Bacteriological and Histopathological Studies on Photobacteriosis in *Tilapia Zillii* and *Mugil Seheli*

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Abstract:

This study was applied on 140 fishes of two species, *T.zillii* as member of Cichlaidae and *M.seheli* as member of Mugilidae collected randomly and seasonally from Lake Temsah to investigate the prevalence of photobacteriosis. Isolates from two fish species were identified by morphological characterization, biochemical tests and API20E as *Photobacterium damselae*. 15 fish of *M.seheli* and 22 fish of *T. zillii* were infected with *Ph. damselae* subspecies *damselae* while 6 fish of *M.seheli* and 9 fish of *T. zillii* were infected with *Ph.damselae* subspecies *piscicida*. Total prevalence of *Ph.. damselae* subspecies *damselae* was 26.42% while prevalence of *Ph. damselae* subspecies *piscicida* was 10.71%. Prevalence of photobacteriosis was the highest in summer season and the lowest in winter season. Histopathological studies in naturally infected fishes revealed that there was necrosis and degeneration in livers and kidneys with congestion of blood vessels and hyperplasia in melanomacrophage centers in spleen.

Introduction:

Cichlidae species are the most popular and highly economic fishes in most lakes in Egypt. *Tilapia zillii* is distinguished by its adaptation to living in fresh, brackish and nearly saline water, and can survive in partially polluted water ***(Zyadah, 1997).*** The mullets are worldwide distributed species in tropical and temperate coastal waters. The keeled mullet *Liza carinata* locally named "Sehlia" is a mugilidae of commercial value for fisheries and aquaculture in Suez Bay and Suez Canal sector ***(El Ganainy and El Boray, 1999)***. Disease is usually the outcome of an interaction between the host, the pathogen and external stressor ***(Austin and Austin, 2007).*** Bacterial diseases represent a major problem among cultured fish in Egypt ***(Abdelaziz and Kamel, 2005).*** Bacterial pathogens considered the main cause of high mortalities and economic losses among fish diseases ***(Eissa et al., 2012)*** .Photobacteriosis is a fish disease that causes enormous losses in fish aquaculture production worldwide ***(Kusuda & Salati 1993 and Romalde & Magariiios1997)***.

The causative agent of fish photobacteriosis is *Ph. damselae* that includes Gram-negative marine bacteria belonging to 2 different subspecies, namely subsp. *damselae* and subsp. *piscicida*. *Ph. damselae* subsp. *piscicida* (formerly *Pasteurella piscicida*) is the causative agent of pasteurellosis or pseudotuberculosis, one of the most important fish diseases in marine aquaculture. it was subsequently transferred to the genus *Photobacterium* according to the phenotypic data ***(Smith et al., 1991)***, and supported by ***Ruimy et al. (1994)*** who carried the phylogenetic analysis. *Ph. damselae* subsp. *damselae* has been reported to cause fatal diseases in a variety of marine animals and human ***(Osorio et al., 2004).*** The aim of this work is studying the clinical picture and P.M. lesions of infected fishes with photobacteriosis ,isolation and identification of the causative agent, studying the total and seasonal prevalence of the disease and investigating the histopathological picture of the disease.

Material and Methods:

Fishes:

A total of 140 fishes of two different species, of different body weights were represented as 80 *Tilapia zillii* and 60 *Mugil seheli* , were selected for this study from Lake Temsah seasonally from August 2015 to July 2016, where these species have a wide range of distribution in the aquatic habitat in Egypt. Fresh fish were collected, and transported immediately to the laboratory in an ice-box for full clinical, post mortem and bacteriological examinations.

Clinical and P.M. examination

All fishes were clinically examined according to the methods described by ***Noga (2011)*** for detecting any abnormalities. For demonstration of the internal abnormalities, the postmortem examination was performed on all freshly dead and moribund fishes according to ***Meyers (2006).***

Bacteriological examination

Samples were taken aseptically from lesions in livers, kidneys and spleen. Bacterial isolation was performed according to ***Gauthier et al. (1995)***. Inoculi were streaked into Brain heart infusion (BHIB) and (BHIA) supplemented with 3% NaCl and incubated at 24 ºC for 1-2 days, recovered colonies were inoculated onto blood agar medium supplemented with 3 % NaCl and Thiosulfate citrate bile salt sucrose agar (TCBS) and incubated at 24 ºC for 1-2 days. The recovered suspected colonies were picked up for further identification according to culture, morphology and traditional biochemical characterization. The biochemical characters for all isolates were determined using the API-20E strip system according to ***Buller (2004).***

Histopathological examination

Specimens were freshly taken from affected organs of naturally infected

fishes. Small pieces of the organs were fixed in 10% neutral formalin, dehydrated and then embedded in paraffin wax. Sections were cut at 5-6 μm and stained with H&E for routine histological examination according to ***Robert and Moeller (2012).***

Results

Clinical and P. M. examination of naturally infected fishes

As shown in (Plate 1), naturally infected fishes with photobacteriosis showed haemorrhage on the abdominal region and the base of operculum with abdominal distension. The P.M. lesions revealed that enlarged and pale with hemorrhagic spots on livers others appeared congested livers, congested gills and kidney.

Results of Bacterial examination:

Isolated colonies from affected organs of naturally infected fishes on BHIA with 3% NaCl appeared as small whitish yellow non-separated colonies, blood agar with 3% NaCl appeared as white colonies at 24 ºC for 1-2 days suspected to be *Photobacterium damselae*. Isolates appeared as white non hemolytic colonies, no growth with BHIA with 6% NaCl, no growth on TCBS, bipolar staining with gram stain, nonmotile and sensitive to vibriostatic agent O/129, It was suspected to be *Photobacterium damselae* subsp. *piscicida*. Isolates appeared as white hemolytic colonies, grew at BHIA with 6% NaCl , pin point green colonies on TCBS, Gram -ve short rod with gram stain, motile and sensitive to vibriostatic agent O/129 , It was suspected to be *Photobacterium damselae* subsp. *damselae* . The result of the conventional and commercial system for biochemical tests revealed that *Photobacterium damselae* subsp. *piscicida* isolates were positive in (ADH), (CIT), (VP), (GLU), (RHA), (MEL), (AMY), (ARA) and (OX). On the other hand, it showed negative results for these tests:(OPNG), (LDC), (ODC), (H2S), (URE), (TDA), (IND), (GEL), (MAN), (INO), (SOR) and (SAC).The code number on API20E strips was 2205057. While *Photobacterium damselae* subsp. *damselae* isolates were positive in these tests: (ADH), (URE), (VP), (GLU) and (OX). Oppositely, they showed negative results for other tests. The code number on API20E strips was 2015004.

Results of prevalence of photobacteriosis in some marine fishes:

As shown in Fig (1-3).

Results of histopathological studies

As shown in **(Plate 2)** livers and kidneys in all examined fishes showed diffuse vacuolation and focal necrosis, severe congestion of blood vessels and focal leukocytic infiltration. Spleen showed severe destruction of hematopoietic tissue and mild hyperplasia of melanomacrophage center.

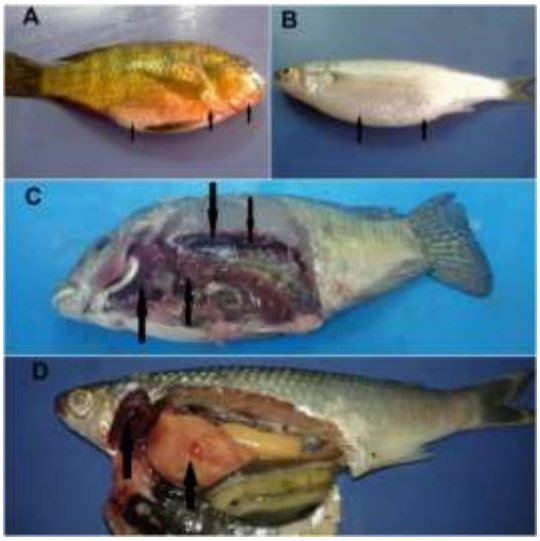

Plate1: Naturally infected with photobacteriosis **Photo(A)** *T. zillii* showing haemorrhage on the abdomen with abdominal distension. **(B)** *M.seheli* showing abdominal distension.**(C)** *T. zillii* showing congested liver and gills with severe haemorrhage in kidney. **(D)** *M. seheli* showing enlarged and pale with haemorrhagic spots on liver and congested gills.

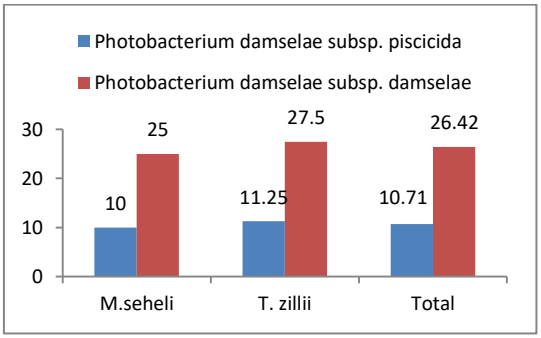

Fig 1: Total prevalence of photobacteriosis in the examined fishes.

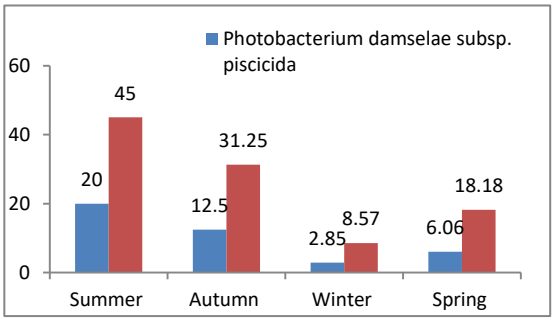

Fig 2: Seasonal prevalence of photobacteriosis in the examined fishes.

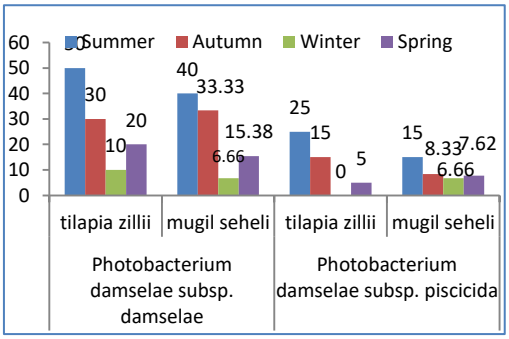

Fig 3: Seasonal prevalence of two *Ph. damselae* subspecies in the examined *T.zillii* & *M. seheli*.

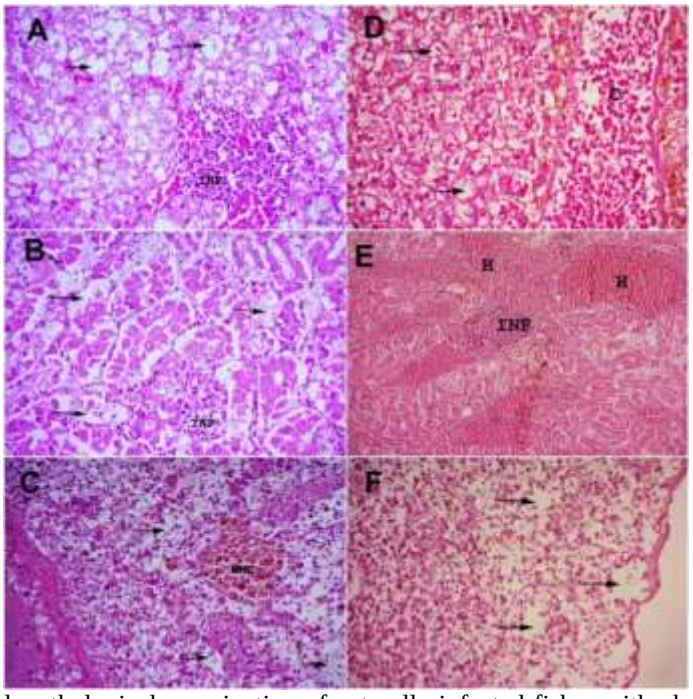

Plate 2: Histolopathological examination of naturally infected fishes with photobacteriosis: in *Tilapia zillii* **(A)** Liver showing degeneration of hepatocytes (arrows) congestion of blood vessels and focal leukocytic infiltration (INF) X400 **(B)** Kidney showing vacuolar degeneration and necrosis of renal tubules (arrows) and focal leukocytic infiltration (INF) X400 **(C)** Spleen showing severe destruction of hematopoietic tissue (arrows) and mild hyperplasia of MMC X400. In *Mugil seheli* **(D)** Liver showing diffuse vacuolation and focal necrosis of some hepatic cells (arrows), with severe congestion of blood vessels (C) X400**. (E)** Kidney showing degeneration of renal tubules, diffuse inter-tubular haemorrhage (H) congestion of blood vessels and focal leukocytic infiltration (INF) X400 **(F)**Spleen showing severe depletion and necrosis of lymphoid tissue (arrows) X400 .

Discussion

Photobacteriosis is currently recognized as one of the most devastating bacterial diseases in mariculture worldwide ***(Costas et al. 2013).*** Concerning the clinical picture and P.M. lesions of photobacteriosis, it was revealed that naturally infected fishes revealed haemorrhages at abdominal and anal regions and abdominal distension. The P.M. lesion revealed congested liver and gills with severe haemorrhage in kidneys. These clinical signs and postmortem alterations resembled with ***Yiagnisis et al. (2004)*** who reported that external skin lesions (hemorrhages and skin ulcers) and fluid in the peritoneal cavity (ascites) of most of the wild marine fish were infected with *P. damselae in* Greece. These results agreed with ***Stephens et al. (2006)*** *and* ***Kanchanopas-Barnette et al. (2009)*** who reported the same signs and P.M. in different fish species and ***Labella et al. (2006)*** who mentioned that gross clinical signs were similar to those previously described for vibriosis and pasteurellosis in other cultured sparid fish. These signs may be attributed to ECP. The main ECP components related to virulence include proteases, hemolysins, and siderophore-mediated iron sequestering systems ***(Wang et al., 2007).*** These mechanisms can provoke host tissue destruction and hemorrhages, playing an important role in colonization, invasiveness, and dissemination of the bacterial pathogen within the host ***(Silva et al., 2003).***

Regarding the morphological and cultural characters of two *Photobacterium* subspecies that had been isolated, our study revealed that *Photobacterium damselae* subsp. *piscicida* were Gram negative with bipolar staining, non-motile. On BHIA with 3% NaCl, showing small pale non separated colonies while no growth on TCBS medium and on blood agar supplemented with 3% NaCl revealed non hemolytic activity. These phenotypic properties were nearly the same reported by ***Austin and Austin (1999); Zorrilla et al. (1999); Korun and Timur (2005)*** *and* ***Essam et al. (2016)***. Bacterial identification was confirmed by API-20E analysis system and was agreed with ***Liu et al. (2011) .***While *Photobacterium damselae* subsp. *damselae* was Gram negative short rods, motile and on TCBS agar were small green colonies, on BHIA 3,6 % NaCl. On blood agar with 3% NaCl, appeared as small hemolytic colonies. These results were in agreement with ***Labella et al. (2006).*** According to the confirmatory diagnostic API-20E system were resembled to that mentioned by ***Toranzo et al., (1991)*** and ***Nicky (2004).*** Regarding the total prevalence of *Ph. damselae* was 26.42% for *Photobacterium damselae* subsp. *damselae,* this result was higher than ***Tanekhy (2013)*** who found the prevalence of

Photobacterium damselae subsp. *damselae* in 200 marine were collected from cages in Wadi-mariut region at Borg -El Arab city at Alexandria governorate was 4.2%. Regarding the total prevalence of *Photobacterium damselae* subsp. *piscicida* was 10.71%. These results were lower than ***Moustafa et al. (2010)*** who reported that the prevalence of *Photobacterium piscicida* was 15.5% among 6 different fish species from Qarun Lake and Suez Gulf. These different in prevalence may be attributed to difference of locality, salinity and temperature**.** Concerning the seasonal prevalence of two subspecies was the highest in summer season in a ratio of (45%) and (20%) and the lowest in winter in a ratio of (8.57%) and (2.85%) for *Photobacterium damselae* subsp. *damselae* and *Photobacterium damselae* subsp. *piscicida* respectively. These results in agreed with ***Magarinos et al. (1996)*** and ***Labella (2010)*** who found that the two subspecies had certain affinity to the high water temperature for inducing fatal disease in fish. Regarding the prevalence of photobacteriosis in marine fishes, our results reported that the highest prevalence of *Photobacterium damselae* subsp. *damselae* and *Photobacterium damselae* subsp. *piscicida* respectively was (27.5%) and (11.25%) in *T. zillii* and the lowest in *M. seheli* in a ratio of (25%) and (10%) respectively, this result agreed with ***Serracca et al. (2011)*** who found that the seasonal prevalence of *Photobacterium damselae* subsp. *piscicida* after examination of wild mullet species collected in the river Magra, Italy was highest in summer season (74%) then autumn season (61.8%) and in spring was (19.6) and ***Moustafa et al. (2010)*** who reported that the highest prevalence of *P.piscicida* in different marine fishes among them *Tilapia zillii* was recorded during the summer season (7.75%) followed by the autumn (5.30%) followed by the spring (2.44%) on the other hand, it was not recorded in winter. This may be due to the highest environmental temperature and these findings were agreed with ***Korun and Timur (2005)*** who observed that, *Pasteurellosis* occurs in cases of bad marine water quality, high temperature (over $23^{O}C$), and high salinity (20-30%), causing daily mortality of 2.8-5% and considerable economic losses. Regarding the seasonal prevalence of photobacteriosis in *T.zillii* was (25 %) in summer season followed by autumn season (15%) then spring season (5%) and winter season (0%) for *P. damselae* subsp. *piscicida* Furthermore, the prevalence in *P. damselae* subsp. *damselae* in tilapia species was in summer season (50%) followed by autumn season (30 %) then (20%) spring season and winter season (10%). In *M. seheli* was summer seasons had the highest seasonal

prevalence of *P. damselae* subsp. *piscicida* (15%) followed by autumn season (8.33%) and spring season (7.69 %) finally winter season (6.67%). On the other hand, the prevalence of *P. damselae* subsp. *damselae* was in summer season (40 %) followed by autumn season (33.33 %) then (15.38 %) spring season and winter season (6.67%). These results agreed with ***Fatemeh Naseri et al. (2014)*** and ***Essam et al. (2016)*** who isolated different *P. damsalae* species from different fish species and was the highest prevalence in summer and the lowest in winter. Concerning the histopathological studies, livers and kidney showed severe degeneration and diffuse vacuolation of hepatocytes, congestion of blood vessels with focal leukocytic infiltration ,degeneration of renal tubules and diffuse inter-tubular haemorrhage . These results agreed with ***Abu-Elala et al. (2015)***. The pathogenicity of the bacterium for hepatocytes and endothelial cells appears to be restricted to a region where the bacterium can proliferate and produce the ECPs ***(Noya et al. 1995 and Romalde 2002)***. Spleen showed severe destruction of hematopoietic tissue and mild hyperplasia of melanomacrophage center. This picture may be because of extensive infiltration of macrophages and neutrophils that occurred in the initial phase of disease, followed by extensive phagocyte depletion in advanced infection ***(Do Vale et al. 2007).***

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الملخص العربى

أجريت هذه الدراسة على 140 سمكة من نوعين مختلفين الشبار الاخضر من عائلة الشبار و السهلىى من عائلة البوريات حيث تم تجميعهم عشوائيا وموسميا من بحيرة التمساح لدراسة نسبة ظهور مرض الفوتوبكتيريا. تم التعرف على العزلات من هذه الاسماك بواسطة الشكل الخارجى والخصائص الكيميائية وايضا بواسطة ا .بى. آى 20 على انها الفوتو بكتيريا دامسيلا . وجد ان حوالى 15 سمكة من اسماك السهلية و22 سمكة من الشبار الاخضر مصابة بالفوتوبكتيريا دامسيلا من نوع الدامسيلا وعدد 6 أسماك من نوع السهيلى و9 من الشبار الاخضر كانوا مصابين بالفوتوبكتيريا دامسيلا من نوع البيسيسيدا . كانت نسبة الاصابة الكلية بميكروب الفوتو بكتيريا دامسيلا من نوع الدامسيلا حوالى 26.42 % بينما كانت بمكيروب الفوتو بكتيريا دامسيلا من نوع البيسيسيدا حوالى10.71% . كانت نسبة الاصابة بمرض الفوتو بكتيريا الاعلى فى فصل الصيف والاقل فى فصل الشتاء. أوضحت الصورة الهستوباثولوجية للأسماك المصابة على وجود تليف واضمحلال في الكبد والكلى مع التهابات في الاوعية الدموية وزيادة عدد خلايا الميلانوميكروفاج فى الطحال.