Bacterial infections affecting marine fishes in Egypt

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Abstract: Marine fishes are suffering from continuous depletion due to bacterial pathogens infections triggered by devastating environmental changes at their native aquatic environment. Qarun Lake and Suez Gulf are among the most vulnerable areas. 600 fish samples of Six different fish species; *Epinephelus tuvina*, *Sisganus rivulatus*, and *Dedlechilus labiosus* native to Suez-gulf at Suez governorate; *Mugil capito* , *Solea vulgaris* and *Tilapia zilli* native to Qarun Lake at El-Fayoum governorate were examined throughout the different year seasons. Gram positive and negative fish pathogenic bacteria were isolated from a total of 245 fish sample. Among those samples, the following bacteria were retrieved in the following percentages respectively, 17.55% (Vibrio*. anguillarum),* 16.73% (*Vibrio. alginolyticus*), 15.51% (*Pasteurella. piscicida*), 15.91% (*Pseudomonas. fluorescens)*, 13.46% *(Streptococus. fecalis*), 11.02% (*Aeromans . hydrophila*), 6.12% (*Aeromans. sobria)* and 3.67% were infected with *Staph. aureus*. The *Siganus rivulatus* was the highest infected fish species with a prevalence of 8.33%, while *Mugil capito* was the lowest infected species (5.67 %). The highest total prevalence of bacterial infection was recorded in summer season (40.81%) while the lowest was recorded in winter (15.91%). The aforementioned bacterial isolates were successfully re-isolated from experimentally infected fish. The retrieved isolates were confirmed by semi-automated (API 20 E) and conventional biochemical tests.
[M. Moustafa, Laila. A. Mohamed, M.A. Mahmoud, W.S, Soliman, A.E. Eissa and M.Y. El-gendy. **Bacterial infections affecting marine fishes in Egypt**. Journal of American Science 2010;6(12):1314-1324]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Marine fishes, Bacterial diseases ,Diagnosis, seasonal variation.

1-INTRODUCTION

Fish is considered as one of the main principal sources of the national income in Egypt. Like all animals, marine fish are subjected to numerous diseases, especially bacterial one, in which bacteria play the main role in producing the disease. Diseases are intensified by climatic changes that reflect negatively on the aquatic environment which is a good media for numerous pathogens **Wedemeyer, 1996)**.

The prevalence of bacterial pathogens have been well documented in several cultured and wild freshwater fish species, however; only a few bacteriological surveys on the prevalence of bacterial pathogens responsible for outbreaks in marine fishes **(Alicia., et al 2005).**

On the long run water resources will be the most limiting factor to be considered in aquaculture development in Egypt, especially freshwater aquaculture. Therefore, marine waters are the immediate alternative sources for water needed for mariculture **(Sadek, 2000).** One of the most promosing regions for mariculture in Egypt are The Gulf of Suez and Lake Qarun .

The present work was planned to isolate and identify the most predominant bacterial pathogens in some marine fishes native to both Suez Gulf, at Suez governorate and Lake Qarun at EL-Fayoum province. Further, work aimed to evaluate the seasonal variation of bacterial isolates among the examined fishes.

2. Materials and methods

2.1. Sampling and processing

Six hundred (600) marine fishes of Six different spp. were examined freshly from two localities in Egypt, (Suez Gulf and Lake Qarun). Through the different seasons of the year.

Twenty-five fish of each species were collected and examined seasonally. Fish species, numbers of fish, average body weights and localities are shown in table **(1).** Clinical and P.M examination were carried out using the methods described by **(Buller, 2004).**

Samples from gills, liver, spleen, kidney and external lesions from fishes were cultured on general and selective media; tryptic soy agar and tryptic soy broth (Difco) supplemented with 1.5% (w/v) NaCl, marine agar (Difco), and thiosulphate–citrate–bile

salt–sucrose agar (TCBS, Difco). aeromonas agar base medium supplemented with Ampicillin, pseudomonas agar base medium supplemented with CFC and 2 % NaCl , R-S agar supplemented with novobiocin and 2 % NaCl, and Azide blood agar supplemented with 2 % NaCl . All the inoculated media were incubated at 22 C for 2–5 days. clinically diseased fish, additional samples were taken from external lesions.

2.2. Identification of the isolates

Pure cultures of the isolates were identified by biochemical characterization following the criteria proposed by those described in the Bergey's Manual of Determinative Bacteriology, (**Holt et al., 1993).**Final confirmation of each strain was achieved using the analytical profile index of API20-E system **(Buller, 2004).**

2-3- Experimental infection

70 apparently healthy *O. niloticus* fish, weighting 50 ± 5 gram were selected after the 15 day of the acclimation period for determination of the pathogenicty of the most prevalent bacterial isolates. Fishes were divided into seven groups each contained 10 fish. The inocula prepared for bacterial isolates as I/P injections were prepared according to **(Austin & Austin, 1999).** Fish were observed daily for 15 days. Six groups were consistently inoculated I/P with bacterial suspension of (*Aeromonas hydrophila*, *Pseudomonas fluorescence*, *Vibrio anguillarum*, *Pasteurella piscicida*, *Streptococcus fecalis* and *Staphylococcus aureus*) at dose of 0.2 ml of (3 X 10^7 CFU) while the control group (group 7) were injected I/P with 0.2 ml of sterile tryptic soy broth according to **Hussain (2002)**.

3. Results

3-1Clinical examination

Symptoms detected in the diseased fish included Haemorrhages widely distributed into many parts of the body (Fig 1). Some fishes showed eye exophthalmia and opacity(Fig 2), scale detachment and darkness of the skin. gills were congested((Fig 3). while in others appeared to be pale and swollen with accumulation of excessive amount of mucus. Abdominal distention was observed in some fishes. Vent inflammation as well as prolapse was also seen in many cases.

Necropsy findings of the naturally infected marine fishes exhibited reddish serous fluid in the abdominal cavity. The liver was pale (Fig 4). in some fishes and congested, haemorrhagic with numerous randomly scattered whitish nodules throughout the parenchyma in others (Fig 5).. Spleen was congested and enlarged in some fishes, while in others appeared to be apparently normal. Kidneys were congested and slightly enlarged. In some fishes, white patches were found in kidneys. The intestines were haemorrhagic, inflamed with congestion of their blood vessels. In other samples, the intestines were seen filled with gases.

3.1. Isolation and identification of the bacterial isolates.

Biochemical characteristics of gram –ve bacterial spp. isolated from examined fishes are shown in Table (2).

Results indicated that 245 naturally collected fishes out of 600 were found to be infected with different types of bacteria. The culture results demonstrated that (203) fishes were found to be infected with Gram-negative bacteria and only (42) fishes were infected with Gram-positive bacteria. 17.50 % of the infected fishes were positive for *V. anguillarum,* (16.73%) for *V. alginolyticus*, (15.51%) for *P. piscicida*, (15.91%) for *Ps. fluorescens,* (13.46%) for *S. fecalis*, (11.02%) for *A. hydrophila*, (6.12%) for *A. sobria* and (3.67%) of the surveyed fishes were infected with *Staph. aureus.* The total prevalence of bacterial isolates is illustrated in tables (**3**)

Prevalence of different types of bacterial infections in the different examined fishes is illustrated in table (**3**). The results revealed that *Siganust rivulatus* was the most infected fish spp (50 %), followed by *E. tuvina* (42 %), *S. vulgaris* (41 %), *Tilapia spp.* (40 %), *M.sahlae* (38 %), while *M. capito* was the lowest infected spp (34 %).

The prevalence of bacterial infections for fishes collected from both Suez Gulf and Lake Qarun was illustrated in table (**4**). The results revealed that: The total Prevalence of bacterial infections for fishes collected from the Gulf of Suez (53.06%) was higher than that recorded for those collected from lake Qarun (46.93%).

The results indicated that, the highest total prevalence of bacterial infections among the naturally infected marine fishes was recorded in the summer season (40.81%), followed by autumn (25.71%), then spring (17.14%). On the other hand the minimal prevalence of infection was recorded in winter (15.91%). table (5).

The highest prevalence of bacterial infection among the naturally infected marine fishes in winter season, was recorded for *Ps. fluorescens* (7.75%) while the lowest one (0.40%) was recorded for *V. anguillarum.* on the other hand *P. piscicida*, *S. fecalis and Staph. aureus* were not recorded. For spring season, the highest prevalence of bacterial infection (3.67%) was recorded for *A. sobria* and *V. anguillarum*. while the lowest one (1.22%) was recorded for Ps. fluorescens and S. fecalis. on the other hand Staph. aureus were not detected. The highest prevalence of bacterial infection (9.38%) in summer season was recorded for *V. anguillarum*, while the lowest one (0.40**%**) was recorded for *A. sobria*. The highest prevalence of bacterial infection (5.30%) in autumn season was recorded for *V.alginolyticus* and *P.piscicida* while the lowest one (0.40%was recorded for *A. sobria*. The Prevalence of different types of bacterial infections in the different seasons is illustrated in table (5).

Results of experimental infection .

Mortality patterns in experimentally infected *O. niloticus* with the different bacterial isolates. Table (**6**).

experimentally infected O. niloticus with the different isolates were characterized by septicemic lesions nearly similar to those of naturally infected fish. Experimentally infected fish showed haemorrhagic patches distributed on different parts of the body surfaces. fin and tail rot (fig7). Some fish exhibited typical ulcers (fig8). Some cases showed inflammation of the vent (fig9).Pnecropsy findings showed, congestion of Liver; in some cases the liver was pale. Spleen and kidneys were congested and enlarged. Gall bladder was distended. The gut was haemorrhagic and filled with yellowish content. Serous to serosanguinous fluid in the abdominal cavity was noticed in some cases.

Re-isolation of all the injected bacterial isolates was obtained from dead and sacrificed experimentally infected fish. Moreover, the results of culture and biochemical characteristics of the re-isolated different bacterial isolates revealed the same morphochemical characteristics of the injected bacterial isolate.

Discussion

Septicemic bacterial infections such as vibrios, aeromonads, pseudomonads, photobacteria, streptococci and staphylococci have been observed in several fingerlings, juveniles, adults and brood stocks of some marine fish species **(Alicia *et al.*, 2005** and **Samuelsson *et al.*, 2006)**.

In regards to bacterial pathogens that have been isolated, results came in this study revealed that Gram-negative bacteria prevailed the Gram-positive ones with *Vibrio anguillarum*, *Vibrio alginolyticus*, *Pasteurella piscicida (photobacterium damsella subspp piscicida), Pseudomonas fluorescens, Streptococcus fecalis, Aeromonas hydrophila, Aeromonas sobria* and *Staphylococcus aureus* were the most common isolated spp. These results are supported by those reported by **Zorrilla *et al.* (2003)** who declared that the main pathogenic microorganisms isolated from diseased gilt-head seabream in the marine water at south western Spain were; *Vibrio* spp, *Pseudomonas* spp, *P. piscicida*, *Flavobacteria maritimus*, *Aeromonas* spp and Gram positive bacteria were also isolated but in low prevalence.

Table (1) Number and Weight

	Locality	Number	weight
E. tuvina	Suez Gulf	100	95±20
S. rivulatus	Suez Gulf	100	70±10
M. sahla	Suez Gulf	100	50±5
S. vulgaris	Lake Qarun	100	75±15
M capito.	Lake Qarun	100	85±10
Tilapia zilli	Lake Qarun	100	655

In concern to the total prevalence of bacterial infections in the naturally infected marine fishes at the present study (40.83 %) which may appear to be lower than those reported by some authors for the freshwater fishes as **Soliman (1999)** who noticed that the total bacterial prevalence was (65%). This difference might be due to the unfavorable effect of the salinity of marine water on the viability of bacterial pathogens.

In regard to the localities of isolation, results revealed that the prevalence of bacterial infections was higher (53.06%) in fishes collected from the Gulf of Suez than that (46.93%) recorded for those collected from Lake Qarun. the prevalence of Lake Qarun may be explaind as the lake Qarun is the largest reservoir of agricultural waste water drainage of Fayoum province as well as the drainage from fish farms established around the lake (**Mansour & Sidky, 2003**).

The high prevalence of isolation recorded from the Gulf of Suez may in part be attributed to the stress induced by high crude oil pollution at the Gulf water which is maintained by the low water

flow, low water exchange rates and daily crowded ship traffic crossing the gulf as well as industrial effluents from oil refineries. All these factors are compromising to the fish immune system ending up with marked increase in the magnitude of bacterial infections.

The study declared that marine fish can succumb MAS, as supported by **Larsen & Jensen (1977)** who isolated *A. hydrophila* from ulcer disease in Cod, *Gadus morhua* L., a strictly marine fish. Authors added that motile aeromonas group especially *A. hydrophila* is considered one of the most important pathogen responsible for haemorrhagic septicemia in a wide variety of marine water fish. Moreover, **Vethaak (1992**) isolated *A. hydrophila* from ulcers, lesions, and blood of ulcerated European flounder.

The results pointed out that the highest prevalence of *A. hydrophila* was recorded in winter season (5.71%) followed by spring (2.85%), in summer and autumn the results were the same (1.22%). These results supported by **Pathak *et al.* (1988)** who suggested that the highest isolation rates of *A. hydrophila* occurred during the late winter followed by a progressive decline in density during the summer and monsoon seasons. Moreover, **Popovic *et al.* (2000)** mentioned that there was clear seasonality in the prevalence of *A. hydrophila* as there were no isolates recovered in the summer months. On contrast, **Meyer (1970)** reported that the most epizootics of motile aeromonase were generally reported in spring and early summer.

In regards to the seasonal prevalence of *A. sobria*, our study recorded that the highest prevalence e of *A. sobria* septicemia was recorded during the spring (3.67%) followed by winter (1.63%) while the minimal prevalence e of infection (0.40%) was recorded during the summer and autumn. These results are in concordance with those obtained by **Wahli *et al.* (2005)** who noticed that mortalities due to *A. sobria* peaked during the low water temperatures of winter time and reached levels of 1% of the total fish on the farm per day. On contrary, the results are not in concordance with those obtained by **Kooj *et al.* (1988**) who demonstrated that the highest prevalence of Aeromonads in marine water have been obtained in the warmer months**.**

The pathogenicty of *A. hydrophila* for experimentally infected *Oeorchromis niloticus* with *A. hydrohila* may be attributed to the production of toxins and extracellular enzymes and lethal toxins including, proteases, amylases , lipases, enterotoxin, cytotoxins and haemolysin **(Saavedra *et al.*, 2004).**

In regards to the total prevalence of pseudomonas septicemia, the study recorded that (15.91%) of infected fish were positive for pseudomonas infection, These results are in concordance with those obtained by **Hussain (2002)** who reported that (15.27 %) of naturally infected marine fishes were positive for *Ps, flourescens* septicemia**.** on contrast, the results are lower than those reported by **Khan *et al.* (1981)** who reported that *Pseudomonas* spp accounted for (72 %) of the mortalities recorded in captive Atlantic cod, *Gadus morhus* L associated with fin rot disease.

The highest prevalence of *Ps. fluorescens* septicemia was recorded during the winter season (7.75%) followed by autumn (4.48%) summer (2.44%) and spring (1.22%), this reveals that *Ps. fluorescens* has certain affinity to low temperature for propagation and wide spreading infection **(El-Moghazy, 2004)**. The results are supported by **Golomazou *et al.* (2006)** who demonstrated that Pseudomonads were isolated mainly in cold months of winter. On contrary, the results are not in concordance with those obtained by **Hoda *et al.* (1999)** who revealed that the prevalence of pseudomonads was lower in winter than summer. This may be also attributed to amplified activity of proteinases produced by pseudomonads at the low temperature (10-25°C) that play the significant role in the pathogenesis of pseudomonas septicemia **(Hoshino *et al.*, 1997)**.

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

In regards to the total prevalence of vibriosis recorded (34.28%), this result are in accordance with those reported by **Khan *et al.* (1981)** who recorded that vibrios accounted for (28%) of mortalities in captive Atlantic cod, *Gadus morhus* L associated with fin rot disease. the results are lower than those recorded by **Zorrilla *et al.* (2003)** who recorded that the prevalence of vibrios among diseased gilthead sea bream, *Sparus aurata* L in southwestern Spain was (69.90%).

V. anguillarum in this study was the *Vibrio* spp most frequently isolated as (17.55%) of the infected cases were positive for *V. anguillarum*. this is in accordance with **Zorrilla *et al.* (2003).** On the other hand *V. alginolyticus* was the cause of (16.73%) of the recorded cases. this high prevalence *of V. alginolyticus* indicates its importance in mariculture as supported by **Zhu *et al*. (2000)** who suggested that *V. alginolyticus* is one of the key diseases that have made great harm to a wide variety of marine fishes.

The highest prevalence e of *V. anguillarum* infection was recorded during the summer (9.38%), followed by autumn (4.08%), spring (3.67%), and only (0.4%) were recorded in winter. On the other hand the highest prevalence e of *V. alginolyticus* infection was recorded in summer (8.57%), autumn (5.30%), spring (2.04%) and (0.81%) in winter. The results of the seasonal prevalence of *Vibrio* spp are in concordance with those reported by **Roberts (2001)** who demonstrated that in wild, vibriosis normally occurs in fish in late summer when the temperatures are high. On the other hand, **(Golomazou *et al.* (2006**) reported that *V. alginolyticus* were not associated with a particular season.

The pathogenicty of *V. anguillarum* for experimentally infected *O. niloticus* may be attributed to the effect of the lethal and toxic effect of the extracellular toxins and enzymes produced by the bacterium **(Nottage & Birkbeck, 1987)**.

In concern to the total prevalence of *P. piscicida* in this study recorded that (15.51%) of diseased fish were positive for *P. piscicida*. The results are higher than those recorded by **Balebona *et al.* (1998)** who declared that (6.7%) of diseased gilt-head sea bream, *Sparus aurata* L. in southwestern Spain were infected with *P. piscicida*. On the other hand, our results are lower than those recorded by **Athanassopoulou *et al.* (1999)** who recorded that the prevalence of *P. piscicida* in diseased Cuvier, *Puntazzo puntazzo* L. collected from marine aquaculture systems in Greece was (80%).

Seasonally, the highest prevalence e of *P. piscicida* in our study 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. These results are in concordance with those reported by **Magarinos *et al.* (1996) who** declared that *P. piscicida* has certain affinity to the high water temperature inducing fatal disease in fish but only when the water is warm and when water quality is low. On the other hand, **Mladineo *et al.* (2006)** suggested that temperature had not a strong influence on the course of pasteurellosis.

In regard to the pathogenicty of *P. piscicida* for experimentally injected fishes may be attributed to the effect of its toxic and harmful effect of the extracellular products produced by *P. piscicida* that possessed strong phospholipase, cytotoxic, and haemolytic activities (**Nakai *et al.*, 1992**).

In regards to the total prevalence of streptococcal septicemia. The present study recorded that (13.46 %) of infected fish were positive for streptococcal infection. These results higher than those recorded by **Zorrilla *et al.* (2003)** who recorded that 7% of bacterial infection affecting cultured gilthead sea bream, *Sparus aurata* L. was attributed to Gram-positive bacteria. **Hussain (2002)** recorded that (6.25 %) of naturally infected marine fish were positive for streptococcal septicemia.

In regards to the seasonal prevalence of streptococcal septicemia, the highest prevalence of the streptococcal infection was recorded in the summer season (6.16%) followed by autumn (4.08%) and spring (1.22 %) on the other hand it was not recorded during the winter. These results are in concordance with those obtained by **Varvarigos (1997)** who revealed that *Streptococcus* spp cause septicemia to all farmed species mainly during late spring and early summer when sea water temperatures are high.

In regards to the experimental infection *of O. niloticus* 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 regards to the total prevalence of *Staph. aureus*, the present study recorded that (3.67%) of infected fish were positive for Staphylococcal infection. These results were lower than those recorded by **Athanassopoulou *et al.* (1999)** who recoded that the total prevalence of *Staph. epidermidis* among diseased *Puntazzo puntazzo* in marine aquaculture systems in Greece was (10 %). Moreover, **Zorrilla *et al.* (2003)** recorded that (7%) of bacterial infections affecting gilthead sea bream

Sparus aurata L. were attributed to Gram-positive bacteria**.**

Seasonally the highest prevalence of Staphylococcal infection was recorded in the present study in the summer season (2.85%) followed by autumn (0.81%) while it was not recorded in spring nor in winter**.** These results are supported by **Varvarigos (2001)** who declared that *Staphylococcus* spp causing septicemia to all farmed species mainly during late spring and early summer during the high temperature of sea water. This may be explained by the high organic matters in water and the stress induced by the high temperature and hence the decrease in DO.

In regards to the experimental infection of *O. niloticus* with *Staph. aureus***.** The results are in accordance with **Huang (2000)** who indicates that staphylococci can be a possible cause of mortalities and losses among fish.

Table (2) Variable

	A. hydrophila	*A. sobria*	*Ps. fluorescens*	*V. anguillarum*	*V. alginolyticus*	*P. piscicida*
B –Galactosidase production (OPNG)	+	+	-	+	-	-
Arginine dihydrolase production (ADH)	+	+	+	+	-	+
Lysine decarboxylase production(LDC)	-	+	-	-	+	-
Ornithine decarboxylase production(ODC)	-	-	-	-	-	-
Citrate utilization (CIT)	-	Variable	-	Variable	+	-
H2S production(H2S)	-	-	-	-	-	-
Urease production(URE)	-	-	-	-	-	-
Tryptophane deaminase production (TDA)	-	-	-	-	-	-
Indole production(IND)	+	+	-	+	+	-
Acetoin production(VP)	+	+	+	+	Variable	+
Gelatinase production(CEL)	+	+	-	+	+	-
Acid from glucose(GLU)	+	+	Variable	+	+	+
Acid from manitol(MAN)	+	+	-	+	+	-
Acid from inositol(INO)	-	-	-	-	-	-
Acid from Sorbitol(SOR)	-	-	-	+	-	-
Acid from rhamnose(RHA)	+	-	-	-	-	-
Acid from sucrose(SAC)	+	+	-	+	+	-
Acid from from melibiose(MEL)	-	-	V	-	-	-
Acid from amygdalin (AMY)	Variable	-	-	-	Variable	-
Acid from arabinose (ARA)	Variable	-	Variable	Variable	-	-
Cytochrome oxidase prod(OX)	+	+	+	+	+	+

Table (**3**): Prevalence of bacterial infections in the examined marine fishes

Type of M.O / Fish spp	No. Of Exam fish	NO inf fish	*A. hydrophila*		*A. sobria*		*Ps.fluorescens*		*V. anguillarum*		*V.alginolyticus*		*P.piscicida*		*S. fecalis*		*Staph. aureus*	
			No.inf	%	No. inf	%	No. inf	%	No. inf	%	No. inf	%	No inf	%	No. Inf	%	No.inf	%
E. tuvina	100	42	2	4.76	4	9.52	7	16.66	10	23.8	8	19.04	6	14.28%	2	4.76%	3	7.14%
S. rivulatus	100	50	4	8	5	10	6	12	7	14	9	18	10	20%	8	16%	1	2%
S. vulgaris	100	41	6	14.63	0	0	5	12.19	8	19.51	7	17.07	4	9.75	9	21.95%	2	4.87%
M capito.	100	34	3	8.82	2	5.88	4	11.76	5	14.7	3	8.82	9	26.47	6	17.64%	2	5.88%
M. sahlae	100	38	7	18.42	1	2.63	8	21.05	6	15.78	10	26.31	3	7.89%	3	7.89%	0	0
Tilapia zilli	100	40	5	12.5	3	7.5	9	22.5	7	17.5	4	10	6	15	5	12.5%	1	2.5%
Total	600	245	27	11.02	15	6.12	39	15.91	43	17.55	41	16.73	38	15.51%	33	13.46%	9	3.67%

•Percentage was calculated according to the total number of infected fish.

Table (4) Prevalence of bacterial infections in Lake Qarun and Suez Gulf

Locality / M.O	Lake Qarun	Prevalence %	Suez Gulf	Prevalence %
A. hydrophila	14	5.71	13	5.30
A. sobria	5	2.04	10	4.08
Ps. fluorescens	18	7.34	21	8.57
V. anguillarum	20	8.16	23	9.38
V. alginolyticus	14	5.71	27	11.02
P. piscicida	19	7.75	19	7.75
S. fecalis	20	8.16	13	5.30
Staph. aureas	5	2.04	4	1.63
Total	115	46.93	130	53.06

Table (5): collective seasonal prevalence of bacterial infections in the examined marine fishes

Type. of M.o / season	*A.hydrophila*		*A.sobria*		*Ps.fluorescens*		*V.anguillarum*		*V.alginolyticus*		*P.piscicida*		*S.fecalis*		*Staph.aureus*		Total
	No inf	%	No. inf	%	No inf	%	No inf	%	No inf	%	No inf	%	No inf	%	No inf	%	
Winter	14	5.71	4	1.63	19	7.75	1	0.40	2	0.81	0	0	0	0	0	0	15.91
Spring	7	2.85	9	4.08	3	1.22	9	3.67	5	2.04	6	2.44	3	1.22	0	0	17.55
Summer	3	1.22%	1	0.40	6	2.44	23	9.38	21	8.57	19	7.75	20	6.16	7	2.85	40.81
Autumn	3	1.22	1	0.40	11	4.48	10	4.08	13	5.30	13	5.30	10	4.08	2	0.81	25.71
Total	27	11.02	15	6.12	39	15.91	43	17.55	41	16.73	38	15.51	33	13.46	9	3.67	

Table (6): Mortality patterns of experimentally infected *O. niloticus* with the different bacterial isolates.

Bacterial isolates	No of mortality /day												Mortality. (%)
	1	2	3	4	5	6	7	8	9	10	11	2-15	
A.*hydrophila*	-	1	-	2	-	-	2	1	-	1	1	-	80
Ps. fluorescens	-	2	-	-	1	1	2	1	1	-	1	1	100
V. anguillarum	1	1	2	1	2	-	-	1	-	1	-	1	100
P. piscicida	2	0	-	1	1	-	-	2	-	-	1	1	80
S. fecalis	1	-	1	-	-	1	1	1	1	2	-	1	90
Staph. aureas	-	1	-	2	-	-	1	1	-	-	2	-	70
Control	-	-	-	-	-	-	-	-	-	-	-	-	0

N.B. The dose of bacteria inoculated per fish were 0.2 ml of 3 X 10^7 CFU Number of I/P injected fishes per each group were 10.

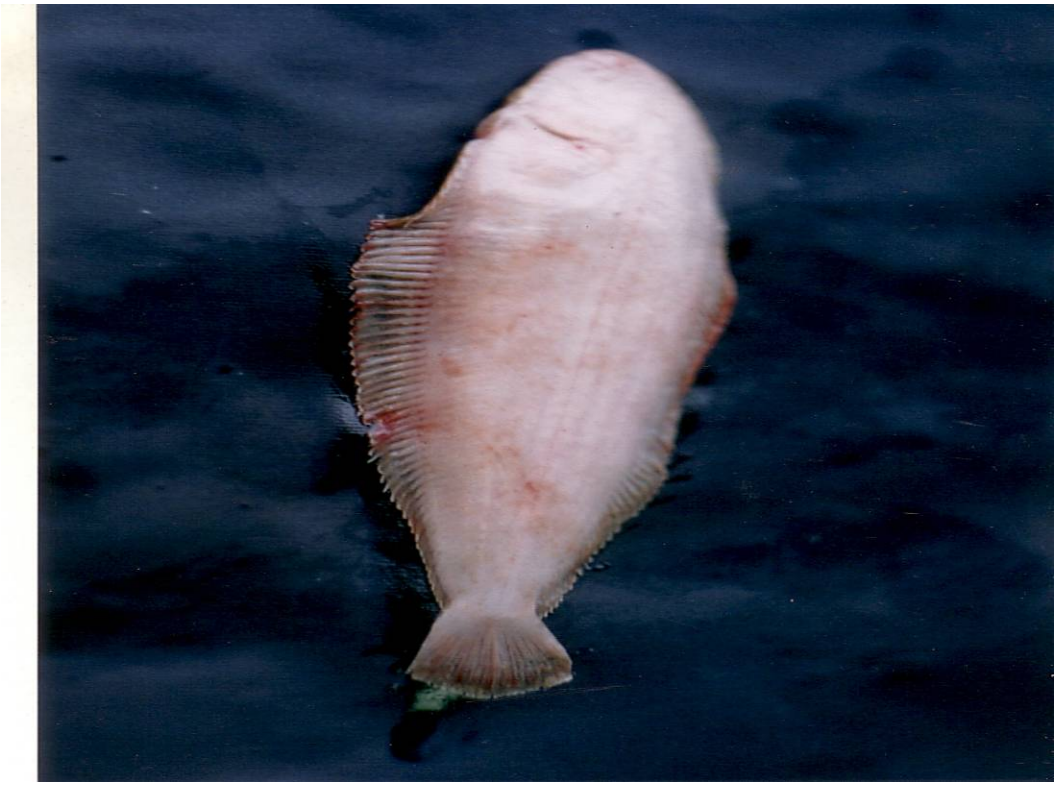

Fig (1) Fish

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10/10/2010