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### Mass Mortalities in Mari-Cultured European Sea Bass (*Dicentrarchus labrax*) at Northern Egypt.

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#### ABSTRACT

Septicemic bacterial diseases represent the most prominent sector of diseases with direct colossal impacts on Egyptian mariculture. Significant mortalities have been recorded among sea bass, *Dicentrarchus labrax* L. cultured in at floating net-cages and earthen ponds located within the North of Egypt during the year of 2012. Moribund and / or freshly dead fish samples showing a picture of clinical septicemia were collected for bacteriological and histopathological studies. A total number of 100 fish samples were inspected through the course of these episodes. *Vibrio alginolyticus* (*V. Alginolyticus*) was the most prevalent bacterial pathogen 32.25 %, followed by *Pseudomonas fluorescens* (*P. fluorescens*) 24.19% , *Tenacibaculum maritimum* (*T. maritimum*) 17.74 % and *Streptococcus agalactiae* (*S. agalactiae*) 14.51 %. *Vibrio vulnificus* (*V. vulnificus*) infections recorded the lowest rate 11.29 %. Bacterial infections were linked with numerous pathological changes. Adverse water quality measures recorded in the investigated fish farms were strongly incriminated in triggering the infections with an ultimate end of mass mortalities.

**Keywords:** Sea bass, Mass mortalities, Bacterial pathogens, PCR, Water quality

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## INTRODUCTION

Nationally, aquaculture has been identified as the best answer to reduce the expanding gap between supply and demand for fish production [1]. Aspects for growing the Egyptian mariculture are highly diversifying and promising. The country has vast aquatic marine resources that potentially support the establishment of wide varieties of aquaculture facilities [2, 3]. Bacterial diseases of cultured fishes are among the influential constraints hindering the establishment and expansion of competent aquaculture programs. It constitutes the greatest proportion of disease problems in various types of fish production to an extent that exceeds all other diseases combined. Virtually, for each type of aquaculture and for every fish species, specific bacterial pathogens are responsible for serious mortalities [4,5]. Outbreaks are exacerbated with the development of intensive marine fish farming which leads to the emergence of lofty of pathologies [6,7]. The potential of a bacterial disease to cause mass mortality in fish is a multi-factorial syndrome [8]. Variable factors related to the host, environment and the pathogen may work in concert to define the nature of the triggered course of infection. Environmental factors such as oxygen level, temperature, salinity and pH might be involved in triggering disease outbreaks especially in intensive aquaculture systems [9,10]. Sea bass, *Dicentrarchus labrax L.* is one of the leading Mediterranean mariculture fish species [11]. Production systems are diverse but mainly run under the category of intensive land based and sea cages mariculture systems [12]. Despite their economic importance, there are no considerable publications on the dynamics of bacterial pathogens and their relationship to unfavorable water quality. In this study, we discuss the bacterial etiologies incriminated in sea bass mass mortalities reported in two Egyptian fish farms throughout the year of 2012.

## MATERIALS AND METHODS

### Fish samples and rearing conditions

A total of 100 fish were investigated through the course of two epidemics that hit the sea bass reared in floating net-cages and earthen ponds located in two different coastal provinces at the North of Egypt. The winter episode attacked the floating net-cages located at the city of King Marriott west of Alexandria province. The summer outbreaks hit the earthen ponds within the vicinity of Muthallath El-Deeba region at Damietta. A total of 50 moribund and / or freshly dead fish presenting the typical picture of clinical septicemia were collected and examined through every outbreak for the causative agent / agents behind these episodic mass mortalities. The body weights of fish samples obtained from Alexandria fish farm ranging between 100-220 g while those collected from Damietta province were 250-400 g respectively.

### Bacteriological examination

### Sampling and sample processing

Samples for bacteriological examination were obtained from gills, liver, kidney, spleen and brain under aseptic condition. Loopfuls from the above mentioned tissues were cultured

onto ordinary and selective media. 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 blood agar supplemented with 1.5 % (w/v) NaCl. The inoculated plates were incubated at 25 °C for 18-48 hours.

### Identification of isolates

Identification of bacterial isolates was performed by studying the phenotypic and biochemical characteristics using both conventional and commercial miniaturized API 20 E and API strept systems following the criteria proposed by those described in [13].

### Molecular identification

#### DNA extraction

DNA from bacterial colonies was extracted using Bacterial Genomic DNA extraction Kit (**Fermentas, Vilnius, Lithuania**) following the manufacturer's instructions. Concentration and purity of DNA samples were measured using a spectrophotometer (Konica Minolta, Tokyo, Japan).

#### Oligonucleotide primers and PCR

The sequence of each set of primers specific for every bacterial isolate was inquired from previous studies references (Table 1). PCR was performed in 25 µl volumes consisting of 5 µM master mix, 100ng 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 follows: Initial denaturation for 5 minutes at 94 °C, consisted total of 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. On the contrary, the thermal profiles of PCR reactions for *V. vulnificus* isolates were, initial denaturation 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). The amplification products were analyzed by running through 1.0 % agarose gel electrophoresis, stained with ethidium bromide and visualized under UV trans-illumination system. A DNA ladder with 100-bp increment (**Fermentas, Vilnius, Lithuania**) was used as a molecular weight marker.

**Table 1: Target genes, oligonucleotide primer sequence and thermal profiles for (PCR) reactions.**

Bacterial species	Target gene	Sequence	Thermal profile of cycling program				PCR products molecular weight	Reference
			No. of cycles	Den.	Ann	Ext.		
<i>V. Alginolyticus</i>	Collagenase	Forward: 5'CGAGTACAGTCACTTGAAAGCC3' Revers: 3' CACAACAGA AACTCGCGTTACC5'	35	94°C\1 min	50°C\1 m	72°C\ 10 min	737	Di Pinto et al., (2005)
<i>V. Vulnificus</i>	16S rDNA	Forward: 5'TCTAGCGGAGACGCTGGA3' Revers: 3'GCTCACTTTCGCAAGTTGCC5'	30	94°C\30 S	54°C\30 S	72°C\10 min	273	kim&Jeong (2001)
<i>P. fluorescens</i>	16S rDNA	Forward: 5'TGCATTCAA AACTGACTG3' Revers: 3'AATCACACCGTGGTAACCG5'	35	94°C\1 m	50°C\1 m	72°C\10 min	850	Scarpellini et al., (2004)
<i>T. maritimum</i>	16S rRNA	Forward: 5'TGTAGCTTGCTACAGATGA3' Revers: 3'AAATACCTACTCGTAGGTACG5'	35	94°C\1 min	50°C\1 m	72°C\10 min	400	Cepeda et al., (2003)
<i>S. agalactiae</i>	16S- 23Sintergenic region	Forward: 5' CCACGATCTAGAAATAGATTG3' Revers: 3'TGCCAAGGCATCCACC5'	35	94°C\1 min	50°C\1 m	72°C\10 min	150	Berridge et al., (2001)

### Histopathological examination

Tissue specimens from gills, liver, spleen, kidney and brain of infected fish were preserved in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with Hematoxylin and Eosin (H&E) according to the method described by [14]. The histopathological alterations of H&E stained tissue sections were microscopically assessed under low / high microscopic powers.

### 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 [15,16]. Temperature, dissolved Oxygen (DO), pH and salinity were measured on spot while un-ionized ammonia (NH<sub>3</sub>), nitrites, nitrates and heavy metals (iron, copper, zinc, cobalt, cadmium and lead) were measured in laboratory according to methods adopted from [16].

## RESULTS

The bacteriological examination revealed a total of sixty two bacterial isolates retrieved from forty nine infected fish. The majority of these isolates were Gram-negative (85.48 %) that were categorized into *Vibrio alginolyticus* (32.25 %), *Pseudomonas fluorescens* (24.19 %), *Tenicibaculum maritimum* (17.74%) and *Vibrio vulnificus* (11.29 %). Interestingly, *Streptococcus agalactiae* was the only Gram-positive bacterial pathogen incriminated in such outbreaks 14.51 %. The full phenotypic and biochemical characteristics of recovered bacterial isolates are illustrated in table (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 bass.**

Item	<i>V. alginolyticus</i>	<i>V. vulnificus</i>	<i>P. fluorescens</i>	<i>T. maritimum</i>
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	-
H <sub>2</sub> S production(H <sub>2</sub> S)	-	-	-	-
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)	+	+	+	+

**Table 3: Morpho-chemical characteristics of *S. agalactiae* isolates retrieved from naturally infected sea bass.**

<b>Colony characters</b>	Onto marine and TSAS* agar	Small pinpoint whitish round and slightly raised
	Onto Blood agar	$\beta$ 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 )	-
	$\alpha$ galactosidase ( $\alpha$ -GAL )	-
	$\beta$ glucuronidase ( $\beta$ GUR )	Variable
	$\beta$ galactosidase ( $\beta$ 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: Incidences of bacterial infections in sea bass during winter and summer outbreaks.**

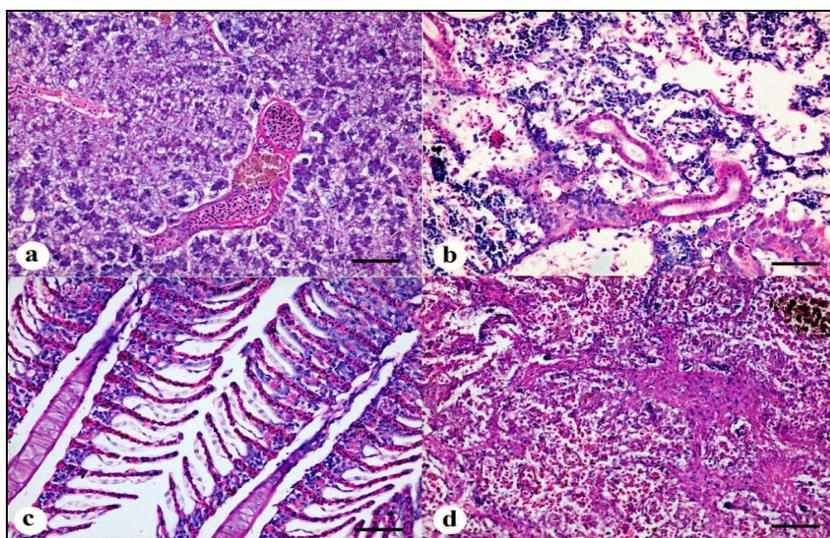
Bacterial strains	Winter outbreak		Summer outbreak		Total	
	No. of isolates	Seasonal incidence	No. of isolates	Seasonal incidence	No. of isolates	Total incidence
<i>V. alginolyticus</i>	8	12.9	12	19.35	20	32.25
<i>V. vulnificus</i>	0	0	7	11.29	7	11.29
<i>P. fluorescens</i>	13	20.96	2	3.22	15	24.19
<i>T. maritimum</i>	3	4.83	8	12.9	11	17.74
<i>S. agalactiae</i>	0	0	9	14.51	9	14.51
The total number of isolates	24	38.7	38	61.29	62	100

The percentage was calculated according to the total number of retrieved isolates (62).



**Figure 1: (a) Net cages at Alexandria governorate, (b,c) Naturally infected seabass showing haemorrhagic patches on the external body surface, (d) Naturally infected seabass showing paleness of liver**

Clinically, infected fish displayed severe external hemorrhages. Scales detachment, fin and tail rot were also noticed. 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, opacity and ocular hemorrhages were also noticed in some cases (Fig 1a-d). Necropsy findings revealed serosanguinous fluid in the abdominal cavity. The liver was congested while in some other cases it was pale and enlarged. The kidney and spleen were congested and enlarged. Hemorrhagic gastroenteritis with enlargement of gall bladder was also reported.



**Figure 2: Histopathological changes in seabass tissues naturally infected with *V. vulnificus* and *P. fluorescens* (H&E, bar = 50  $\mu$ m)**

a: Hepatopancreas of seabass naturally infected with *V. vulnificus* showing congestion of main blood vessels and sinusoidal spaces with diffuse vacuolar degeneration and hepatocytic necrosis revealing densely basophilic stained hepatocytes with activation of melanomacrophage centers. b: Posterior kidney of seabass naturally infected with *P. fluorescens* showing diffuse necrosis of the interstitial tissue with discrete tubular necrosis leaving cyst like spaces with moderate mononuclear cell infiltration in-between necrotic tissue. c: Gills of seabass naturally infected with *P. fluorescens* showing 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. d: Spleen of seabass naturally infected with *T. maritimum* showing multifocal necrosis with discrete depletion of white pulp with activation of melanomacrophage centers.

PCR yielded specific amplicons identical to the size of target gene sequence for each bacterial strain. In particular, *V. alginolyticus* as well as *V. vulnificus* primers produced specific amplicons 737-bp and 273 bp respectively. Furthermore, *P. fluorescens* primers amplified a fragment of 850 bp. Also *T. maritimum* primers yielded specific 400 bp amplicon. On the hand, *S. agalactiae* primers produced specific 150-bp fragments ( Fig. 3).

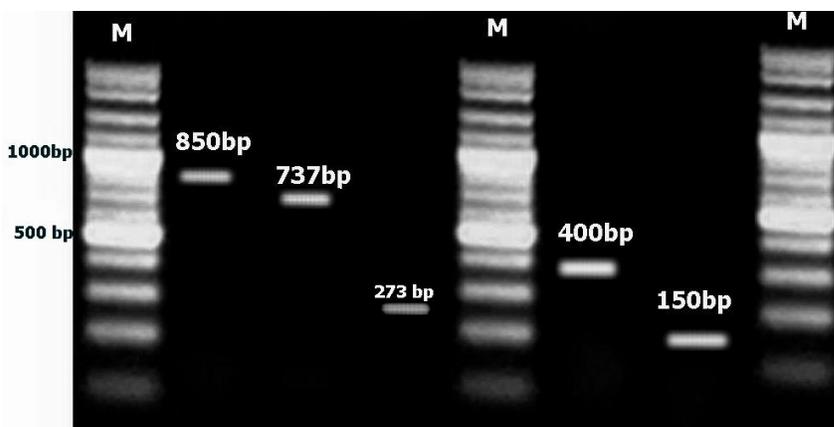


Figure 3: Identification of bacterial strains by PCR

Improper values of water quality parameters were recorded in both investigated fish farms (Table 5). Average water temperatures were 8.2 °C and 33.1 °C in Alexandria and Damietta farms respectively. Dissolved oxygen levels were lower than the optimal values 4.3 and 3.8ppm. The pH values of water in Alexandria and Damietta farms were 7.1 and 8.4 respectively. Salinity was within the expected range 31 ‰ and 37 ‰ respectively.

The 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<sub>3</sub>, NO<sub>2</sub> and NO<sub>3</sub> were far from the optimum recommended levels 0.8 mg/l, 0.75 mg/l and 1.3 mg/l respectively. The same measures in Damietta farm were also more noxious 1.3 mg/l, 0.99 mg/l and 1.69 mg/l respectively. Levels of some detected metals were higher than the recommended marine high reliability trigger value. The measures recorded for Cobalt,

zinc, lead, iron and copper recorded in Alexandria farm were, 0.068 mg/l, 0.058 mg/l, 0.099 mg/l, 0.821 mg/l and 0.062 mg/l, respectively, while the same measures noticed in Damietta farm were, 0.118 mg/l, 0.0881 mg/l, 0.0362 mg/l, 1.07 mg/l and 0.066 mg/l respectively. It is worthy to mention that cadmium was not detected in both farms.

**Table (5): Water quality measures in examined fish farms during outbreaks.**

Sampling sites	Season	Temp °C	Salinity %	D.O. mg/l	pH	NH <sub>3</sub> mg/l	NO <sub>2</sub> mg/l	NO <sub>3</sub> mg/l	Heavy metals (mg/l)					
									Cd	Co	Zn	Pb	Fe	Cu
Site 1	Winter	8.2°C	31	4.3	7.1	0.9	0.85	1.1	ND	0.058	0.054	0.091	0.721	0.052
Site 2	Summer	33.1°C	37	3.8	8.4	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.

Histologically, cardiovascular degenerative, proliferative and necrotic changes were evident. Some bacterial agents were detected in histological sections (Fig. 2). Extensive hemorrhages were noticed in hemopoietic tissues with congestion of hepatic, renal, splenic and cerebral blood vessels. Moreover, diffuse vacuolar degeneration and discrete necrotic changes were commonly evident in hepatocytes, pancreatic acinar cells, glomerulo-tubular and interstitial renal tissue. The necrotic areas were replaced by empty spaces and tissue debris. Furthermore, multifocal depletion of hemopoietic elements was eminent concurrent with hyper-activation of melanomacrophage centers and multifocal infiltration of mononuclear cells. Ultimately, 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 the proliferation of malpighian cells concurrently with some infiltration by eosinophilic granular cells.

### DISCUSSION

Sea bass is one of the predominant mari-cultured fish species at the majority of Mediterranean countries [17]. The high commercial value of Sea bass has encouraged aquaculturists to invest in its mass production [18]. However the rapid development of intensive culture systems has been faced in many incidences with infectious disease epidemics with consequent serious economic losses [6]. Virtually, diseases outbreaks are recognized as a significant constraint to aquaculture production and its economic development in many countries [4].

In agreement with [8,19], the improper water quality measures recorded in the investigated fish farms are strongly incriminated in provoking the high mortalities noticed in theses farms. This is justified by the fact that pathogenesis of bacterial fish disease is a multi-factorial process .Such process necessitates that interaction of variable factors related to

invading pathogens, hostile environment and fish host, which work together in synergism to shape the nature of the triggered course of infection [20].

In the current research, the bacteriological and molecular studies have confirmed that 49 % of the investigated sea bass were found to be infected. *V. alginolyticus* was the most prevalent bacterial pathogen (32.25 %) retrieved from moribund / freshly dead fishes followed by *P. fluorescens* (24.19%), *T. maritimum* (17.74 %) and *S. agalactiae* (14.51 %) with the lowest infection rate (11.29) for *V. vulnificus* . The high magnitude of vibrios infection (43.54 %.) during the epidemics confirms the potential of such pathogen in implementing serious impacts on cultured marine fish [21,6]. This may partly attributed to the ubiquitous existence of vibrios in seawater, sediment as well as alimentary tract of marine fishes [22-24]. Thus, the emergence of deteriorated environmental conditions would predictably drive them to vigorously attack fish with consequent eruptions of mass mortalities [19, 25].

*V. alginolyticus* have been involved in many events of mass mortalities noticed in several aquaculture facilities along the Mediterranean sea-coast [26-28,6]. The frequent mass mortalities associated with *V. alginolyticus* infections have alarmed fish farmers to the immense threat of such pathogen onboth cultured marine fish [29] as well as wild fish populations [2].

In respect to *V. vulnificus*, the results emphasized the great impacts of this pathogen on the health status of sea bass, especially during the summer period. For several decades, *V. vulnificus* has been considered as a primary fish pathogen particularly in immune-compromised fish [30,31]. Numerous epizootics relevant to *V. vulnificus* have been recorded in a variety of cultured marine fishes during the past two decades [28,32]. Some studies ignored the relationship between vibrio infections and season of occurrence as vibrios attack cultured fish year-round without reliance to a particular season [33]. Studies in wild marine habitat also proved the association between unfavorable extreme water temperature and the flourishing of vibrio infections in fish [2].

The relatively low prevalence of vibriosis reported during winter episode in comparison to summer epidemics can be justified by the fact that vibrios may survive in a dormant state in cold-water circumstances. The unfavorable conditions of low temperature and nutrient deprivation are the main triggering causes of induction for dormant stages of such pathogen [34, 35]. When appropriate environmental conditions are established, these microorganisms are nevertheless able to recover from this dormant state and infect fish [36].

Results also indicated that the recorded *P. fluorescens* infections approached 24.19 %. This pathogen has long been considered to be among the most prevalent bacterial pathogens attacking variety of marine fish; in particular, stressed and immunocompromised species [19,37,38]. Some other studies showed extremely higher frequencies approaching 88% in gilthead sea bream larvae [39] and 72 % in captive Atlantic cod, *Gadus morhua* [40]. The maximal activity of its proteases is recorded during lower water temperatures (winter season) [9]. In agreement with our results, several reports has concluded that lower water temperatures have triggered robust attacks during winter period [41].

*T. maritimum* infection was recorded in a considerable rate of infection (17.74 %). This relatively higher rate is justified as *T. maritimum* represents a part of the autochthonous bacterial population of the marine fish skin. Moreover, it remains viable in the aquatic environment for a long time, utilizing fish mucus as a reservoir [42]. The highest prevalence of *T. maritimum* infections were recorded during summer outbreaks attacked Damietta farm fishes 12.9% in comparison to 4.83% noticed in winter outbreaks of Alexandria farm fishes. The pathogenicity of *T. maritimum* was possibly maximized at higher temperatures above 15°C together with inferior water quality [43]. However, many other studies reported outbreaks of tenacibaculosis during winter period [44-46].

Interestingly, our results were in agreement with the previous studies that correlated exacerbated streptococcal septicemic infections in fish with the summer period [2, 47]. The *S. agalactiae* recorded prevalence was 14.51 % during summer epidemics. It is well known that *S. agalactiae* has been considered as one of the most devastating pathogens accused for several mass mortalities among different varieties of marine fishes [48, 49]. Deleterious impacts of long exposure to unfavorable high temperature typical to that detected in the studied farms. The potentially stressed farmed fish were susceptible to vigorous streptococcal septicemic infections [50].

From environmental prospectus, the achieved results have confirmed that water quality is an integral part of any aquaculture system. It plays a major role in fish health. Fish diseases are initiated by complex interactions of host, pathogen and environment [8,51]. Maintaining good water quality is critical to successful aquaculture [52]. Deterioration in water quality stresses cultured fishes with consequent increase in the chance of opportunistic pathogens to invade fish causing disease condition [19]. Virtually, there is a close relationship between environmental stress and emergence of outbreaks of fish diseases [53].

In respect to water temperature, the unfavorable cold temperature circumstances 8.2 °C detected in net-cages could be strongly incriminated in predisposing sea bass to surrender these epizootics. Extreme cold temperatures completely halt the activity of immune system, subsequently eliminate fish defense against invading pathogens [54]. Numerous epizootics of bacterial septicemia have been erupted after long exposure to low water temperatures [6, 55].

It is pertinent to denote that drop in temperature causes cold-induced fasting, thermal stress and metabolic depression. Furthermore, Low temperature is selectively suppressive to T cell responses and antibody synthesis [56,57]. The end result is a lower immune capacity, which rendered fish more susceptible to infections [58]. Noticeable mortalities have been repeatedly recorded during such periods [59,60]. Furthermore, the pathogenicity of some bacterial fish pathogens exacerbated at lower temperature. Typically, *P. fluorescens* was the most prevalent bacterial pathogen recorded in winter outbreaks in comparison to the negligible frequencies noticed during summer episodes [9]. This phenomenon, attributed mainly to the temperature dependence of pseudomonads infection. Multiplication, virulence and invasiveness of pseudomonades augment in cold water circumstances [61, 62]. On the other hand regarding summer outbreaks, the relatively high temperature (33.1 °C) at Damietta farms also accused for

these septicemic infections. Numerous septicemic bacterial infections affecting marine fish have been correlated with exposure to long periods of extreme temperature [2].

In respect to dissolved oxygen levels (DO), unfavorable levels lower than the optimal recommended values were recorded (4.3 mg/l) and (3.8 mg/l) in Alexandria and Damietta farms respectively. These relatively low DO levels synergized with other viable environmental aquatic components in both aquaculture facilities to produce the eminent cases of mortalities [63]. Impaired immune mechanisms triggered by these hostile conditions are accused for the establishment of these outbreaks [2, 64]. Furthermore, the virulence of the causative pathogens is exacerbated by exposure to reduced dissolved oxygen levels [53].

Regarding the pH, the recorded values (7.1 and 8.4) seem to be conducive to the higher prevalence of vibrios infections recorded in the study. The pH affects some virulence characteristics of many fish pathogens. Vibrios withstand higher pH values. Moreover, the adhesion of vibrios to fish mucus is greatly influenced by pH values [65, 66].

Results demonstrated that ammonia and nitrite are involved in modulating fishes to surrender these episodes recorded in land based or cage culture systems. Unionized ammonia ( $\text{NH}_3$ ) should not exceed 0.1 mg/L for saltwater fish species [67]. These levels were far from those recorded in our investigations (0.9 and 1.2 mg/l) during winter and summer outbreaks respectively pointing to ammonia in disturbing the general health status of sea bream and sea bass in several ways. High ammonia levels enhance microbial infections through suppressing the immune capacity of fish. Phagocytic and clearance efficiency are diminished. As an ultimate fate for the colossal immuno-suppression of fishes, bacterial invasion will be the most probable event [2, 39, 68, 69]. On the other hand, prolonged exposure to low levels irritates gills and skin creating proper environment for parasitic infestations and opens new portal of entry for opportunistic bacterial infections [70].

For the heavy metals, achieved results also confirmed the synergistic effect of some heavy metals, particularly, copper and iron in disturbing the health status of cultured sea bass which speed up the course of outbreaks. The mean of some detected metal values were higher than levels recommended by [67]. The recorded levels of Co, Zn, Pb, Fe and Cu in Alexandria Fish farm were 0.058 mg/l, 0.054 mg/l, 0.091 mg/l, 0.721mg/l and 0.052 mg/l compared to 0.115 mg/l, .0781mg/l, 0.0359 mg/l, 1.03 mg/l, and 0.046 mg/l respectively in Damietta farm. Achieved results indicated the possible involvement of these metals in triggering such epizootics. According to ANZECC (2000) the marine high reliability trigger value recommended for ; copper, iron, lead, zinc, cobalt and cadmium are; 1.3  $\mu\text{g/L}$ , 300  $\mu\text{g/L}$ , 4.4  $\mu\text{g/L}$ , 15  $\mu\text{g/L}$ , 1  $\mu\text{g/L}$  and 5.5  $\mu\text{g/L}$  respectively. It is well established fact that polluted water weakens the fish host defenses, allowing increased opportunities for epizootic diseases to affect fish populations. Juveniles collected from polluted environments are more vulnerable to variety of microbial infections than those from non-contaminated habitats a result of immune-suppression. The role of heavy metals in rendering fish more susceptible to bacterial fish diseases is documented. Fish reared in copper and iron contaminated habitats are vulnerable to a variety of bacterial pathogens such as vibriosis and tenicibaculosis [42].

Ultimately, the histopathological lesions detected in investigated fishes included circulatory, degenerative, proliferative and necrotic changes. Destruction of the critical components of the circulatory and immune system by toxic bacterial extracellular products (proteases and hemolysins) is thought to be the cornerstone behind these pathological alterations [71, 72].

### CONCLUSION

*V. alginolyticus*, *V. vulnificus*, *P. fluorescens T. maritimum*, and *S. agalactiae*, are considered to be the most threatening bacterial pathogens involved in the septicemic bacterial infections of sea bass and therefore should be included in the future strategies of prophylaxis and treatment of farmed sea bass. The results also clearly point out that maintaining a favorable fish culture, environment and good husbandry practice are crucial for growing healthy fish.

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