolate from diseased *Sparus aurata* fishes was *V. alginolyticus*. On other hand, Moustafa *et al.* [21] who recorded the prevalence of *V. alginolyticus* in *Siganus rivulatus* was 18%, while in *Tilapia zilli* was 10% from Qarun Lake. Whereas Eissa *et al.* [24] found the prevalence of *V. alginolyticus* in *Sparus auratus* was (46 %), followed by *Tilapia zilli* (34%) then *Siganus rivulatus* (25.9 %) in Temsah Lake in Ismailia governorate, Egypt. Moreover, the total prevalence of *V. alginolyticus* among naturally infected Gilthead seabream and European seabass, were 82.19% (120/146) and 87.28% (103/118) respectively from some Egyptian coastal provinces [25].

*A. sobria* is involved in the mortality of the farmed perch and that this bacterium has the potential to act as a primary pathogen producing surface lesions, fin rot, local hemorrhages and clear ascites. Mortalities due to *A. sobria* reached levels of 1% of the total fish on the farm per day [26]. Outbreaks of motile

aeromonad septicemia have often been related to changing water temperatures, overcrowding, rough handling, transfer of fish, low dissolved oxygen, poor nutritional status and fungal or parasitic infections [6,26].

The results of water analysis showed increase in concentration of iron reached to 2.7 mg/l which is higher than the permissible limit (1.0 mg/l). Iron is an essential nutrient for most bacteria, and is one of the important factors for growth and proliferation of bacterial species. It serves as a cofactor for metabolic processes, such as redox reactions, nucleic acid synthesis, and electron transfer [27]. Other works have supported the role and importance of iron in the virulence of Vibrio sp. Low iron levels in nutrient-rich ocean environments not only reduce the bacterial growth rate, survival and ability to persist, particularly at the lower end of the ocean pH range. The addition of ferric oxide to water increased the survival of *V. cholera*, even in the absence of nutrients [28]. Iron plays a major role in the pathogenesis of *V. vulnificus.* The $LD_{50}$ of *V. vulnificus* strains isolated from different sources of Cuddalore coastal waters was in the range of $10^4$–$10^7$ cells in normal mice, but was $10^1$–$10^2$ cells in iron-injected mice [29]. As reviewed before, Intra-peritoneal injection of mice with *V. parahaemolyticus* in the presence of ferric ammonium citrate increased the bacterial proliferation and enhanced the lethality. Also, patients with liver diseases and hemochromatosis, iron overload states, been reported to increase the virulence of *V. vulnificus* and considered as predisposing factors to recurrent infections, septicemia, and high mortality [30]. Also, *A. salmonicida* Siderophores inhibit the defence action of the host's transferrin, giving greater resistance to the bactericidal activity of the host phagocytes [31]. Also, *In vivo* colonization assays revealed that the ability to acquire iron from heme-containing molecules is critical for *A. veronii* to colonize the leech gut and to grow in blood [32].

## CONCLUSION

The results in this study showed that the mass mortality among fish species in Qarun Lake were multifactorial including changing physicochemical parameters such as iron, heavy infestation with isopodosis and bacterial infections. These results were in complete accordance with other studies in that the sharp increase in water parameters (ammonia, salinity temperature and ability to scavenge iron) are the most possible triggering factors for initiation and propagation of infections. This study recommend regular monitoring and treatment of water drainage, find a good way to treat or reduce the parasites infestations to reduce outbreaks as the Lake serves as source of fish for local inhabitants in that area.

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