Iron in water and some marine fishes in relation to vibriosis at Lake Temsah

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Abstract: This study have been applied on 300 marine fishes of three different species represented as *Sparus auratus, Siganus rivulalus*, and *Tilapia zillii* (each 100). They were collected randomly and seasonally from Lake Temsah in ismailia governorate from May 2012 to April 2013.The clinical picture revealed the signs and lesions of septicemia. Isolation and identification of yellow pigmented colonies on TCBS and creamy coloured on TSA media with different NaCl concentration (1.5-8 %), resistant to vibriostat O/129-10 µg and sensitive to vibriostat O/129-150 µg. The causative agent was identified as Vibrio The results revealed that the highest prevalence of vibriosis was recorded in *Sparus auratus* (46 %), *Tilapia zillii* (34%) then *Siganus rivulatus* (25.9 %) while the total prevalence was 36%. The highest seasonal prevalence was recorded in summer (56 %) followed by spring (48%) then autumn (26.67%) and winter (13.33%).The highest seasonal prevalence of vibriosis in all examined fishes was in summer followed by spring. The highest prevalence of was in liver, kidney then spleen and gills The results of iron estimation in tissues revealed that its concentration in musculature was the lowest and it was less than the permissible limits while it was the highest in liver followed by kidneys and spleen. The highest iron concentration in water of Lake Temsah in two different locations was in summer, autumn, spring and the lowest in winter. The histopathological studies of the examined marine fishes showed hyperactivation of the melanomacrophage centers, hemosiderosis, necrosis and mononuclear cell aggregates in the liver, kidneys and spleen.

[Eissa I. A. M., Derwa H. I., Maather El-Lamei, Amina Desuki, Mona S. Zaki, Hasna El-Sheshtawy. **Iron in water and some marine fishes in relation to vibriosis at Lake Temsah.** *Life Sci J* 2013;10(3):2520-2528]. (ISSN: 1097-8135). http://www.lifesciencesite.com**.** 367

Key words: Iron, Prevalence, Lake Temsah, Marine fishes, Vibriosis, Hemosiderosis.

1.Introduction

Owing to excessive use of water resources, it will be the most limiting factor to be considered in aquaculture development in Egypt, especially freshwater resources. Therefore, seawater is the immediate alternative source for water needed for mariculture (**Eissa *et al,* 2012)**.Fortunately, Egypt has vast marine resources of the Mediterranean and Red seas. Aquaculture is an expanding industry in which diseases of bacterial origin, generally due to Gram negative bacteria, are one of the most significant causes for economic losses**.** Vibriosis is one of the most serious bacterial diseases in cultured marine fish worldwide ***(*Lee *et al.* 2002*&*Alcaide 2003*)***

Vibrionacae include marine bacteria that can constitute up to 60% of the heterotrophic bacteria population. Some vibrio species are associated with plankton, suggestion that these bacteria may play an important role in the cycling of elements in the marine environment through mineralization processes. It has been recognized that different species of genus vibrio may cause a fatal disease in marine fishes and invertebrates. Vibrio species are the most dominant heterotrophic bacteria in the marine environment and are widely distributed in the coastal seawaters and /or brakish waters.They are also found on the surface and /or gasterointestinal tract of marine animals. Due to the rapid expansion of the intensive mariculture and the consequent deterioration of culture condition, vibriosis caused by occurs frequently worldwide, which affects a large numbers of fish and shellfish species (**Martinez *et al.*, 2003 and Thompson *et al.*, 2006, Eissa *et al,* 2011 and Albert and Ransangan, 2013).**

This study was planned to investigate the clinical picture of vibriosis, isolation and identification of the causative agents, the total and seasonal prevalence and the histopathological view of the disease among some marine fishes in Lake Temsah. Besides, the estimation of iron in Lake Temsah water and fish tissues was carried out.

2. Materials and Methods

Fishes

A total of 300 marine fishes of three different species, of different body weights were represented as *Sparus auratus*, *Siganus rivulatus, Tilapia zillii* (each 100)**.** They were collected randomly, seasonally and examined freshly from Lake Temsah in Ismailia governorate from May 2012 to April 2013. Fishes

were transferred immediately as soon as possible to the Lab in Dept of Fish diseases and Management, Fac. of Vet. Med, Suez Canal University.

Clinical examination

All fishes were clinically examined according to the method described by **Conroy and Herman (1981)** for detecting any abnormalities.

Bacteriological examination

Samples were taken aseptically from lesions in the external body surface, gills, liver, kidneys and spleen. Inoculi were streaked on TSA supplied with different concentrations of sodium chloride (1.5-8%) and incubated for 24 hrs at 30º C. The recovered suspected colonies were picked up and purified for further identification. Identification was carried out by determining their morphology, culture and traditional biochemical characteristics according to **Baumann and Furniss (1994).**The biochemical characters for all isolates were determined using the API-20E strip system.

Histopathological examination

Specimens were freshly taken from affected organs and tissues of naturally infected fishes.Specimens were trimmed and fixed in 10% phosphate buffered formalin. Then washing in running tap water for 24 hours, dehydrated in different concentration gradients of alcohol and cleared in xylol then embedded in paraffin wax and associated into thin sections 5 micron thickness. Sections were stained with H&E stain and examined microscopically according to **Roberts (2012).**

Estimation of iron in tissue:

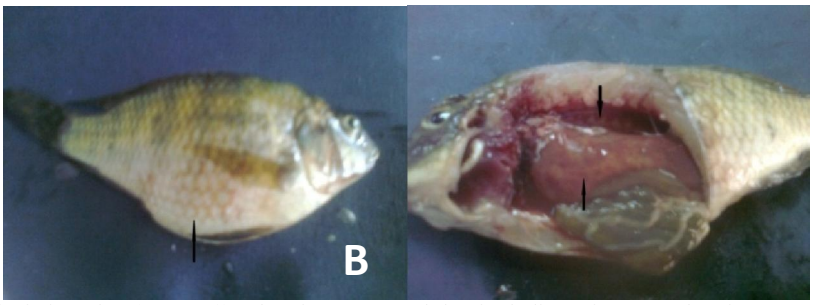
1-In fish tissue: Samples from musculature and liver of infected and non infected fishes were kept frozen at -20 C ^{0} till extraction at deep freezer.Dry ashing was carried out according to **Analytical methods for Atomic absorption Spectrophotometry (AAS), (1982**)

2-In water samples: A total of 10 water samples collected from 2 different areas in Lake Temsah from the same place where the fishes collected every season. The flasks, 2 liter volume was equipped with cork stopper and opened hand prides under water surface then equipped again and fixed with nitric acid 2% (**Chau and Chan 1974**).The treated water samples were filtered through Whattman filter paper No.1. The obtained clear filtrate was kept in clean dry bottles of 100 ml capacity and stored at room temperature till the time of the analysis. The samples were analyzed by Atomic absorption spectrophotometer according to **Clowley (1978).**

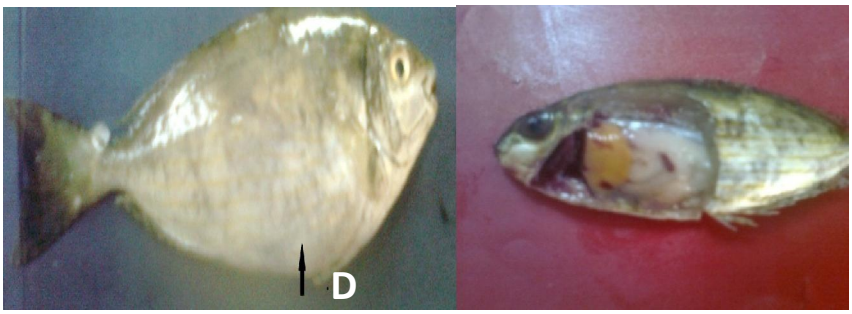
3. Results

Clinical examination of naturally infected fishes

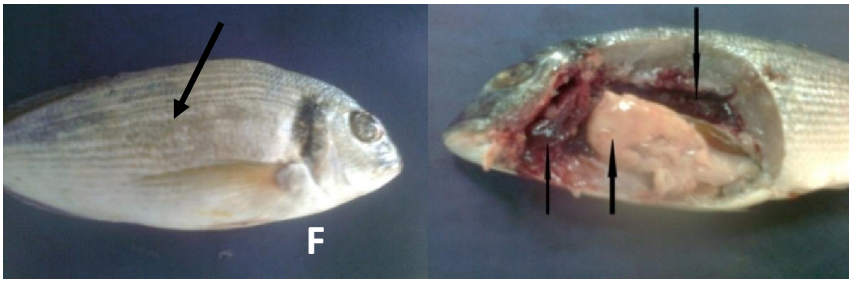
The clinical examination of naturally infected fishes was recorded in Photo 1 to 3.



(Photo.1) A. Naturally infected *Tilapia zillii* showing severe abdominal distention. **B.** Showing congested gills, liver with hemorrhagic spots & slight congestion in the body cavity, intestinal distension with serous fluid.



(Photo.2) C. Naturally infected *Siganus rivulatus* showing abdominal distension **D.** Showing pale liver with hemorrhagic spots & congested gills.



(Photo.3): **E**.Naturally infected *Sparus auratus* showing corneal opacity.**F.** sShowing pale liver and congestion of body cavity & gills

Results of Bacterial examination:

The bacterial colonies isolated from gills, liver, spleen and kidneys of naturally infected fishes on TSA supplemented with different concentrations of NaCl (1.5 to 8 %) were medium sized (2-3 mm in diameter) with creamy colour. On TCBS media were yellow colonies. They grew at 20, 30 and 40ºC after inoculation into TSB showing visible turbidity. Also, showed turbidity after inoculation into tryptone broth with different NaCl (0, 3, 8 %) (W/V) and were resistant to vibriostat O/129-10 µg and sensitive to vibriostat O/129-150 µg. On blood agar containing 1% sodium chloride overnight incubation showed clear area of hemolysis. Isolates were Gram-negative, comma shaped and motile. The result of the conventional and commercial system for biochemical tests revealed that isolates were LDC, ODC, CIT, AMY (+/-) while it was positive for oxidase, catalase, IND, GEL, GLU, MAN, SEC tests and negative for OPNG, ADH, H2S, URE, TDA, VP, INO, SOR, RHE,MEL, ARA tests. The code number on API20E strips was **4146125**, **4246124,4046125.** These results confirmed that the isolated bacteria were related to *Vibrio alginolyticus*

Results of prevalence of vibriosis in some marine fishes:

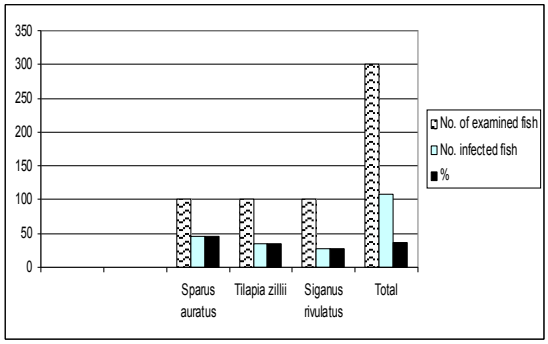


Fig. (1): Showing the prevalence of vibriosis in infected marine fishes.

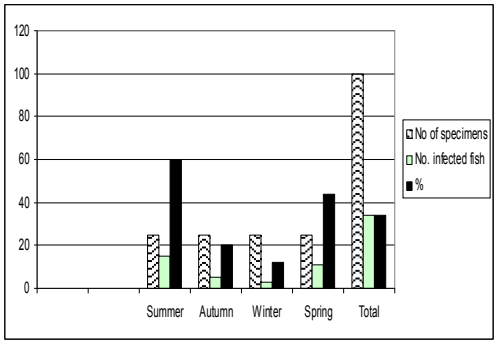


Fig. (3): Showing the seasonal prevalence of vibriosis among examined *Tilapia zillii*.

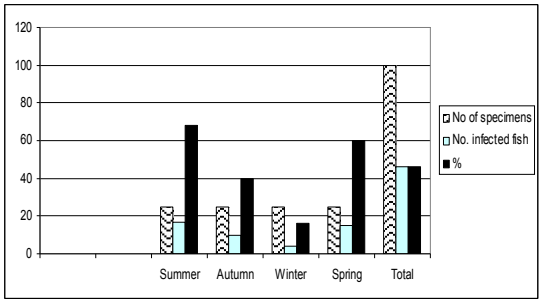


Fig. (2): Showing seasonal prevalence of vibriosis among examined *Sparus auratus*.

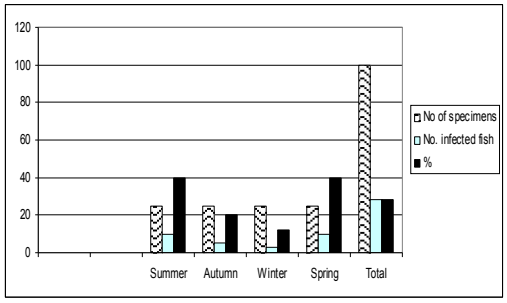


Fig. (4): Showing the seasonal prevalence of vibriosis among examined *Siganus rivulatus*.

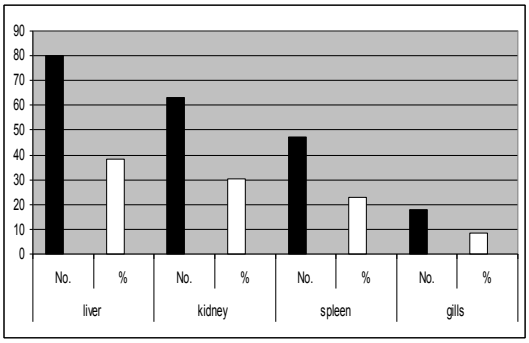


Fig (5): Showing numbers and percentages of vibrio isolates from (liver, kidney, spleen & gills) in examined fish.

Result of estimation of Iron in water and in fish tissues

1) Fish tissues

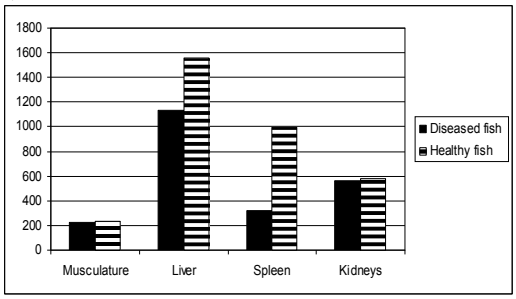


Fig (6): Showing the concentration of iron (µg/g) in musculature, liver, spleen & kidneys in examined *Siganus rivulatus*.

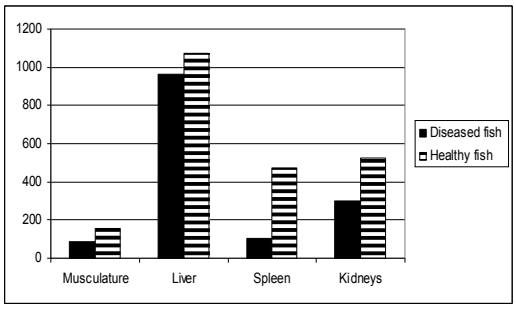


Fig (7): Showing the concentration of iron (µg/g) in musculature, liver, spleen & kidneys in examined *Tilapia zillii*.

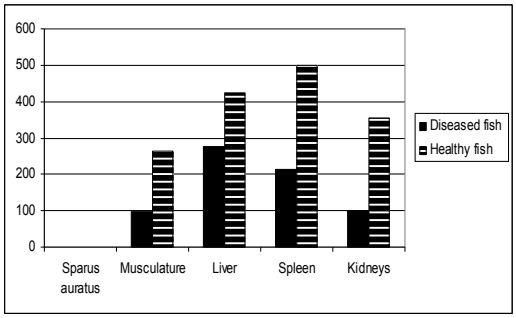


Fig (8): Showing the concentration of iron (µg/g) in musculature, liver, spleen & kidneys in examined *Sparus auratus*.

2) Estimation of iron in water

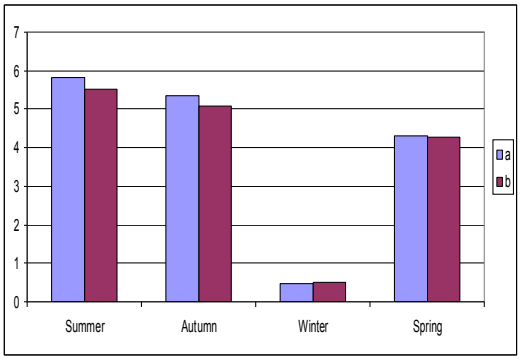


Fig (9): Showing the mean concentrations of iron in ppm in water of Lake Temsah in two areas (a& b) in different seasons.

Results of histopathological studies

The liver and kidneys in all examined fishes showed congestion, necrotic foci, sinusoidal and bile duct deformation was observed. Severe vacuolar degeneration, necrosis, mononuclear cell aggregates and hyperplasia of hepatopancrease with degenerative changes of tubules, damage of haemopoietic tissue. The spleen revealed depletion of white pulp, hemosidrosis and activation of melanomcrophage centers (**Photo 4 to 12).**

4. Discussion

Marine fishes are more used in food than the freshwater one, as about 60% of fish species live in sea water and 40% in fresh one. Concerning the clinical picture of the vibriosis it was revealed that naturally infected fish with vibriosis showed signs and lesions of septicemia. These results are in agreement with those obtained by **Moustafa *et al* (1990), Zorrilla *et al*., (2003 b); Ping *et al*. (2004)**, **Alicia *et al*., (2005)**; **Golomazou *et al*., (2006)** and **Robert *et al*., (2012)**.

This may attributed to that *V alginolyticus* is capable of producing toxins as serum proteases **(Lee *et al*., 1997 and Chen *et al*., 1999)** and production of multiple virulent extracellular products (ECP) mainly protease, haemolysin and siderophore might be characteristics of the virulent strains of *V. alginolyticus* (**Aguirre-Guzman *et al*., 2004 and Gomez-Leon *et al*. 2005)**. Also, **Jun *et al*., 2003; Balebona *et al*., 1998)** attributed the pathological lesions produced by *V alginolyticus* in fish to the effect of ECPs especially their hydrolytic and haemolytic components were toxic and responsible for the invasive and proliferative processes of these bacteria. Also, melanomacrophage related iron levels in affected fish are related to high requirement for iron. Pathogenic vibrio strains have a well developed iron sequestering mechanism based on siderophore,

which induce separation of plasma and tissue iron from its transferrin or ferritin binding proteins. These complexes to the sidrophore and attaches to specific complex transporting outer cell membrane proteins for absorption into the bacterial cells (**Acit *et al.* 1985).**

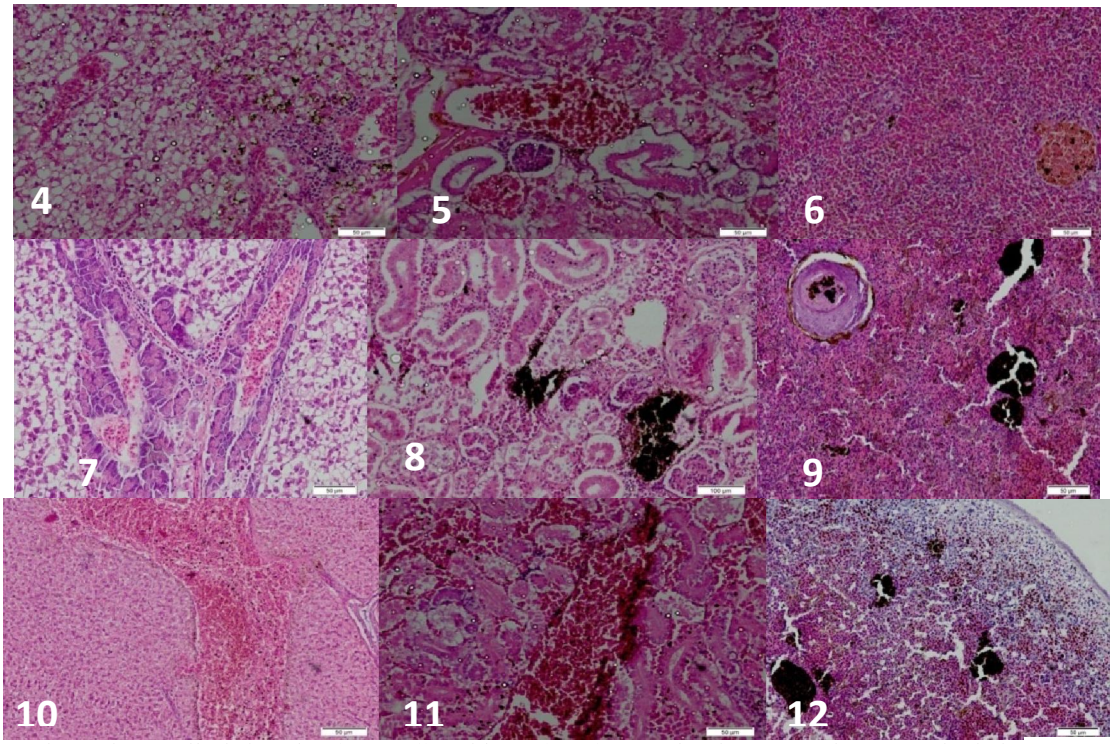


Photo.4: Liver of naturally infected *Tilapia zillii* with *V.alginolyticus* showing congestion, necrotic foci, severe vacuolar degeneration and mononuclear cell aggregates. **Photo.5:** Kidney of naturally infected *T zillii* with *V.alginolyticus* showing congestion, haemorrhage, degeneration and necrosis in the tubular epithelium. **Photo.6:** Spleen of naturally infected *Tilapia zillii* with *V.alginolyticus* showing depletion of white pulp, hemosidrosis and activation of melanomcrophage centers. **Photo.7:** Liver of naturally infected *Sparus auratus* with *V.alginolyticus* showing diffuse vacuolar degeneration, necrosis and hyperplasia of hepatiopancrease. **Photo.8:** Kidney of naturally infected *Sparus auratus* with *V.alginolyticus* showing degeneration of tubules, damage of haemopoietic tissue and activation of melanomacrophage centers. **Photo.9.:** Spleen of naturally infected *Sparus auratus* with *V.alginolyticus* showing degeneration of tubules, damage of haemopoietic tissue and activation of melanomacrophage centers. **Photo.10:** Liver of naturally infected *Siganus rivulatus* with *V.alginolyticus* showing severe congestion of hepatic blood vessels, hemorrhage, degeneration and necrosis. **Photo.11:** Kidney of naturally infected *Siganus rivulatus* with *V.alginolyticus* showing degenerative changes of renal tubular epithelia, damage of haemopoietic tissue, congestion. **Photo.12:** Spleen of naturally infected *Siganus rivulatus* with *V.alginolyticus* showing depletion and necrosis of white pulp with hemosidrosis and activation of melanomcrophage centers. (All are stained with H&E. and X 20).

Regarding isolated vibrio species, it was revealed Gram-negative, comma shape and motile. Also it grew on TSA supplemented with NaCl (1.5-8 %) and highly selective media TCBS medium at 37ºC for 18-24 hours. Colonies appeared as medium sized creamy colored on TSA while on TCBS appeared as yellow colonies. The biochemical characters revealed that they were positive for cytochrome oxidase and catalase, resistant to vibriostat O/129 -10µg and sensitive to vibriostat O/129-150µg as well as API system. It is belonged to *V. alginolyticus*. These results were similar to those recorded by **Austin & Austin (2007), Adeleye *et al* (2010) and Sabir *et al*., (2013).**

The present investigation indicates that the total prevalence of vibriosis among naturally infected marine fishes (36%). This result is lower than that obtained by **Adebayo-Tayo *et al*., (2011)** who recorded that it was about (44.2%) of examined seafoods samples obtained from Oron creek infected with vibrio bacteria and **Balebona *et al*., (1998)** who reported (67.8%) in three fish farms with intensive culture of seabream, *Sparus aurata* in Spain infected with vibrios. On other hand, this result is nearly close to that obtained by **Moustafa *et al.*, (2010)** who found that the prevalence of vibrios, fish samples from Qarun Lake and Suez gulf as (34%). This variation may be attributed to the differences in the

range variation of salinity level, the sample sizes, different climate.

Concerning prevalence of *V.alginolyticu* in naturally examined fishes, the highest was recorded in *Sparus auratus* (46 %), followed by *Tilapia zilli* (34%) then *Siganus rivulatus* (25.9 %).These results agreed with **Balebona *et al*., (1998)** who recorded that the main isolate from diseased fishes was *V.alginolyticus* in *Sparus aurata*. Also, **Zorrilla *et al. (*2003 b)** who recorded a high percentage of Vibrio spp, in seabream. On other hand, **Moustafa *et al., (*2010)** who recorded in *Siganus rivulatus* a prevalence of *V. alginolyticu*18% while in *Tilapia zilli* was 10% from Qarun Lake. Furthermore, **Balebona (1994)** who declared that several species of Vibrio have been involved in epizootic of seabream and the most important species involved in outbreak mortalities was *V alginolyticus*. This difference may be attributed to the different localities as mentioned that Lake Temsah receive wastewater drainage from Ismailia province, there was oil pollution expelled from ships in addition to the industrial and domestic effluents from ships that continually travel through it, and increase human activities considered the main causes of pollution. Excessive oil losses can clog the gills of small fish and/or stress the bigger one. Moreover suspended solids, heavy metals, and organic compound at sufficiently high concentrations can cause gill damage and may trigger diseases as a result of fish stress. Furthermore wastewater drainages also contain high microbial load that contribute to increasing the microbial pathogens in the lake. These results were supported by **Doukas *et al*., (1998)** who mentioned that outflow from wastewater discharges as stress factor potentiate the opportunistic bacterial pathogens.

Regarding the seasonal prevalence of vibriosis among naturally infected marine fishes this results found that the highest seasonal prevalence was recorded in summer (56 %) followed by spring (48%) then autumn (26.67 %) and winter (13.33%). While seasonal prevalence of *V alginolyticus* in *Sparus auratus* was (68 %) in summer followed by in spring (60 %) then autumn season (40 %) and (12%) in winter (16 %). In *T zilli* was (60%) in summer followed by spring (44 %) then (20 %) in autumn and winter. In *Siganus rivulatus*, spring and summer had the highest seasonal prevalence (40%) followed by autumn (20%) then winter (12%). This result showed that summer was the highest infection by vibrio while winter is the lowest. These results were in agreement with **Austin and Austin *(2007)* and Moustafa *et al., (2010).*** mentioned that outbreak of vibriosis only occurs when water temperature exceeds 15ºC.

Regarding the isolation of *V. alginolyticus* from internal organs, it was found in liver, kidney, spleen and gills respectively. These results can be explained as the liver and kidney are the main target organs of infection. These results agreed with **Zorrilla *et al*.,(2003 b)** who reported that vibrio was the dominant group, accounting for about 69% of the total number of isolated strains after bacteriological survey of *Sparus aurata* in Spain where strains were isolated mainly from the spleen, liver and kidney of diseased fish. Also, **El-Bassiony (2001)** reported that liver was the highest organ followed with kidney and spleen.

Concerning the histopathological studies, the liver and kidneys of naturally infected fishes with *V. alginolyticus* showed congestion, necrotic foci, and mononuclear cell aggregates with vacuolar degeneration, necrosis and hyperplasia of hepatopancrease and renal tubular epithelium. Spleen showed depletion of white pulp, hemosidrosis, activation of melanomcrophage centers and damage of haemopoietic tissue. These results agreed with **Stephens *et al*., 2006**; **Korun and Timur. 2009** and **Rebort *et al.*, (2012)**. The liver is an organ that may be used as an indicator of alterations in nutritional or physiological status as commented by **Segner and Juario (1986).** However, general metabolism of fish is compromised in cases of infectious diseases. *V alginolyticus* is capable of producing toxins as serum proteases (**Chen *et al*., 1999)** which may be responsible for alterations in the kidney, liver and spleen in infected fishes. Also, it was shown in liver and spleen hyperactivation of melanomacrophage centers. These may be due to rapid clearance and elimination of bacteria from the blood by macrophages which subsequently settle in the haemopiotic tissues which activate melanomacrophage centers.Later on, the macrophage that contain organisms may lyse and liberate bacterial toxins and result in excessive damage (**Soliman, 1988)**.

Iron is one of the important factors for growth and proliferation of vibrio species, our investigation revealed its concentrations in musculature, liver, spleen and kidney in diseased and healthy fishes. They were 221.06, 1133.7, 319.7, 561.3 µg/g respectively in diseased *Siganus rivulatus* while in healthy ones; they were 229.58, 1559.9, 998.5 and 576.5 µg/g. They were 87.23, 960.6, 105.33, 300.23 µg/g 960.6mg/g, 105.33mg/g, 300.23mg/g respectively in diseased *T zilli* while in healthy ones they were 153.45, 1075, 475.166, 524.667 µg/g. In *Sparus auratus* muscles, liver, spleen & kidney of diseased fish 96.47, 276.98, 212.412, 100.53 µg/g respectively while in healthy fish 264.98, 423.8, 499.25, 356.25 µg/g. These

results indicated that there was accumulation of iron in healthy and diseased fishes because metals contaminants can enter the environment in excess amounts from industrial and mining effluents, from the combustion of fossil fuels, discharge of sewage and sewage sludge; also from fertilizer and pesticide residues. Pathways for metal input to the marine environment include transport via rivers and streams, direct discharge and atmospheric fallout are well known **(Forstner and Wittmann., 1979)**. These results are in agreement with that obtained by **(EI-Nemr *et al.*, 2003)** who found fluctuation of iron concentration in *Siganus rivulatus* and *Tilapia zillii* obtained from Lake Edku. These differences may be related to localities, temperature and the surrounding environment. Also results indicated that the highest iron concentration was in livers of healthy and diseased fishes while the musculature was of the lowest. This agreed with that obtained by **Ambedkar and Muniyan, (2012)** who found that the mean levels of iron(0.56 + 0.021 mg/kg dry wt.) in the liver of *Heteropnustes fossilis* while the least level (0.10 + 0.003 mg/kg dry wt.) in the muscle tissues of *Chanos chanos*. Metal concentrations were always lowest in the muscle and highest in the gill and liver. This probably due to their physiological roles in fish metabolism. It had been shown that target tissues of heavy metals are metabolically active ones, like the liver, kidney and gill. Therefore, metal accumulation in these tissues occur higher level compared to some other tissues like muscle, where metabolic activity is relatively low **(Canli and Atli., 2003**).

Regarding the sharp decrease of iron in healthy fishes (not infected with *V. alginolyticus*) than the infected fishes, this may be due to these bacteria need iron for growth in their hosts through their iron-sequestering systems **(Actis *et al., 1999***). These systems center upon the production of iron-scavenging compounds known as siderophores and the subsequent transport of the ferric-siderophore complex back into the cell cytosol. Also iron chelation is an important property both for environmental, non harmful and for pathogenic microorganisms. Due to redox properties, iron is mostly bound in insoluble complexes, and the concentration of free, accessible iron in most environments is low. Thus, iron limitation is a major constraint for microbial growth in ocean environments **Church *et al.*, (2000)**, and many aquatic microorganisms produce efficient iron chelators (**Reid *et al*., 1993).**

So, according to **Anzaldi and Skaa (2010); Kustusch *et al., (*2011) and Naka *et al* (2012)** who found that vibrio species contain iron-scavenging compounds siderophores known as vibrioferrin these proteins is the base of **i**ron-sequestering systems in these bacteria helping them to uptake of iron from tissues of the hosts to their cytsol. So we can investigate that bacteria used iron into infected fishes and lead to decrease their concentration in the different tissues.

This investigation revealed that the water in two areas (under the bridge and near the ships) showed increase concentration of iron in summer followed by autumn then spring and winter. This result agreed with that obtained by **El Nemr *et al*., (2003)** who found that after examination of water and fish samples from south Mediterranean, samples of study were of high concentration in summer and lowest in winter. This may be due to the water temperature may influence water chemistry, metal solubility, metal uptake by plants and plant growth. Cool water contains more dissolved oxygen than warm water. Thus, metal concentration in the interstitial water of the sediment may decrease with decreasing temperature, as more metals are bound to sediment colloids at high rather than low redox potentials **(Forstner, 1979).**

From the present work, it was concluded that the highly prevalence of vibriosis in the examined marine fishes was recorded in *Sparus auratus* followed by *Tilapia zillii* then *Siganus rivulatus*. Vibriosis was highly prevalent in summer and the lowest was in winter. The liver had the highest prevalence of vibriosis followed by kidney then spleen and gills. *V. alginolyticus* in the examined marine fish is an alarm not only as fish pathogen but as human hazard. Estimation of iron in water act as alarm to the increase of pollutants especially industrial ones in Lake Temsah that lead to its increase for acting as motive factors for *V. alginolyticus*. Estimation of iron in fish tissues indicated that musculature of fish which are edible for human consumption has the lowest iron concentration and it does not exceed the permissible limits in fish.

References

1. **Actis, L.A; Potter, S. A and Crosa, J.H (1985):** Iron regulated outer protein OM2 of *Vibrio anguillarum* is encoded by virulence plasmid PJMI.J.Bacteriol.,161:736-42
2. **Actis, L. A.; Tolmasky, M.E. & Crosa, J. H. (1999)**: Vibriosis. In: Woo,P.T.K., Bruno, D.W. (Eds.), Fish Diseases and Disorders. CAB Intern., Publ., United Kingdom, 3: 523– 558.
3. **Adebayo-Tayo, B. C.; Okonko, I. O.; John, M. O.; Odu, N. N.; Nwanze, J. C. and Ezediokpu, M. N. (2011):** Occurrence of Potentially Pathogenic VibrioSpecies in Sea Foods Obtained from Oron Creek.Advances in Biological Res. 5 (6): 356-365.

4. **Adeleye, I. A.; Daniels, F. V. and Enyinnia, V. A. (2010):** Characterization And Pathogenicity of *Vibrio Sp*. Contaminating Seafoods In Lagos, Nigeria. Internet Journal of Food Safety 12: 1-9.
5. **Aguirre-Guzman,G.; Ruiz, H. M. and Ascencio, F. (2004)**: A review of extracellular virulence product of Vibrio species important in diseases of cultivated shrimp. Aqua Res 35: 1395–1404.
6. **Albert, V. and Ransangan, J. (2013):** Effect of water temperature on susceptibility of culture marine fish species to vibriosis. International Journal of Research in Pure and Applied Microbiology 3(3): 48-52
7. **Alcaide, E. (2003):** Numerical taxonomy of Vibrionaceae isolated from cultured amberjack (Seriola dumerili) and surrounding water. Current Micro. 46: 184–189.
8. **Alicia, E. T.; Beatriz, M. and Jesus, L.R. (2005):** A review of the main bacterial fish diseases in marine culture systems. Aquaculture, 246: 37 – 61.
9. **Ambedkar, G and Muniyan, M. (2012):** Analysis of heavy metals in waters, sediments and selected freshwater fish collected from Gadilam river Tamilnadu, India. International Journal of Toxicology and Applied Pharmacology 2 (2): 25-30.
10. **Analytical Methods for atomic absorption Spectrophotometry (1982):** Analysis of fish and sea food.Perkin. Elmer, Norwalk, Connecticut, USA.
11. **Anzaldi, L. L. and Skaar, E.P. (2010):** Overcoming the heme paradox: heme toxicity and tolerance in bacterial pathogens. Infect Immun 78: 4977–4989.
12. **Austin B. & Austin D.A. (2007)** Characteristics of the diseases: Vibrionaceae representatives. In: Bacterial Fish Pathogens, 4th edn (ed. by B. Austin & D.A. Austin), pp. 40–44. Praxis Publishing Ltd, Chichester.
13. **Balebona, M. C. (1994):** Caracterizacion y meccanismos de virulencia de capas Del ge nero Vibrio aisladas de patologi as de dorada (Sparus aurara L.) cultivadas., Uni. Malaga. Spain.
14. **Balebona M.C., Andreu M.J., Bordas M.A., Zorrilla I., Morin˜igo M.A. & Borrego J.J. (1998)**: Pathogenicity of *Vibrio alginolyticus* for cultured gilt-head sea bream (*Sparus auratus* L.)". Applied and Environmental Microbiology 64: 4269–4275.
15. **Baumann, P. and Furniss, A. L. (1994**): Vibrionaceae, *in Hensyl W. R., ed,* Bergey's Manual of Determinative Bacteriology, Williamd and Wilkins, Baltimore.
16. **Canli, M. and Atli, G. (2003)**: The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb Zn) levels and the size of six Mediterranean fish species. Environmental Pollution 121: 129–136.
17. **Chau,Y. K and Chan, K.(1974):** Determination of labiles strongly bound metals in lake water. Water Research J. 8: 383-388.
18. **Chen, F. R.; Liu, P. C. and Lee, K. K. (1999):** Purification and partial characterization of a toxic serine protease produced by pathogenic *Vibrio alginolyticus*. Microbios, 98 (390): 95-111.
19. **Clowely, K.M. (1978):** Atomic absorption development is food analysis in kind RD Science Publisher I T D Ripple –Rood borkind essexengland.
20. **Conroy D.A. and Herman L.R. (1981):** Textbook of fish diseases.T.F.H. Publ,West Sylvania
21. **Doukas, V.; Athanasssopoulou, F.; Karagouni, E. & Dotasika, E. (1998)**: *Aeromonas hydrophila* infection in cultured sea bass, dicentrarchus labrax l. and puntazzo puntazzo from the Aegean Sea, J fish dis., 21: 317-360.
22. **Eissa I.A.M, Derwa H.I, Abd-Elrehim, A.(2011)** Epizootiological studies on vibriosis in marine shrimp *Penaeus japonicus* in Suez-Bay. Suez Canal Vet Med J,XV1(2) 81-88
23. **Eissa I.A.M, Derwa H.I, Amina Desuki, Mabrok M. (2012)** Studies on enterococcosis among some marine fishes in Lake Temsah, Ismailia. *The Global J of Fisheries and Aquaculture Re, V. 5, No. 5, 115-127.*
24. **El-Bassiony (2001):** Study on vibriosis in some cultured fish in Egypt. PhD., Fish Dis. & Hyg., Fac. Vet. Med., Alex. Uni**.**
25. **El Nemr, A.; El-Sikaily, A. & Khaled, A.(2003):** Heavy Metals Concentrations in some fish tissues from south Mediterranean waters,. Egypt- / Aquat Bid & Fish., Vol 7(3):155 -172
26. **Forstner, U.(1979):** Metal transfer between solid and aqueous phases. Fo¨ rstner, U., Wittman, G.T.W. (Eds.), Metal Pollution in the Aquatic Environment. Springer.Verlag, Berlin, 197–207
27. **Forstner, U. and Wittmann, G. T. W. (1979):** Metal pollution in the aquatic environment. Springer-Verlag, Berlin, pp. 30-61.
28. **Golomazou, E.; Athanassopoulou, F.; Vagianou, S.; Sabatakou, O. Tsantilas, H.; Rigos, G. & kokkokiris, L. (2006):** Diseases of White Sea Bream (*Diplodus sargusL.*) Reared in Experimental and Commercial Conditions in Greece Turk. J. Vet. Anim. Scin., 30: 389-396.

29. **Gomez-Leon, J.; Villamil, L.; Lemos, M.L.; Novoa, B. and Figueras, A. (2005)**: Isolation of *Vibrio alginolyticus* and *Vibrio splendidus* from aquacultured carpet shell clam (*Ruditapes decussatus*) larvae associated with mass mortalities. Appl Environ Microbiol 71: 98–104.
30. **Jun, L. I.; Zhou. L. & woo. N. Y. (2003):** Invasion route and pathogenic Mechanism of V. alginolyticus to Silver Sea Bream Sparus sarba. J. Aquac. Anim. Health, 15: 302-313.
31. **Korun, J. and Timur,G. (2008):** marine vibriosis associated with diseased sea bass (*Dicentrarchus labrax*) in Turkey. Jor. Fishiers and Science 2(1): 66-76.
32. **Kustusch, J. R.; Kuehl, J. C. and Crosa. H. G (2011):** Power plays: iron transport and energy transduction in pathogenic vibrios. Biometals. 24(3): 559–566.
33. **Lee, K. K.; Yu, S. R. and Liu, P. C. (1997):** Alkaline serine protease is an exotoxin of *Vibrio alginolyticus* in kuruma prawn, *Penaeus japonicus*. *Current Microbiology*, vol. 34, no. 2,110-17.
34. **Lee, K.K., Liu, P.C. & Chuang, W.H. (2002**): Pathogenesis of gastroenteritis caused by *Vibrio carchariae* in cultured marine fish. Marine Biotechnology 4: 267–277.
35. **Martinez, J.S.; Carter-Franklin, J.N.; Mann, E. L.; Martin, J. D; Hay-good, M. G & Butler A (2003)**: Structure and membrane affinity of a suite of amphiphilic siderphores produced by a marine bacterium.ProcNatl Acad Sci USA 100:3754-59.
36. **Moustafa, M. Eissa I.A.M. and Mervat S. Hanafi. (1990)** Vibriosis in marine fishes of lake Qarun. Zagazig Vet. J., 18, 5, 94-105
37. **Mosustafa, M.; Laila, A.M.; Mahmoud, M. A.;Soliman, W. S. & El-gendy, M. Y.(2010):** Bacterial infections affecting marine fishes in Egypt, j of American Science: 6(11): 603-612.
38. **Naka, H.; Chen, Q.; Mitoma, Y.; Nakamura, Y.; McIntosh-Tolle, D.; Gammie, E. A.; Tolmasky, E. M. and Crosa, H. G. (2012):** Two replication regions in the pJM1 virulence plasmid of the marine pathogen *Vibrio anguillarum*. Micro.Pathog. 67: (2) 95–101.
39. **Ping, C. L.; Lin, J. Y.;Hsiao. P.T.;Lee, K. K. (2004)**: Isolation and characterization of pathogenic *Vibrio alginolyticus* from diseased cobia *Rachycentron Canadum* L.J. Basic Microbiol., 44:23-28.
40. **Reid, R. T.; Live, D. H.; Faulkner, D. J. and Butler, A. (1993)**: A siderophore from a marine bacterium with an exceptional ferric ion affinity constant.Nature366:455 58
41. **Robert, B., Moeller, Jr. (2012):** Bacterial fish Diseases California Animal Health and Food Safety Laboratory SystemUniversity of California.
42. **Sabir, M.; Ennaji, M. and Mustapha, C. (2013):** *Vibrio Alginolyticus*: An Emerging Pathogen of Foodborne Diseases. Inter. Jour. Sci &Tech. 2:302-309.
43. **Segner, H. and Juario, J. V.(1986):** Histological observations on the rearing of milkfish, *Chanos chanos*, fry using different diets. J of Applied Ichthyology. 4: 162-173.
44. **Soliman, M. K. (1988):** the pathogensis of A. hydrophila isolates in fish with especial emphasis on their control. Ph.D. Thesis Fac. Med. Alex. Uni.
45. **Stephens, F. J.; Raidal, S. R.; Buller, N. and Jones, B. (2006).** Infection with *Photobacterium damselae* subspecies *damselae* and *Vibrio harveyi* in snapper, *Pagrus auratus*with bloat. Austrilian Veterinary J, 84(5): 173-177.
46. **Thompson,F.; Austin,B. and Swings, J. (2006):** The biology of vibrios.ASM Press; Washington.
47. **Zorrilla, M.; Chabrillon, A. S.; Rosales, P. D.; Manzanares, E. M.; Balebona, M. C. & Morinigo, M.A. (2003 b):** Bacteria recovered from diseased cultured gilthead sea bream, *sparus aurata* L. in south western Spain. Aqua., 218:11-20.

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