



Impact of Climate Change on Some Seasonal Bacterial Eruptions among Cultured Marine Fishes from Egyptian Coastal Provinces

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ABSTRACT

Climate change is now considered one of the greatest challenges and is expected to have a drastic impact on mariculture. The present study aimed to evaluate the impact of climatic changes on the emergence of bacterial pathogens among cultured marine fish in northern Egyptian provinces. 135 samples of three marine fish species, represented as 45 of *Dicentrarchus labrax* (700 ±25 g), 45 of *Sparus aurata* (350 ±25 g), and 45 *Argyrosomus regius* (1 kg ±50 g) were collected from private marine fish farms located in Deeba Triangle, Shataa Damietta (Damietta governorate) and Ismailia province, Egypt. Moribund fishes exhibited erratic swimming behaviour, haemorrhage, erosion and ulcers on the skin. Necropsy findings of infected fish revealed congested liver or pale with engorged gall bladder, congested kidney and spleen. With the presence of serous to hemorrhagic ascetic fluid. *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, and *Photobacterium damsela* subspecies *piscicida* were the most retrievable bacterial strains from moribund fish. *V. alginolyticus* was the most prevalent isolated bacterial strain and represented 50%, 50% and 40% of the total isolates recovered from *Sparus aurata*, *Dicentrarchus labrax* and *Argyrosomus regius*, respectively. Retrieved isolates were morphologically and biochemically identified using the API 20E system, followed by further confirmation by sequencing of 16S rRNA genes. The histopathological examination revealed severe inflammatory reactions together with melanomacrophage center alterations within the examined splenic, hepatic, and renal tissues. Data analysis has shown that poor water quality and severe climatic change, especially during the summer, were implicated in the emergence of bacterial infections among cultured marine fish.

Keywords: Climate change, Deeba Triangle, Marine fishes, *Photobacterium damsela* subspecies *piscicida*, *Vibrio alginolyticus*.

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INTRODUCTION

Egyptian Aquaculture is the largest aquaculture business in Africa, is currently considered the primary source of fish supply (Kaleem and Sabi, 2021). Egypt ranks ninth globally in terms of aquaculture production, with an annual production of 1.5 billion tons, and farmed fish

comprise almost 79.6% of the total production (Abdelsalam *et al.*, 2023).

Egyptian mariculture is mostly practiced in the northern cities of Alexandria, Port Said, Damietta, and the Suez Canal region. Marine fish is about 564.39 thousand tons, representing about 22.82% of

the total fish produced in Egypt (Mahmoud *et al.*, 2023), and the Deeba Triangle produces a significant portion of this percentage (Eissa *et al.*, 2021).

European seabass (*Dicentrarchus labrax*), Gilthead sea bream (*Sparus aurata*), and meagre (*Argyrosomus regius*) are the three main marine species cultured in Egypt (Aly *et al.*, 2021). Six nations account for almost 90% of seabream and seabass production worldwide: Turkey (37%), Greece (25%), Egypt (14%), Spain (9%), Tunisia (4%), and Italy (4%) (Muniesa *et al.*, 2020). The production of meagre began in 2008 with an estimated 2000 tons and reached 5,884 tons in 2014. Aquaculture of meagre is preferred due to their high growth rates and tolerance to higher salinity levels (FAO, 2018).

Deeba Triangle (a triangle region between Damietta and Port Said Province) is currently regarded as one of the most exposed areas to the drastic impact of climate change (Eissa *et al.*, 2021). Climatic changes, including fluctuations in temperature, rainfall patterns and deteriorated water quality are the main triggers of bacterial infection eruptions among the cultured marine fish in these areas (Cianconi *et al.*, 2020). In Egypt, bacterial infections are the main cause of severe mortalities and morbidities in a variety of cultured marine fish and the increase in water temperature in summer has a significant effect on mortality rates, increasing physiological stress and rendering fish more vulnerable to bacterial infection (Kaleem and Sabi, 2021).

Vibriosis is one of the devastating bacterial diseases affecting cultured marine fish, caused by pathogenic *Vibrio* species, and is regarded as one of the most serious bacterial infections that have zoonotic and financial significance in mariculture (Cascarano *et al.*, 2021). *Vibrio alginolyticus* and *Vibrio parahaemolyticus* are responsible for mass mortalities among cultured marine fish on many farms throughout the Mediterranean area (Snoussi *et al.*, 2008; Rowley *et al.*, 2014). Vibriosis is one of the diseases that may be severely affected by climatic changes. Vibrios grow efficiently in temperate water (> 15° C) and at low salinity (<25 ppt) (Vezzulli *et al.*, 2013). Thus, the anticipated rise in temperature and decrease in salinity in temperate regions will affect the epidemiological patterns of vibriosis (Rowley *et al.*, 2014).

Photobacterium damsela subsp. *piscicida* is a common bacterium responsible for photobacteriosis (pasteurellosis), a significant bacterial fish disease in the Mediterranean affecting both cultured and wild marine fish species (Pećur Kazazić *et al.*, 2019;

Bagni, 2021; Colloca and Cerasi, 2021). The disease is characterized by the existence of several white nodules on the surface of the internal organs (particularly the kidney and spleen) (Labella *et al.*, 2019; Morick *et al.*, 2023). The eruptions of photobacteriosis are known to arise in summer, and severe mortalities typically happen when water temperatures rise above 18– 20° C. Under this temperature, fish can harbour the pathogen as a subclinical infection for extended periods of time (Woo *et al.*, 2020).

Climatic changes, such as high water temperatures, especially during summer season, are usually a risk factor for disease outbreaks (Islam *et al.*, 2022). Other environmental stressors, including high salinity, increased ammonia, and physical damage, can all be exacerbated by the climatic changes rendering fish more susceptible to bacterial infections (Mehrim and Refaey, 2023).

The current study aimed to evaluating the impact of climatic changes on the eruption of bacterial diseases among marine fish in the northern Egyptian provinces.

MATERIALS AND METHODS

1. Study area

The study area includes private marine fish farms located in the Deeba Triangle which is bordered by (the Mediterranean Sea to the north, the Damietta Estuary to the west, and Lake Manzala to the south), Shataa Damietta (Damietta governorate) and Ismailia province. The main water supply is coming from the Mediterranean Sea.

2. Fish Sampling

135 samples of three marine fish species represented as 45 of *Dicentrarchus labrax*, 45(700 ±25 g), 45 *Sparus aurata* (350 ±25 g), and 45 *Argyrosomus regius* (1 kg ±50 g) were collected seasonally from private marine fish farms located in Deeba Triangle, Shataa Damietta (Damietta governorate) and Ismailia province, Egypt. The collected fish were transferred immediately in an insulated icebox to the Lab of Aquatic Animal Medicine and Management, Cairo University. The collected fish were subjected to clinical, postmortem and bacteriological investigations according to Eissa (2016).

3. Water sampling

Water samples were collected from the fish farms using sterile glass bottles, transported in isolated ice box, and analyzed following the standard procedures described by APHA (2005). Samples

were collected in August (summer), and October (autumn). Water samples were physio-chemically analyzed for temperature, dissolved oxygen levels, pH values, and water salinity using a portable multi-parameter water quality meter (HI98194, Hanna Instruments) and ammonia using (HI4101, Hanna Instruments).

4. Bacteriological isolation and identification

Loopfuls from internal organs including (liver, kidney, and spleen) and skin ulcers under complete aseptic conditions were streaked onto tryptic soy agar supplemented with 2% NaCl (TSA, Difco), sheep blood agar with 2% NaCl, and thiosulfate citrate bile salt sucrose agar (TCBS, Oxoid). All inoculated plates were incubated at 28° C for 24–48 h. For purification purposes, single colonies were selected from the plates and re-streaked onto TSA plates with 2% NaCl. Pure colonies were phenotypically identified using Gram staining and Motility test. Conventional biochemical identification was carried out using oxidase, catalase test and sensitivity to a vibriostatic agent (O/129) according to **Buller (2004)**. Furthermore, the biochemical identification of bacterial strains was confirmed using API 20E test (Biomérieux, France), according to the guidelines of the manufacturer. Eventually, the purified strains were kept in tryptic soy broth with 15% (vol/ vol) glycerol and kept at – 80° C for further molecular identification.

5. 16S rRNA gene sequencing

Representative purified bacterial isolates were selected for molecular identification by partial sequencing of 16S rRNA gene. DNA was extracted from pure colonies using QIAamp DNA Mini kit (Qiagen) according to the manufacturer's protocol. PCR for 16SrRNA gene was done using the universal primer (16S-F: 5'-AGAGTTTGATCCTGGCTCAG-3') and (16S-R: 5'-GGTACCTTGTTACGACTT-3'), described by **Weisburg et al., (1991)**. The PCR reaction was performed in a final volume of 25 µL, using 1X PCR mix consisting of 0.25 µM of both primer, 200 mM dNTP, 1 U Taq polymerase and 250 ng genomic DNA. PCR reaction conditions were adjusted as follows: initial DNA denaturation at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55°C for 60 s, and extension at 72°C for 1 min, and a final extension at 72°C for 10 min.

Purified PCR amplicons were sequenced using ABI 3730xl DNA sequencer (Applied Biosystems™) at Sigma. The assembled 16S rRNA genes were compared against other 16S rRNA genes sequences using BLAST® search in the database of GenBank.

6. Phylogenetic analysis

The sequences of the 16S rRNA gene of bacterial strains were deposited in GenBank. To calculate genetic distance, the phylogenetic trees were constructed using MEGA X 11 using the neighbor-joining method, and the level of confidence was verified by bootstrap analysis for each branch at 1000 repeats (**Kumar et al., 2018**).

7. Histopathological Examination

Autopsy samples were taken from the liver, kidney, spleen, swim bladder and gills of fish fixed in neutral buffered formalin 10%, washed, dehydrated, cleared and embedded in paraffin. The paraffin embedded blocks were sectioned at 4-microns thickness and stained by Hematoxylin and Eosin stain (**Bancroft et al., 2013**) for histopathological examination by a light microscope (Olympus BX50, Japan).

RESULTS

1. Clinical and Necropsy findings

Moribund fish showed erratic swimming behaviour and decreased appetite. The common gross pathological lesions recorded were skin haemorrhages, erosions, ulcerations, and detached scales. Necropsy findings of the spleen and kidney revealed severe congestion. The liver was congested and some cases, pale and friable, with an enlarged gall bladder. The gills were congested (**Fig.1**). The swim bladders showed congestion, enlargement, thickening, and inflammation of their walls. With the presence of serous to hemorrhagic ascetic fluid.

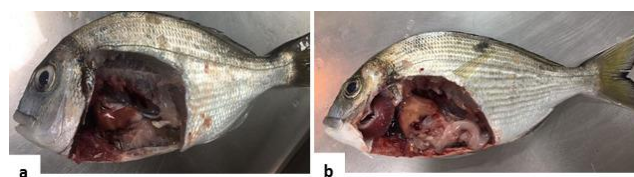


Fig.1: Dissected Gilthead seabream showing degenerated liver and mottled spleen.

2. Water quality analysis

The water quality analysis indicates abrupt increase in toxic ammonia (0.3 mg/L), water temperature (28 –31° C), water pH (9.5 - 10), with concurrent decrease in dissolved oxygen (3 –3.5 mg/L) and salinity (20 –33 g/L).

3. prevalence of infection among the examined fish

The total number of clinically diseased fish was 71 with 52.6 % prevalence rate. For *Sparus aurata*, the total number of clinically diseased fish was 27 from 45 with 60% prevalence rate. For *Dicentrarchus labrax*, the total number of clinically

diseased fish was 29 from 45 with 64.5 % prevalence rate. For *Argyrosomus regius*, the total number of clinically diseased fish was 15 from 45 with 30.3%

prevalence rate. The prevalence of infection among the examined fishes (apparently healthy and clinically diseased) is presented in (Table 1).

Table 1: Prevalence of infection among the examined fishes (apparently healthy and clinically diseased)

| Fish species | Total No. | Apparently healthy fish | | Clinically diseased fish | |
|-----------------------------|-----------|-------------------------|------|--------------------------|------|
| | | No. | % | No. | % |
| <i>Sparus aurata</i> | 45 | 18 | 40 | 27 | 60 |
| <i>Dicentrarchus labrax</i> | 45 | 16 | 35.5 | 29 | 64.5 |
| <i>Argyrosomus regius</i> | 45 | 30 | 66.7 | 15 | 30.3 |
| Total | 135 | 64 | 47.4 | 71 | 52.6 |

4. Bacteriological Examination

4.1. Phenotypic characteristics of retrieved isolates

Three different bacterial isolates were identified using conventional and semi-automated biochemical tests. The first identified bacterium was *Vibrio alginolyticus* that appeared as 2-3 mm smooth yellow colonies on TCBS, and large swarming colonies on TSA + 2% NaCl. The bacterial isolates were gram-negative straight to slightly curved rods, motile, oxidase and catalase positive, and susceptible to vibriostatic agent (O/129). The API E 20 profile of *V. alginolyticus* isolates is presented in Table 2.

The second identified bacterial pathogen was *Vibrio parahaemolyticus* that appeared 1-2 mm pin-point green colonies with a dark blue center on TCBS, and small, white, non-swarming on TSA + 2% NaCl. The bacterial isolates were gram-negative short rods, motile, oxidase and catalase positive, and susceptible to vibriostatic agent (O/129). The API E 20 profile of *V. parahaemolyticus* isolates (Table 2).

Table 2: Phenotypic and biochemical characteristics of the retrieved isolates

| Criteria | <i>Vibrio alginolyticus</i> | <i>Vibrio parahaemolyticus</i> | <i>Photobacterium damsela</i> <i>subsp. piscicida</i> |
|--|---|---|--|
| Culture characters on TCBS | 2-3 mm Smooth yellow-colored colonies | 1-2 mm pin-point green-colored colonies with dark blue center | Don't grow |
| Culture characters on TSA with 2% NaCl | Swarmed creamy colonies | Non- Swarmed white colonies | Small grayish colonies |
| Sheep blood agar 2% NaCl | Non-hemolytic | Beta- hemolysis. | Non-hemolytic |
| Gram staining | Gram-negative Straight to slightly curved rods | Gram-negative Straight to slightly curved rods | Gram-negative short bipolar rods |
| Motility test | Motile | Motile | Non- Motile |
| Catalase | + | + | + |
| Oxidase | + | + | + |
| vibriostatic agent (O/129) | Sensitive | Sensitive | Sensitive |
| ODC | + | + | - |
| LDC | + | + | - |
| ADH | - | - | + |
| OPNG | - | - | - |
| CIT | + | - | + |
| H2S | - | - | - |
| URE | - | - | - |
| IND | + | + | - |
| VP | - | - | + |
| GLU | + | + | + |
| MAN | + | + | + |
| INO | - | - | - |
| SOR | - | - | - |
| RHA | - | - | + |
| SAC | + | - | - |
| MEL | - | - | + |
| AMY | + | + | + |

The third identified bacterial isolate was identified as *Photobacterium damsela* subsp. *piscicida*, that appeared small grayish colonies on TSA + 2% NaCl, and did not grow on TCBS agar. The bacterial isolates were gram-negative bipolar coccobacilli short rods, non-motile, non-hemolytic, oxidase and catalase positive, and sensitive to vibriostatic agent (O/129). The API E 20 profile of *P. damsela* subsp. *piscicida* isolates (Table 2).

5. Sequencing analysis

The assembled sequences have been deposited in GenBank under the accession numbers: OR785488, OR785723 and OR785475. The GenBank accession number (OR785488) was 1400 -bp and showed 100% - 99.8% similarity to the accession number of *V. parahaemolyticus* (OL619052, OL619053, OL619054, NR041838 and MW182301), while the GenBank accession number (OR785723) was 1434 bp and showed 100% - 99.93% similarity to the accession number of *V. alginolyticus* (MK102583, MF101235, KX904708, MN945277 and MN938893). Further, the GenBank accession number (OR785475) was 1077-bp and showed 100%-99.9% similarity to the accession number of *P. damsela* subsp. *Piscicida* (MN186608, MW063536, ON564502, ON564500 and ON564499) (Fig.2).

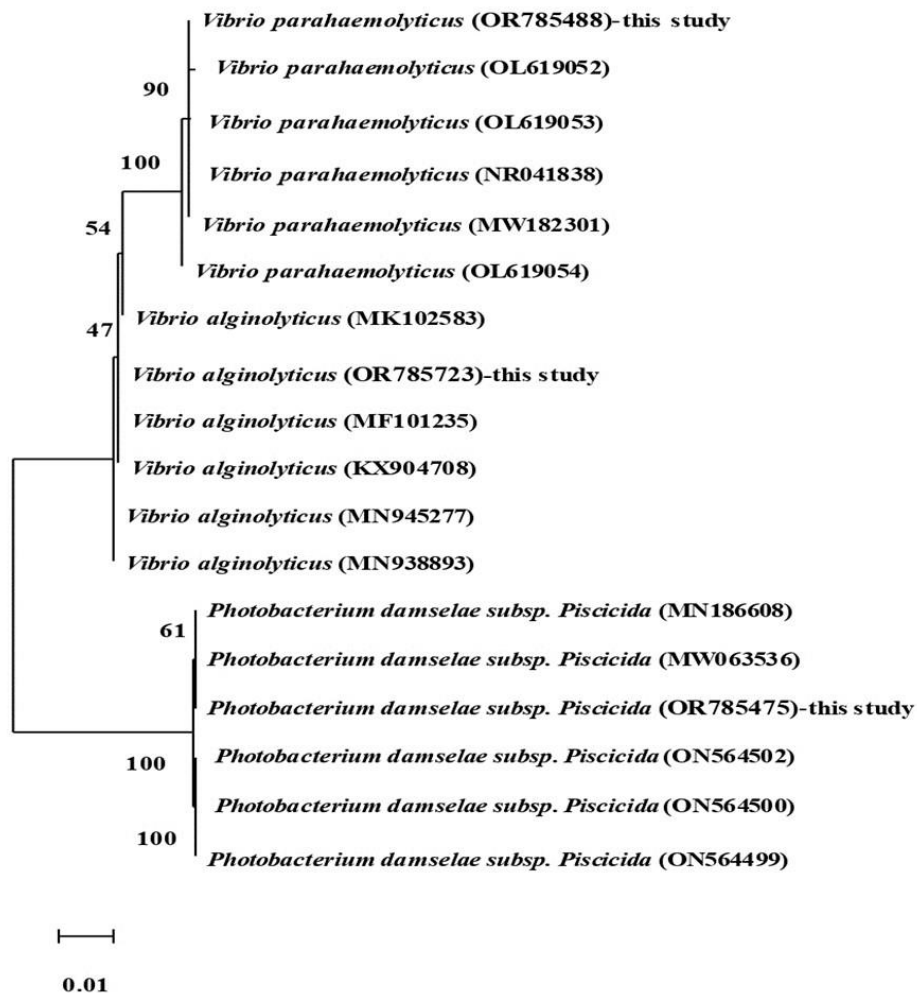


Fig.2: Phylogenetic analysis based on the 16S rRNA gene sequences of *V. alginolyticus*, *V. parahemolyticus* and *P. damsela* subsp. *Piscicida* isolated in this study. The tree was constructed and analyzed by the neighbor-joining method.

6. Seasonal prevalence of bacterial isolates in *Sparus aurata*

Fifty suspected strains were isolated from the clinically diseased fishes. A total of 20 bacterial isolates were retrieved from moribund *Sparus aurata*. The most prevalent isolated bacterial strains were *V. alginolyticus* with 50% of the total isolates. Other bacterial pathogens belonging to *V. parahaemolyticus*, and *P. damsela* subsp. *piscicida* were also identified with 35%, and 15% of the total isolates, respectively. The seasonal prevalence of bacterial isolates among moribund *Sparus aurata* of this study (Table 3).

Table 3: Seasonal prevalence of recovered isolates from moribund *Sparus aurata*

| Retrieved bacterial isolates | Summer | | Autum n | | Winter | | All isolates | |
|--|--------|------|------------|------|--------|------|--------------|------|
| | No. | % | No. | % | No | % | No. | % |
| <i>V. alginolyticus</i> | 5 | 25 % | 3 | 15 % | 2 | 10 % | 10 | 50 % |
| <i>V. parahaemolyticus</i> | 3 | 15 % | 2 | 10 % | 2 | 10 % | 7 | 35% |
| <i>P. damsela</i> subsp. <i>piscicida</i> | 2 | 10% | 1 | 5 % | 0 | 0 % | 3 | 15% |
| Total | 1 | 50% | 6 | 30 % | 4 | 20 % | 20 | 100 |

7. Seasonal prevalence of bacterial isolates in *Dicentrarchus labrax*

A total of 20 bacterial isolates were isolated from diseased *Dicentrarchus labrax*. The most prevalent isolated bacterial strains were *V. alginolyticus* with 50% of the total isolates. Other bacterial strains belonging to *V. parahaemolyticus*, and *P. damsela* subsp *piscicida* were also identified with 30%, and 20% of the total isolates, respectively. The seasonal prevalence of bacterial isolates among diseased *Dicentrarchus labrax* of this study (Table 4).

Table 4: Seasonal prevalence of recovered isolates from moribund *Dicentrarchus labrax*

| Retrieved bacterial isolates | Summer | | Autumn | | Winter | | All isolates | |
|--|--------|------|--------|-----|--------|-----|--------------|------|
| | No. | % | No. | % | No. | % | No. | % |
| <i>V. alginolyticus</i> | 6 | 30 % | 2 | 10% | 2 | 10% | 10 | 50 % |
| <i>V. arahaemolyticus</i> | 4 | 20 % | 1 | 5% | 1 | 5% | 6 | 30 % |
| <i>P. damsela</i> subsp. <i>piscicida</i> | 3 | 15% | 1 | 5% | 0 | 0% | 4 | 20% |
| Total | 13 | 65% | 4 | 20% | 3 | 15% | 20 | 100% |

8. Seasonal prevalence of bacterial isolates in *Argyrosomus regius*

A total of 10 bacterial isolates were retrieved from moribund *Argyrosomus regius*. The most prevalent isolated bacterial strains were *V. alginolyticus* with 40% of the total isolates. Other bacterial strains belonging to *V. parahaemolyticus*, and *P. damsela* subsp *piscicida* were also identified with 30%, and 30% of the total isolates, respectively. The seasonal prevalence of bacterial isolates among moribund *Argyrosomus regius* of this study (Table 5).

Table 5. Seasonal prevalence of recovered isolates from moribund *Argyrosomus regius*

| Retrieved bacterial isolates | summer | | Autumn | | winter | | All isolates | |
|--|--------|------|--------|-----|--------|-----|--------------|------|
| | No. | % | No. | % | No. | % | No. | % |
| <i>V. alginolyticus</i> | 2 | 20 % | 1 | 10% | 1 | 10% | 4 | 40 % |
| <i>V. parahaemolyticus</i> | 2 | 20 % | 1 | 10% | 0 | 0% | 3 | 30 % |
| <i>P. damsela</i> subsp. <i>piscicida</i> | 2 | 20% | 1 | 10% | 0 | 0% | 3 | 30% |
| Total | 6 | 60% | 3 | 30% | 1 | 10% | 10 | 100% |

9. Histopathological findings

Histopathological examination of Gilthead seabream hepatic tissues showed focal haemorrhages were detected in the parenchyma associated with dilatation and congestion of the portal vein (**Fig.3 a**) as well as dilatation and congestion of the central vein with activation of melanomacrophage centre (**Fig.3 b & C**). Concerning kidneys, there was activation of the melanomacrophage centre with severe diffuse vacuolar degeneration of tubular lining epithelium (**Fig.3 d**) and lymphoid proliferation in between the tubules (**Fig.3 e**). Spleen showing activation of melanomacrophage center in between the follicles (**Fig.3 f**).

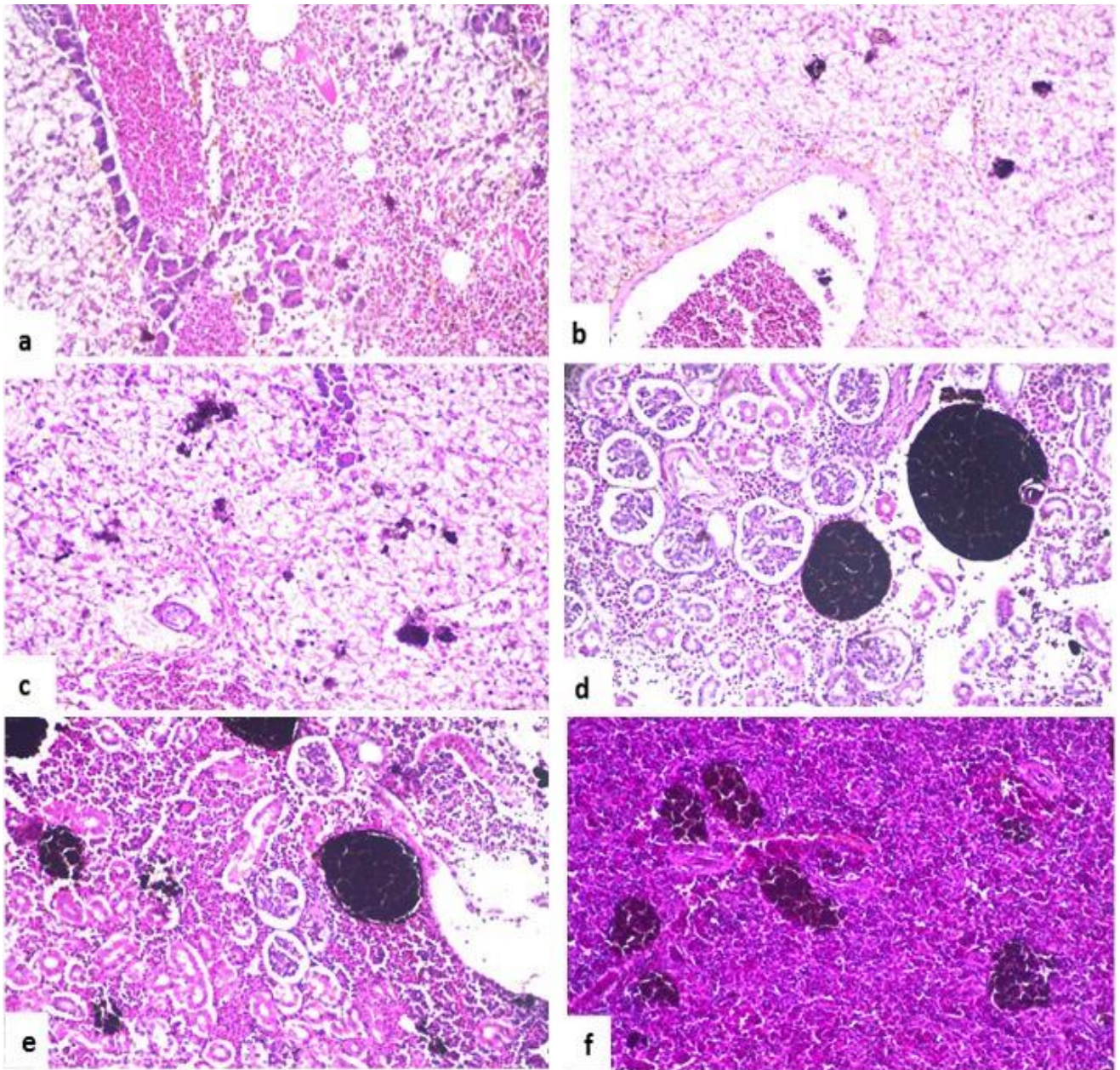


Fig.3: photomicrograph, Gilthead seabream (a) liver showing focal hemorrhages with dilatation and congestion of the portal vein (black arrow) (H&EX200). (b) as well as dilatation and congestion of the central vein (black arrow) and (c) activation of melanomacrophage center (black arrows) (H&EX200). (d) kidneys showing diffuse vacuolar degeneration of tubular lining epithelium and activation of melanomacrophage center (black arrows) (H&EX200). (e) higher magnification of the previous photo showing lymphoid proliferation in between the tubules (black arrow) (H&EX400). (f) Spleen showing activation of melanomacrophage center in between the follicles (black arrows) (H&EX400).

Histopathological examination of European seabass liver parenchyma revealed inflammatory cells infiltration surrounding and adjacent the dilated central vein (**Fig.4 a**). Spleen showing activation of melanomacrophage center (**Fig.4 b**). Concerning kidneys, congestion of renal blood vessel with hemorrhage and inflammatory mononuclear cell infiltrations in the renal interstitial tissue (**Fig.4 c**). Slight hyperplasia of epithelial cells at the primary lamellae (**Fig.4 d**).

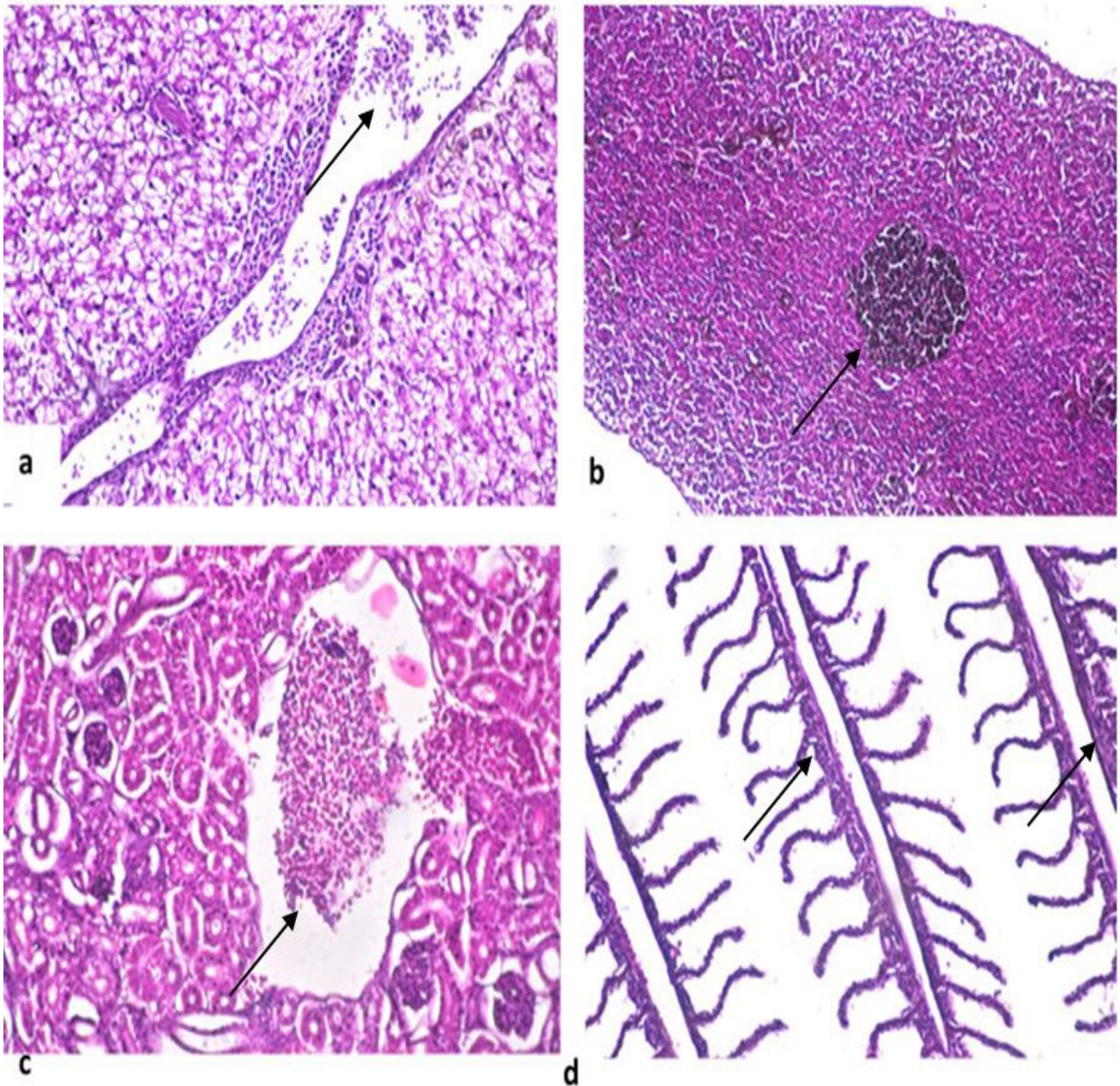


Fig.4: photomicrograph, European seabass (a) liver parenchyma showing inflammatory cells infiltration (black arrow) (H&EX200). (b) Spleen showing activation of melanomacrophage center (black arrow) (H&EX400). (c) Kidneys showing sever dilatation and congestion in the blood vessels between the tubules and glomeruli (black arrow) (H&EX400). (d) Gills showing slight hyperplasia of epithelial cells at the primary lamellae (black arrows) (H&EX400).

Histopathological examination of meagre swim bladder revealed inflammatory cells infiltration and fibrosis in the bladder wall (**Fig.5 a**) associated with congestion in the blood vessels (**Fig.5 b**). liver, there was hyperplasia of collagen fibers (**Fig.5 c**) Vacuolar degeneration of hepatocytes (**Fig.5d**). kidneys, there were sever dilatation and congestion in the blood vessels between the tubules and glomeruli (**Fig.5 e**). Concerning Spleen, there was ischemia in the pulps (**Fig.5 f**).

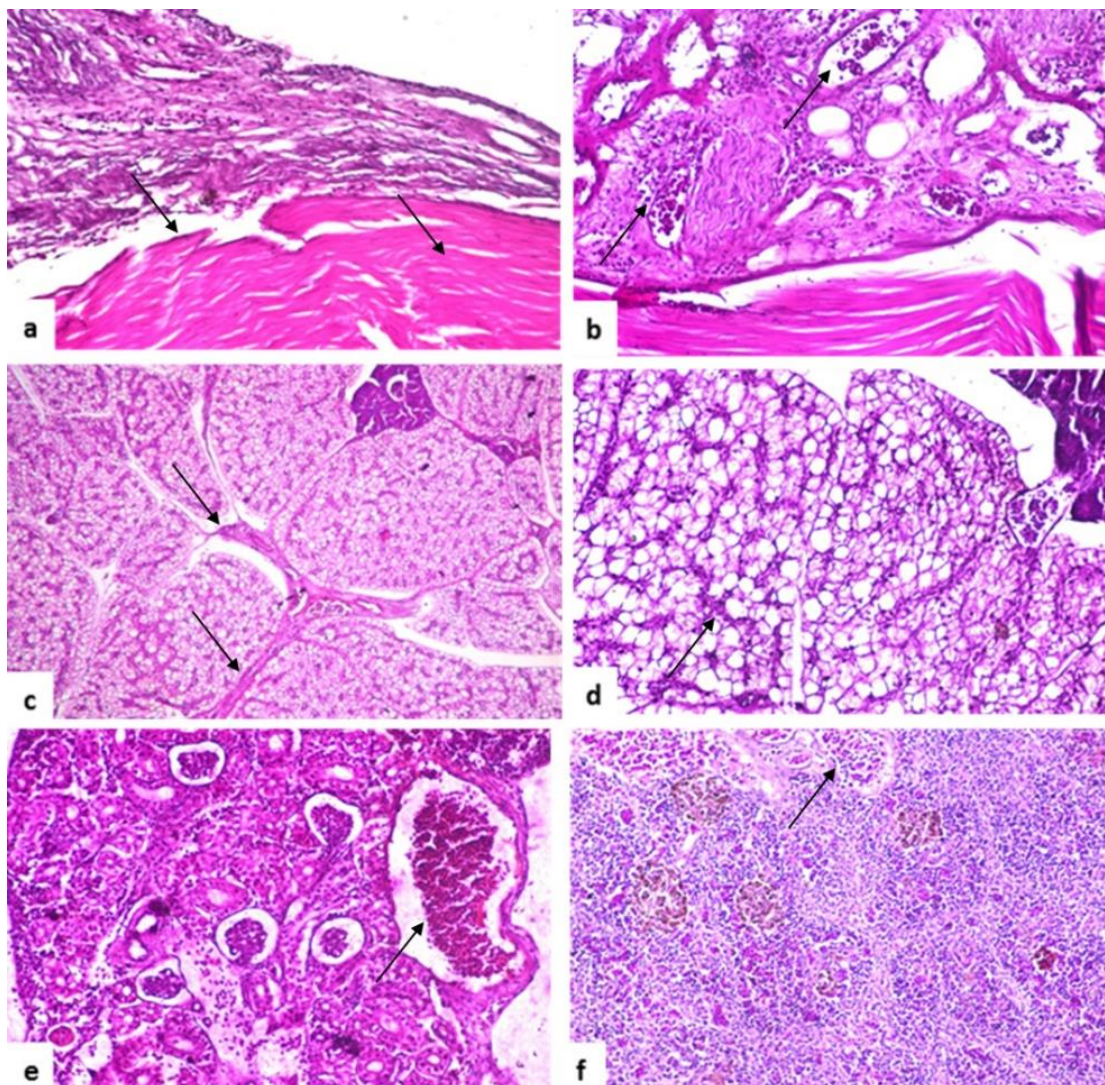


Fig.5: photomicrograph, Meagre (a) swim bladder wall showing inflammatory cells infiltration and fibrosis (black arrows) (H&EX200). (b) as well as congestion in the blood vessels (black arrows) (&EX200). (c) Liver showing hyperplasia of collagen fibers (black arrows) (H&EX200). (d) Higher magnification of liver showing Vacuolar degeneration of hepatocytes (black arrow) (H&EX400). (e) Kidneys showing sever dilatation and congestion in the blood vessels (black arrow) (H&EX200). (f) Spleen showing ischemia in the pulps (black arrow) (H&EX200).

DISCUSSION

Climate change is now considered one of the biggest challenges facing the national, regional, and global societies. Fisheries represent a significant industry in the Egyptian national income (**Abd El Tawab et al., 2018**). In aquaculture economy, marine fish represent the main investment options for northern coastal fishermen (**GFARD, 2020**). There are varieties of environmental stressors, such as improper water quality characteristics and increasing water temperature that might affect marine fish. In mariculture, these stressors, particularly declining water quality characteristics in Deeba Triangle, could promote the spread of infectious diseases, resulting in outbreaks in Egyptian mariculture (**Liu and Chen, 2004; Islam et al., 2022**).

Additionally, the water's chemical composition on these northern Egyptian coastlines is high in iron, which promotes the pathogenicity of vibrios (siderophores), particularly during the hot summer months (**El-mezayen et al., 2018; Eissa et al., 2020**).

In the present study, stressful aquatic environments, such as low dissolved oxygen, elevated water temperatures, inconsistently high salinity and a remarkably high increase in un-ionized ammonia, together with severe climatic conditions, have staggeringly compromised the fish immune status and increased their vulnerability to infection by ubiquitous bacteria, including *Vibrio* spp. and highly invasive bacteria (*Photobacterium* spp.) (**Rebl et al.,**

2020). There were similarities between our results and those described by **Abdelaziz et al. (2013)**, who recorded an abrupt increase in un-ionized ammonia, water temperature, and salinity during recurrent episodes of mortalities in European seabass and gilthead seabream.

In the current study, a total of 135 marine fishes were collected from several private marine farms located in Deeba Triangle, Shataa Damietta (Damietta governorate), and Ismailia province. In respect to the prevalence of infection, the total number of infected fish was 71 from 135 with a 52.6 % prevalence rate. These results nearly agreed with those of **El-Gendy (2013)** who recorded 44.1% prevalence rate among infected fish. In contrast, our result was slightly lower than that of **Moustafa et al. (2010)** who recorded 69.9% prevalence rate among infected fish. This variation in prevalence may be attributed to differences in sampling season, isolation locations, and species diversity.

The disease outbreaks in fish may be linked to the suppression of fish immune system. Such immune-suppression is positively correlated with severe climatic conditions such as an abrupt rise in water temperature and a subsequent decrease in dissolved oxygen (**Abdelaziz et al., 2013**). Immunologically, the aforementioned climatic changes are well known to have a significant impact on adaptive, innate and cellular immune responses (**Cascarano et al., 2021**). The clinical examination of the moribund fishes revealed the presence of skin haemorrhages, erosion, and ulcers. These clinical signs are predominantly noticed in septicemic bacterial infections, such as *Vibrio* and *Photobacterium* species (**Moustafa et al., 2010; Essam et al., 2016**). While the postmortem examination of the necropsied fishes revealed clear signs of septicemia manifested by congestion in the kidney, abdominal ascites, gill congestion, an enlarged liver with an engorged gall bladder, and splenomegaly. Our findings were in concordance with those reported by **Abdelaziz et al. (2017)**, **Abd El Tawab et al. (2018)**, and **Khalil and Abdel-latif, (2022)**.

Isolation and identification of *Vibrio* and *Photobacterium* isolates depend on the colonial characteristics of enriched and selective media as well as phenotypic and biochemical identification. Based on phenotypic, biochemical, and API 20 E identification systems, the bacterial isolates recovered from moribund fish were presumptively identified as *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Photobacterium damsela* subspecies *piscicida*. The phenotypic and biochemical characteristics of these

isolates were nearly the same as those reported by (**Essam et al., 2016; Eissa et al., 2018; Patel et al., 2018; Khalil and Abdel-latif, 2022**). Notably, the API 20 E system is usually used to investigate the biochemical reactions of bacterial strains. However, several molecular methods have been designed for accurate and quick identification of infectious bacteria in cultured and wild fishes (**Abdelslam et al., 2017**). The molecular identification is mainly dependent on 16S rRNA sequencing (**Chatterjee and Haldar, 2012**). Although sequencing the 16S rRNA genes has been shown to be effective in confirming the identification of the aforementioned pathogenic bacteria, the high equipment costs associated with this technique make it less favourable for diagnosing diseases in fish. These findings coincide with the results obtained by **Essam et al. (2016)** **Eissa et al. (2020)** and **Eissa et al. (2021)**, who used 16S rRNA gene to identify *Vibrio* and *Photobacterium* strains from moribund fishes.

In the current study, three different bacterial pathogens have been isolated from moribund fishes. The isolated bacteria were *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Photobacterium damsela* subspecies *piscicida*. These bacterial pathogens are highly recognizable fish bacteria that infect cultured marine fish and have been linked to mass mortalities in farmed seabass (*Dicentrarchus labrax*), seabream (*Sparus aurata*) and meagre (*Argyrosomus regius*) in numerous Egyptian regions (**Abdelaziz et al., 2013; Eissa et al., 2020; Eissa et al., 2021**).

In respect to seasonal prevalence, the highest seasonal prevalence was recorded during summer season, followed by autumn and the lowest during winter. These recorded results indicate that the high seasonal prevalence of bacterial infections was correlated with the high temperature recorded in summer season and the lowest was recorded in winter season, as reported by **Moustafa et al. (2010)**, **Mustapha et al. (2012)** and **Essam et al. (2016)**. This can be explained that the higher temperatures as a result of severe climatic change reduced immunity and decreased resistance to disease, so fish became more vulnerable to septicemic infection.

The histopathological examination of various organs of moribund fishes revealed that the liver showed focal hemorrhages in the parenchyma associated with severe congestion of blood vessels and severe degeneration in the hepatocytes. While kidneys showed mononuclear cell infiltration around renal tubules, diffuse vacuolar degeneration of tubular lining epithelium, severe dilatation and congestion in the blood vessels between the tubules and glomeruli. Spleen showed focal proliferation of

melanomacrophage center. The presence of histopathological alterations in the internal organs clearly explained the septicemic nature of *Vibrio* and photobacterium infections. The obtained results are consistent with **Mladineo et al., (2006) and Ragab et al., (2022) who** found the same alteration in different fish. The lesions in the hemopoietic tissue including kidney and spleen indicated that, the *Vibrio* and Photobacterium infection may suppress the immune system and give a chance to infection with other bacteria. The lesions in the splenic cells and the blood vessels may indicate the effect of the bacterial toxins on the endothelial lining of the blood vessels (**Hassan et al., 2015**). The tissue damage in various organs may also be attributed to the production of bacterial extracellular products, which reveal a critical role in the pathogenesis of vibriosis and photobacteriosis (**Eissa et al., 2018; Aly et al., 2020; Ragab et al., 2022**).

CONCLUSION

Vibriosis caused by *V. parahaemolyticus* and *V. alginolyticus* and photobacteriosis caused by *Photobacterium damsela* subspecies *piscicida* are significant bacterial diseases affecting cultured marine species. Vibriosis and Photobacteriosis eruptions are triggered with hot climate with consequent deterioration of pond's water quality. Local and systemic immunities are key factor in progression of these bacterial eruptions. Thus, enhancing immunity will decisively minimize the possibilities of such bacterial eruptions.

Availability of data and materials

All data are included in the manuscript.

Conflicts of interest

All authors declare that they have no conflict of interest.

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Author's Contributions

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