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Investigations into the Potential Causes of Mass Kills in Mari-Cultured Gilthead Sea Bream (*Sparus aurata*) at Northern Egypt.

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ABSTRACT

Infectious agents are potential causes of mass kills among cultured fish populations worldwide. The study was carried out through the course of two outbreaks targeted gilthead sea bream, *Sparus aurata,* cultured in two models of mari-culture systems at northern Egypt, floating net-cages and earthen ponds during 2012. Affected fish farms showed unique threatening seasonal episodes of high mortalities. Disease problems recorded during this period were investigated, focusing on phenotypic, molecular characterization and histopathological alterations. Total number of 100 moribund and ⁄ or freshly dead fish samples showing picture of clinical septicemia were inspected through the course of these epidemics. *Vibrio alginolyticus* was the most predominant bacterial pathogen 28.57 % followed by *streptococcus agalactiae* 24.48 %, *Pseudomonas fluorescens* 18.36 % and *Vibrio vulnificus* 16.32 %. Infections attributed to *Tenacibaculum maritimum* recorded the lowest rate 12.24 %. PCR yielded specific amplicons identical for the size of the target gene sequence characteristic for each bacterial isolate. Circulatory, degenerative, proliferative and necrotic changes were evident in histopathological examination. Disease conditions were exacerbated with the existence of unfavorable water quality measures.

Keywords: Mass kills, Gilthead sea bream, Bacterial pathogens, PCR, Water quality.

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INTRODUCTION

The fisheries collapse and the worldwide escalating demand for animal protein have led aquaculture to become the best substitute for wild fisheries. Unfortunately, the majority of the world's fishing areas have reached their maximum sustainable yield while additional supplies for seafood are still progressively required [1]. Interestingly, mariculture sector has great potentials for enormous expanding in Egypt. Several incentives and necessities significantly motivate national authorities to develop this industry including, extensive available aquatic marine resources, obvious constraints to growing freshwater aquaculture, rapid growth of population, stagnation of fish catch and the eager interest of the private sector [2, 3, 4, 5].

Contemporary, disease outbreaks are still the major dilemma affecting the economic structure of aquaculture causing unpredictable colossal mortalities worldwide [1, 5, 6]. Globally, bacterial diseases devastating cultured fish represent a stumbling stone on the expansion and realization of full aquaculture potentials. It constitutes the most important sources of disease problems in all various types of fish production to an extent that exceeds all other causes combined. Virtually, in each type of aquaculture and for every fish species, specific bacterial pathogens are responsible for serious mortalities [7, 8]. Numerous bacterial pathogens have been involved in several destructive epizootics in various mariculture facilities worldwide led by, Vibrios, Pseudomonads, *Tenacibaculum maritimum,* Streptococcus, *Photobacterium damselae* spp. *piscicida* and some Aeromonads [1, 5, 9, 10, 11].

The pathogenesis of bacterial diseases affecting aquatic animals is typically a multi-factorial. Variable factors related to the environment, host and the pathogen should work synergistically to delineate the nature of the triggered course of infection. Array of environmental factors like, unfavorable dissolved oxygen, nitrogenous waste products and presence of some noxious metals have long been incriminated in triggering numerous disease outbreaks particularly in intensively cultured fishes [8, 12, 13, 14].

Gilthead sea bream, *Sparus aurata,* is the predominant marine fish species cultured in the Mediterranean [1, 9]. Its high commercial values attracted great interests [6]. Regionally, there has been a scarcity in literatures discussing its health problems. The current study was originally planned to investigate the bacterial causes stand behind sea bream mass mortalities detected in the Northern Egypt during 2012. Moreover, our investigation aimed to assess environmental measures relevant to these outbreaks.

MATERIALS AND METHODS

Fish and rearing conditions

The study was carried out during the course of two disease outbreaks targeted gilthead sea bream, *Sparus aurata,* cultured in two models of mari-culture systems, floating net-cages and earthen ponds both located in the northern of Egypt during 2012. Winter episode attacked the floating net-cages sited in Marriott west Alexandria province while summer epidemics hit the earthen ponds within Muthallath El-Deeba region at Damietta.

Colossal fish losses were terrible. Mortalities approached 90 % in Marriott floating net-cages and 100 % in Damietta (Figs.1a, b). Total number of one hundred fish specimens, 50 moribund and ⁄ or freshly dead fish were examined through every outbreak for the presence of the etiological agents of diseases. The body weights of fish samples obtained from Alexandria fish farm ranged between 80-200 gr while those collected from Damietta province were 150-300 gr.

Bacteriological examination

Sampling and processing

Samples were obtained from gills, liver, kidney, spleen and brain under complete aseptic condition for bacteriological examination. Loopfuls from the above mentioned tissues were cultured onto marine agar, tryptic soy agar supplemented with 1.5 % (w/v) NaCl, thiosulphate citrate bile salt sucrose agar (TCBS), pseudomonas agar base medium supplemented with CFC (cetrimide, fusidin and cephaloridine supplement and 1.5% (w/v) NaCl, aeromonas agar base supplemented with ampicillin and 1.5 (w/v) % NaCl, and sheep

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blood agar supplemented with 1.5 % (w/v) NaCl. The inoculated plates were incubated at 25 °C for 18-48 hours. Representative numbers of the different colonial types detected on the media were collected from plates and streaked on TSA supplement and 1.5% (w/v) NaCl plates for purity and identification.

Identification of isolates

Identification of pure bacterial isolates was performed by studying their morphological and biochemical characteristics using traditional as well as commercial API 20 E and API strept kits following the criteria described in [15].

Molecular identification

DNA extraction

DNA from bacterial colonies was extracted using Bacterial Genomic DNA extraction Kit (Fermentas, Vilnius, Lithuania) following the manufacturer's instructions. Briefly, bacterial colonies were grown into trypticase soy broth (Oxoid) containing 1.5% NaCl and incubated at 25 °C for 24 hr. The broth cultures were then transferred into 1.5 ml tubes and centrifuged for 5 min at 13,000 g at room temperature the resulting pellet was used for nucleic acid extraction. Concentration and purity of DNA samples were measured using spectrophotometer (Konica Minolta, Tokyo, Japan) then the eluted DNA was used as a template in the PCR assay.

Oligonucleotide primers and PCR:

The sequence of each set of primers specific for every bacterial isolate was inquired from previous studies Table 1. PCR were performed in 25 µl volumes consisting of 5 µM mastermix, 100 ng of genomic DNA template and 1 pMol of each specific primer for different bacterial isolates.

The thermal cycling profiles of PCR reactions for the different bacterial isolates (*V. alginolyticus, P. fluorescens, T. maritimum* and *S. agalactiae*) were as follow: Initial denaturation for 5 minutes at 94 °C. Further cycling for 35 cycles at 94 °C for 1 minute, 50 °C for 1 minute, 72 °C for 1 minute and a final extension at 72 °C for 10 minutes. Differently, *V. vulnificus* isolates were initially denaturated for 5 minutes at 94 °C. The reaction mixtures were cycled 30 cycles at 94 °C for 30 second, 54 °C for 30 second, 72 °C for 30 second and a final extension at 72 °C for 10 minutes Table 1.

Table 1: Target genes, oligonucleotide primers sequence and thermal profiles for PCR reactions

Bacterial species	Target gene	Sequence	Thermal profile of cycling program				PCR Amplicon base pair (bp)	Reference
			No. of cycles	Den.	Ann	Ext.		
V. alginolyticus	Collagenase	Forward: 5′ CGAGTACAGTCACTTGAAAGCC 3′ Revers: 3′ CACAACAGAACTCGCGTTACC 5′	35	94°C\1 m	50°C\1 m	72°C\10 m	737	[16]
V.vulnificus	16S rDNA	Forward: 5′ TCTAGCGGAGACGCTGGA 3′ Revers: 3′ GCTCACTTTCGCAAGTTGGCC 5′	30	94°C\30 S	54°C\30 S	72°C\10 m	273	[17]
P. fluorescens	16S rDNA	Forward: 5′ TGCATTCAAAACTGACTG 3′ Revers: 3′ AATCACACCGTGGTAACCG 5′	35	94°C\1 m	50°C\1 m	72°C\10 m	850	[18]
T. maritimum	16S rRNA	Forward: 5′ TGTAGCTTGCTACAGATGA 3′ Revers: 3′AAATACCTACTCGTAGGTACG 5′	35	94°C\1 m	50°C\1 m	72°C\10 m	400	[19]
S. agalactiae	16S-23S intergenic region	Forward: 5′ CCACGATCTAGAAATAGATTG 3′ Revers: 3′ TGCCAAGGCATCCACC 5′	35	94°C\1 m	50°C\1 m	72°C\10 m	150	[20]

The amplification products were analyzed by running through 1.0 % agrose gel electrophoresis, stained with ethidium bromide and visualized under UV trans-illumination system. DNA Ladder (Fermentas, Vilnius, Lithuania), consisting of DNA fragments ranging in size from 100 bp to 1500 bp was used as a molecular weight marker.

Water quality examination

Water samples were obtained from different locations within each aquaculture facility in sterile plastic bottles and stored according to standard methods described by [21, 22] then Physico-chemically analyzed. Temperature, dissolved Oxygen (DO), pH and salinity were measured on spot in fish culture systems while un-ionized ammonia (NH_3), nitrites, nitrates and heavy metals (iron, copper, zinc, cobalt, cadmium and lead) were measured in laboratory according to methods adopted from [22].

Histopathological studies

Tissue specimens for histopathological techniques were taken from gills, hepatopancreas, spleen, kidney and brain of infected fish. Samples were preserved in 10 % buffered formalin, then dehydrated by a series of upgraded ethanol solution, embedded in paraffin, and sectioned at 5 µm thick. Tissue sections were routinely processed and stained with Hematoxylin and Eosin (H & E) then examined by light microscopy according to [23].

RESULTS

Clinical examination

High mortalities noticed in the investigated farms were striking. Haemorrhagic patches extensively distributed on the external body surfaces. Skin darkness, scales detachment, erosions, fin and tail rot were also noticed occasionally. The gills were congested with accumulation of excessive amounts of mucus. Some fish demonstrated abdominal distention, ascites and protrusion of the anal opening. Moreover, exophthalmia as well as ocular haemorrhages were infrequently noticed in some cases (Fig.1c). Necropsy findings revealed serosanguinous fluid in the abdominal cavity. Additionally, the liver was congested and haemorrhagic (Fig. 1d). In some other cases it was pale. The kidney and spleen were congested and enlarged. The majority of investigated fishes showed gall bladder distention.

Fig.1. (a, b) High fish mortalities during summer episode attacked earthen ponds at Muthalath El-Deeba Damietta province. (c) Naturally infected sea bream showing haemorrhages on the external body surface. (d) Naturally infected sea bream showing congestion and enlargement of liver

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Isolation and identification of field bacterial isolates

The phenotypic characteristics of loopfuls obtained from different fish organs revealed a total number of forty nine bacterial isolates retrieved from thirty seven infected fish. Most of the isolates were Gram-negative 75.51 % represented as; *Vibrio alginolyticus* 28.57 %, *Pseudomonas fluorescens* 18.36 %, *Vibrio vuln*ificus 16.32 % and *Tenicibaculum maritimum* 12.24 %. On the other hand *Streptococcus agalactiae* was the sole Gram-positive bacterial pathogens involved in such outbreaks 24.48 %. Full phenotypic and biochemical characteristics of recovered isolates are illustrated in tables (2, 3) and the frequency of isolation of the different bacterial species is summarized in table (4).

Table 2: Phenotypic and biochemical characteristics of gram- negative bacterial isolates retrieved from naturally infected sea bream

Item	*V. alginolyticus*	*V. vulnificus*	*P. fluorescens*	*T. maritimum*
Colony characters Onto marine agar and TSA	smooth, convex, white to creamy, round, raised and shiny	smooth, convex, white to creamy, round, raised and shiny	circular, smooth, convex and yellow-green and glistening	Pale yellow flat with uneven edges, Spreading and strongly adherent to agar surface. Absorb Congo red with red-colour development of colonies
Colony characters Onto TCBS	yellow-colored	green-colored	-Ve	-Ve
Colony characters Onto Pseudomonas agar base medium	-Ve	-Ve	yellow-green colored colonies	-Ve
Microscopical characters	Gram -Ve, straight to slightly curved rods.	Gram -Ve, straight to slightly curved rods.	Gram –Ve, short bacilli.	Gram –Ve, long, slender, pleomorphic bacilli
O/129 sensitivity (150 mg)	+Ve	+Ve	-Ve	-Ve
Motility	Motile	Motile	Motile	Motile
Biochemical characters				
B –Galactosidase production (OPNG)	-	-	-	-
Arginine dihydrolase production (ADH)	-	-	+	-
Lysine decarboxylase production(LDC)	+	Variable	-	-
Ornithine decarboxylase production(ODC)	-	Variable	-	-
Citrate utilization (CIT)	+	-	Variable	-
H2S production(H2S)	-	-	-	-
Urease production(URE)	Variable	-	-	-
Tryptophane deaminase production (TDA)	Variable	-	-	-
Indole production(IND)	+	Variable	-	-
Acetoin production(VP)	-	-	+	-
Gelatinase production(CEL)	Variable	Variable	-	+
Acid from glucose(GLU)	+	Variable	-	-
Acid from manitol(MAN)	+	Variable	-	-
Acid from inositol(INO)	-	-	-	-
Acid from Sorbitol(SOR)	Variable	-	-	-
Acid from rhamnose(RHA)	-	-	-	-
Acid from sucrose(SAC)	+	-	-	-
Acid from melibiose(MEL)	-	-	Variable	-
Acid from amygdalin (AMY)	-	+	-	-
Acid from arabinose (ARA)	-	-	-	-
Cytochrome oxidase prod(OX)	+	+	+	+

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Table 3: Phenotypic and biochemical characteristics of *S. agalactiae* isolates retrieved from naturally infected sea bream

Colony characters	**Onto marine and TSAS* agar**	**Small pinpoint whitish round and slightly raised**
	Onto Blood agar	β Haemolysis
Microscopical Examination	Gram- stain and cell form	Gram positive cocci arranged in short chains
Biochemical characteristics obtained from API 20 Strept	Voges-Proskauer (VP)	+
	Hippurate (hip)	+
	Aesculin (ESC)	-
	pyrrolidonyl arylamidase (PYRA)	-
	α galactosidase (α-GAl)	-
	β glucuronidase (β GUR)	Variable
	β galactosidase (β GAL)	-
	Alkaline phosphatase (PAL)	+
	leucine arylamidase (LAP)	+
	Arginine dihydrolase (ADH)	+
	Ribose (RIB)	+
	Arabinose (ARA)	-
	Mannitol (MAN)	-
	Sorbitol (SOR)	-
	Lactose (LAC)	Variable
	Trehalose (TRE)	Variable
	Inulin (INU)	-
	Raffinose (RAF)	-
	Amygdalin (AMD)	Variable
	Glycogen (GLYG)	-

*TSAS: Tryptic Soy Agar Supplemented with NaCl

Table 4: Prevalence of bacterial infections in sea bream during outbreaks

	winter outbreak		summer outbreak		Total	
	No. of isolates	Seasonal prevalence	No. of isolates	Seasonal prevalence	No. of isolates	Total prevalence
V. alginolyticus	4	8.16	10	20.4	14	28.57
V. vulnificus	2	4.08	6	12.24	8	16.32
P. fluorescens	9	18.36	0	0	9	18.36
T. maritimum	1	2.04	5	10.20	6	12.24
S. agalactiae	0	0	12	24.48	12	24.48
Total number of isolates	16	32.65	33	67.34	49	100

Percentage was calculated according to the total number of retrieved isolates (49)

Molecular identification

All PCR tests yielded definite amplicons identical for the size of the target gene sequence characteristic for each bacterial isolate (Fig. 2). In particular, *P. fluorescens* primers amplified a fragment of 850 bp. *V. alginolyticus* as well as *V.vulnificus* primers produced specific amplicons 737-bp and 273 bp respectively in all strains tested. Furthermore, *T. maritimum* primers yielded specific 400 bp amplicons. On the other hand, *S. agalactiae* primers produced a specific 150-bp fragment.

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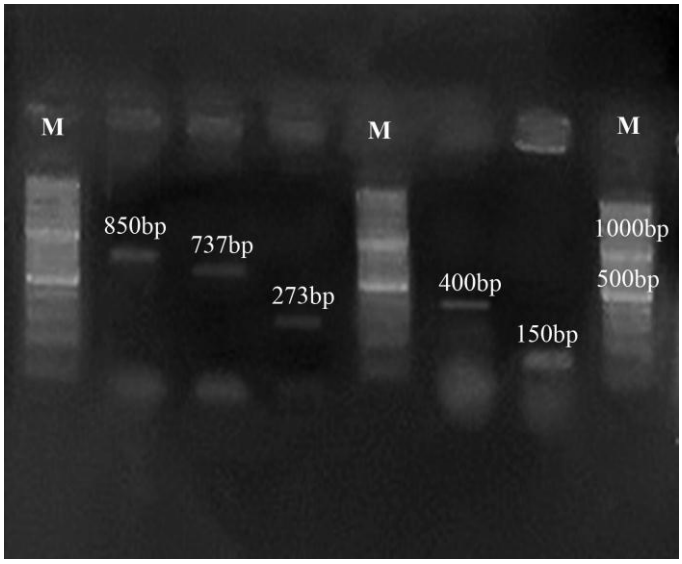

Figure 2: Identification of bacterial strains by PCR

Water quality measures

Disease conditions were linked to existence of stressful environmental circumstances in investigated fish farms table (5). Water temperature approached values improper for cultured sea bream, 9.5°C and 31.5°C in Alexandria and Damietta farms respectively. Dissolved oxygen levels were lower than the optimal values 4.1 and 3.6. Farm waters were slightly alkaline as the pH values reached 7.3 and 8.2 respectively. On the other hand, salinity was within the expected rang 31 ‰ and 37 ‰ respectively.

Table 5: Water quality measures in examined fish farms during outbreaks

	Season	Temperature °C	Salinity ‰	D.O. mg/l	pH	NH_3 mg/l	NO_2 mg/l	NO_3 mg/l	Heavy metals (mg/l)					
									Cd	Co	Zn	Pb	Fe	Cu
Sampling Site 1	Winter	9.5	31	4.1	7.3	0.9	0.85	1.1	ND	0.058	0.054	0.091	0.721	0.052
Sampling Site 2	Summer	31.5	37	3.6	8.2	1.2	0.96	1.48	ND	0.115	0.0781	0.0359	1.03	0.046

Sampling Site 1: Floating Net cage El-hwarria, Marriott- Alexandria, Sampling Site 2: Fish ponds Muthallath El deeba – Damietta. (ND): not detected.

*Average of readings was calculated in all measured parameters.

Results also demonstrated that nitrogenous waste products may be significantly accused for predisposing fish to surrender these outbreaks. The values in Alexandria farm recorded for NH_3, NO_2 and NO_3 were far from the optimum recommended levels 0.9 mg/l, 0.85 mg/l and 1.1 mg/l respectively. On the other hand, the same measures in Damietta farm were also more noxious 1.2 mg/l, 0.96 mg/l and 1.48 mg/l in sequential. Achieved results also indicated the possible involvement of some heavy metals in rendering fishes more susceptible to such outbreaks. Levels of some detected metals were higher than the marine high reliability trigger value recommended. The measures recorded for Cobalt, zinc, lead, iron and copper recorded in Alexandria farm were, 0.058 mg/l, 0.054 mg/l, 0.091 mg/l, 0.721 mg/l and 0.052 mg/l respectively. On the other hand the same measures noticed in Damietta farm were, 0.115 mg/l, 0.0781 mg/l, 0.0359 mg/l, 1.03 mg/l and 0.046 mg/l respectively while cadmium was not detected in both farms.

Histopathological lesions

Circulatory, degenerative, proliferative and necrotic changes were evident. Moreover, some bacterial agents were microscopically detected in histological sections (Fig. 3).

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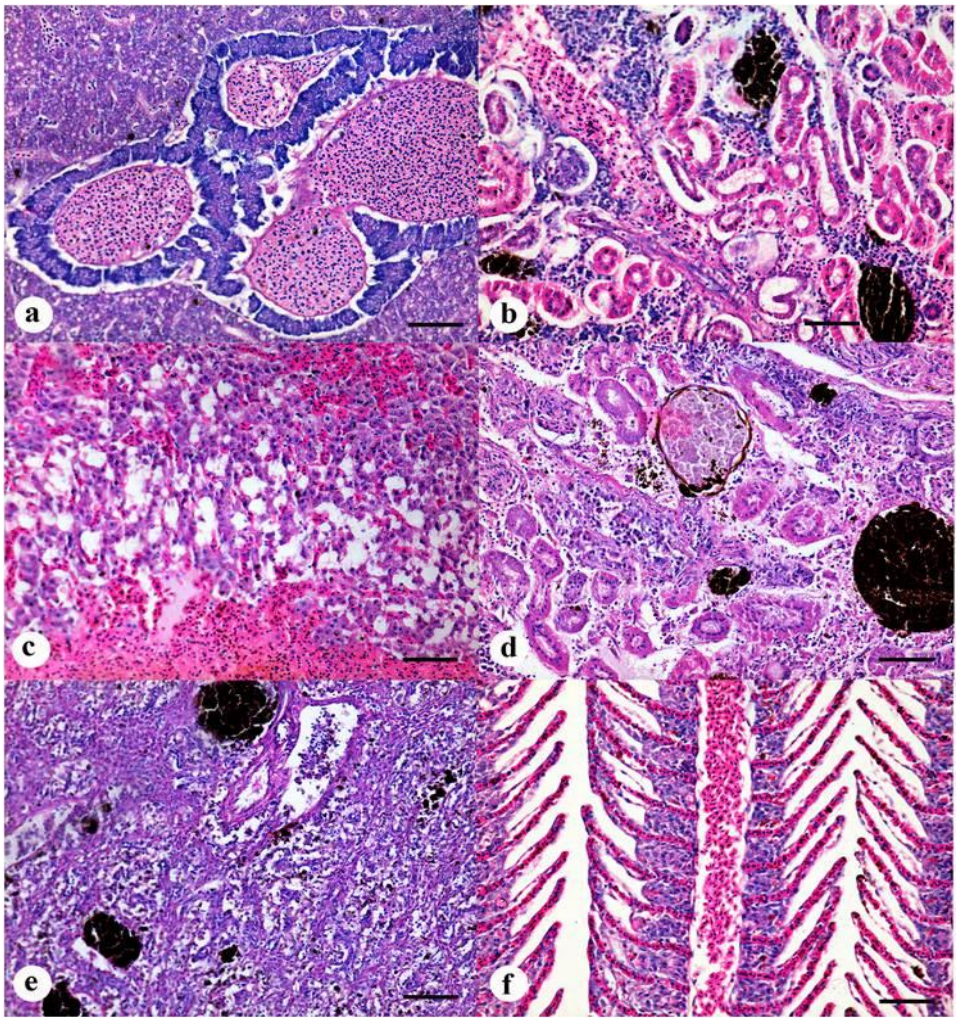

Fig. 3.(a) Hepatopancreas of a sea bream naturally infected with *Streptococcus agalactiae* showing congestion of main blood vessels and sinusoidal spaces with degenerative changes of hepatocytes and pancreatic acinar cells. Bar = 50µm
(b) Posterior kidney of a sea bream naturally infected with *Pseudomonas fluorescence* showing congestion of blood vessels and vacuolation of tubular epithelial cells and their separation from the basement membrane concurrent with focal areas of tubular necrosis, and apparent multifocal mononuclear cell infiltrations and activation of melanomacrophage centers. Bar = 50µm
(c) Hepatopancreas of a sea bream naturally infected with *Tenacibaculum maritimum* showing heamorrhages and congestion of sinusoidal spaces with diffuse vacuolar degeneration. Bar = 50µm
(d) Posterior kidney of a sea bream naturally infected with *Streptococcus agalactiae* showing multifocal areas of tubular and interstitial necrosis, concurrent with circumscribed basophilic areas (may be bacterial colonies and tissue debris) encapsulated with connective tissue fibers and melanin pigment nearby activated melanomacrophage centers. Bar = 50µm
(e) Spleen of sea bream naturally infected with *Tenacibaculum maritimum* showing multifocal necrosis with discrete depletion of white pulp revealing empty spaces with activation of melanomacrophage centers. Bar = 50µm
(f) Gills of a sea bream naturally infected with *Vibrio alginolyticus* showing congestion of the main lamellar blood vessels with separation in-between the epithelial cell lining of the secondary gill lamellae and the underlying capillary bed with hyperplasia of the epithelial lining at the base of the secondary gill lamellae. Bar = 50µm

Haemorrhages were noticed extensively in haemopoeitic tissues. Moreover, diffuse vacuolar degeneration and discrete necrotic changes were commonly evident in hepatocytes as well as pancreatic acinar cells.

Congestion of hepatic, renal, splenic and cerebral blood vessels also detected. Moreover, diffuse vacuolation of tubular epithelial cells and necrotic changes were frequently disseminated in glomerulo-tubular and interstitial tissues. These necrotic areas were replaced by empty spaces and tissue debris. Furthermore,

multifocal depletion of renal and splenic heamopoeitic elements was eminent concurrently with hyper-activation of melanomacrophage centers and multifocal infiltration of mononuclear cells.

The gills showed severe congestion of lamellar blood vessels with frequent telangiectasis, diffuse and multifocal hyperplasia of epithelial lining of the secondary lamellae as well as proliferation of malpighian cells concurrently with immense infiltration by eosinophilic granular cells**.**

DISCUSSION

Egyptian mariculture is an emerging industrial sector requires continued research with scientific and technical developments and innovation. The appearance and development of a fish disease is the result of the interaction among pathogen, host and environment. As an ultimate jeopardy, the environmental and climatic changes are two eminent threats to an optimal development of mari-culture industry. Most recently, an increase in the number of outbreaks associated with several emergent fish pathogenic bacteria became a phenomenal case [5, 11, 24]. Gram-negative bacteria are considered the most critical causes of disease epizootics in mari-culture [10] while gram positive bacteria come next on the critical list of dangerous agents in marine environment [25, 26]**.** Moreover, some bacterial pathogens which were considered as classical fresh water agents have currently become well documented disease agents in mari-culture [11, 13]**.**

The present study confirmed that sea bream reared under captive conditions are prone to several devastating bacterial pathogens capable of causing massive losses. In agreement with other studies concerning marine fish bacterial diseases [5, 10,13,24] most of retrieved bacterial isolates were Gram-negative 75.51 % %. On the other hand gram-positive pathogens accounted for 24.48 %. Results of bacteriological and molecular examination demonstrated that *V. alginolyticus* 28.57 %*, S. agalactiae* 24.48 %*, P. fluorescens*18.36 %*, V. vulnificus* 16.32 % and *T. maritimum* 12.24 % are the destructive Gram-negative bacterial pathogens incriminated in such septicemic infections. On the other hand, *S. agalactiae* 24.48 % is the only Gram-positive pathogens appreciably involved in these episodes.

Vibrios, *Photobacterium damsela* subsp. *Piscicida*, Pseudomonads, *T. maritimum,* Streptococci and Aeromonads species have long been considered to be the main threatening bacterial agents causing high mortalities in cultured marine fish [1, 9, 11, 24, 27].

Vibriosis has long been reported at the top of the most threatening bacterial diseases drastically distress sea bream farming as well as capable of causing substantial economic losses [10, 11]. The current bacteriological and molecular studies definitely confirmed that vibrios were the most frequently retrieved microorganisms 44.8%. Actually *V. alginolyticus* has been involved in many of mass mortalities noticed in several aquaculture facilities along the Mediterranean seacoasts [28, 29]. Numerous epizootics relevant to *V. vulnificus* have also been recorded globally in variety of marine fish [24, 30, 31]. The high recorded rates may partly interpreted by the normal inhabitance of vibrios in sea water, sediment as well as alimentary tract of marine fishes [32, 33] once unfavorable aquatic environmental conditions are established it robustly attacks fish causing epizootic of high mortalities [34, 35].

Pseudomonads septicemic infections are worldwide. In particular, *P. fluorescens* and P. *anguilesiptica* have gained eminent clinical significance for being responsible for high mortalities in numerous cultured and wild marine fish [13, 36, 37]. *P. fluorescens* recorded 18.36 % in winter episode while it was completely missing in summer outbreak. The maximized activity of its proteases particularly at cooler temperatures, 10°C, circumstances trigger the vigorous attacks noticed during winter period [38].

Results definitely call attention to the jeopardy of *T. maritimum* on the health status of sea bream since it recorded considerable rates 12.24 %. Nowadays, tenacibaculosis is one of the main devastating problems in mariculture [11]. *T. maritimum* form a part of the autochthonous bacterial populations of the fish skin. Moreover, it remain viable in the aquatic environment for a long time, utilizing fish mucus as a reservoir [39].The uppermost frequency of infection was recorded in summer outbreak 10.2% compared to 2.04% noticed in winter episodes since infections relevant to *T. maritimum* exacerbate at higher temperatures above 15°C concurrently with adverse water quality [40].

Results came in our study also confirmed the seriousness of *S. agalactiae* expressed by the eminent infection rates during summer episode 24.48 %. *S. agalactiae* has long been considered as one of the upsetting pathogens accused for several epizootics noticed in many of marine fish [41, 42]. Deleterious impacts of long exposure to unfavorable high temperature typical to that detected in the studied farms potentially stress farmed fish predisposing them to vigorous streptococcal septicemic infections [43 , 44, 45].

In respect to recorded water quality measures, in agreement with [5, 8, 46, 47] disease conditions were found to be associated with the existence of stressful environmental circumstances expressed as unfavorable recorded values of water quality parameters. This is justified that the pathogenesis of bacterial fish disease is multifactorial and variable factors related to invading pathogens, hostile environment and fish host should work together in synergism to define the nature of the triggered course of infection [5, 8, 14].

Winter episodes erupted at lower water temperature approaching 9.5°C. These unfavorable cold temperature circumstances predisposed sea bream to surrender these epizootics. Extreme cold temperatures completely halt the activity of immune system, subsequently eliminate fish defense against invading pathogens [48]. Numerous epizootics of bacterial septicemia have been erupted after long exposure to drop off water temperatures [49]. On the other hand**,** temperature profile recorded during summer episode in the investigated fish farms ranged around 31.5°C. This relative high temperature also impairs the antibody response of fish predisposing them to these epizootics [50]. Numerous septicemic bacterial infections in marine fish have been correlated with exposure to long periods of extreme temperature [5,13].

In respect to dissolved oxygen (DO), detected levels were lower than the optimal recommended values, 4.1 mg/l and 3.6 mg/l in Alexandria and Damietta farms respectively. These relatively low DO levels synergize with other viable components present in the aquaculture facilities to produce substantial fish losses [14, 51]. Impaired immune capacity triggered by theses hostile conditions are strongly accused for the establishment of these outbreaks [5, 13, 14, 46]. Furthermore**,** virulence of several fish pathogens is exaggerated by exposure to reduced dissolved oxygen circumstances [52].

The recorded pH values ranged around 7.3 and 8.2 which seem to be conductive to the higher incidence of vibrios infections recorded in this study. The high incidence of vibrio septicemia recorded within our previous studies in wild marine fish has been interrelated with the relative alkaline pH noticed in the sites of fish sampling [4, 13]. On the other hand, high pH indirectly distress cultured fishes through augmenting the noxious effect of non-ionized ammonia [53].

Regarding nitrogenous waste products, results demonstrated that un-ammonia (UIA) and nitrite are involved in predisposing cultured marine fishes to surrender the episodes recorded in land-based or cage culture systems. UIA should not exceed 0.1 mg/L for saltwater fish species [54] which is far from those recorded in our investigations 0.9 and 1.2 mg/l during winter and summer outbreaks respectively laying a great blame on ammonia in disturbing the general health status of sea bream in several ways [36, 55, 56].

In regard to nitrite, a mean of 0.85 and 0.96 mg/l was detected in fish farms during winter and summer outbreaks respectively which are far from the optimum, less than 0.1 mg /L [54]. Nitrite exposure disturbs the health status of fish through various pathways; osmoregulatory imbalances reduce blood oxygen carrying capacity and upset thyroid hormone function [57,58].

Achieved results also definitely incriminate some heavy metals particularly, cupper and iron, in worrying the health status of cultured sea bream exacerbating these epizootics. According to [54] the marine high reliability trigger value recommended for copper, iron, lead, zinc, cobalt and cadmium are, 1.3 µg/L, 300 µg/L, 4.4 µg/L, 15 µg/L, 1 µg/L and 5.5 µg/L respectively in sequential which is far from those detected for the same metals in the studied farms except for cadmium which was completely absent. The role of heavy metals in rendering fish more susceptible to bacterial fish diseases is documented [59]. Fish reared in copper and iron contaminated habitats are vulnerable to variety of bacterial pathogens such as vibriosis and tenicibaculosis [39,60,61].

Regarding histopathological lesions noticed in infected fishes, destruction of the vital components of the circulatory and immune system by toxic bacterial extracellular products is thought to be the corner stone

behind the recorded pathological alterations. Among these ECPs, proteases and haemolysins are at the forefront, contributing significantly to the ruinous nature of these diseases [4, 62,63].

CONCLUSION

Results definitely confirm that marine fishes reared under captivity are liable to variable number of stressors, including chemical, physical and biological invaders. *V. alginolyticus, S. agalactiae, P. fluorescens, V. vulnificus* and *T. maritimum* are found to be the threatening bacterial pathogens involved in mass mortalities noticed in the studied fish farms. Furthermore, disease conditions were found to be associated with the existence of stressful environmental circumstances expressed by the recorded improper values of water quality parameters in investigated fish farms.

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