Ectoparasites and Bacterial Co-infections causing Summer Mortalities Among Cultured Fishes at Al-Manzala with Special Reference to Water quality parameters

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Abstract: Ectoparasites and bacterial co-infections were identified from fish kills during summer 2016 from pond cultured fishes at Al-Manzala fish farms. Diseased Nile tilapia, Common carp, Silver carp, and African catfish were examined clinically, bacteriologically and surveyed for ectoparasites to determine the mortality causes. Additionally, pond water was sampled during the survey period to explore any physicochemical abnormalities. Examined fishes were off food, lethargic, and have generalized septicemic signs; hemorrhages over the opercula, eye, and at the base of all fins with ulcerations over the skin, dorsal musculature and around the anus with evident high mortalities. Internally, congestion and hemorrhages over all the internal organs with bloody-tinged ascitic abdominal fluid. The bacterial isolates retrieved were *Vibrio alginolyticus*, *V. harveyii*, *Aeromonas hydrophilia*, *Enterococcus faecalis*, and *Edwardseilla tarda*. The identified parasites were; Monogenetic fluke (*Gyrodactylus* sp), ciliated protozoan parasites (*Icthyophithirius multifiliis* and *Trichodina* sp), and ectoparasitic crustacean parasites (*Lernaea cyprinacea,* and *Argulus* spp). Water examination revealed elevated organic matter content, un-ionized ammonia % (NH_3) (0.9 mg/l), Iron (2.45 mg/l), Copper (1.55 mg/l) over the permissible limits. In this context, it was found that the identified ectoparasites may act as a portal of entry or vehicle for secondary bacterial invaders together with bad water quality parameters, and all those factors were combined for the occurrence of mortalities of cultured fishes at Al-Manzala fish farms.

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1. Introduction

In Egypt, Fish farms are clustered in the Northern Nile Delta Region surrounding the four Delta Lakes (Maruit, Edko, Boruls, and Manzala) (**GAFRD, 2010**). Lake Manzala can be considered as the main source of cheap fish for human beings in Egypt, but there are several constraints for its fish production, mainly the heavy metal pollutants (**Zahran, 2008**).

Fish pathogen co-infections frequently occurred in the field, whereas two or more different fish pathogens were actively infecting and harm the same host. They can be defined as concurrent mixed polymicrobial infections that caused serious impacts on the affected fish (**Kotob *et al.*, 2016**). Fish parasites help to raise the susceptibility of fish for secondary bacterial invaders because they act as a vehicle, or carrier for their transmission to fish (**Holzer *et al.*, 2006**). The parasitism caused fish stress which will reduce the fish resistance to the bacterial co-infections, and they caused a portal of entry to secondary invaders (**Bowers *et al.*, 2000**).

The synergistic effects of parasitic and bacterial concurrent infections in different fish species were frequently reviewed in several studies. Monogenean flukes enhanced the invasion of several bacterial fish pathogens, whereas, **Xu *et al.* (2007)** assumed that *Gyrodactylus niloticus* served as a portal of entry and a mechanical vehicle to *Streptococcus iniae* invasion to Nile tilapia especially under intensification in culture ponds. Additionally, *Dactylogyrus intermedius*, enhanced the *Flavobacterium columnare* invasion to the goldfish susceptibility to (**Zhang *et al.*, 2015**).

The ectoparasitic ciliated protozoans (especially *Ichthyophthirius multifiliis* and *Trichodina* sp), were similarly can facilitate the bacterial co-infections to several fish species, where they can damage the epithelium of fish gills and skin (**Matthews, 2005**). Concerning the *I. multifiliis* co-infections with fish bacterial pathogens; it was found that heavy mortalities of Channel catfish were demonstrated when they were co-infected with *I. multifiliis* before being experimentally infected with *Edwardseilla tarda* (**Shoemaker *et al.*, 2012** and **Xu *et al.*, 2012a, b**). Additionally, significant fish kills were also

defined in co-infection of *I. multifiliis* with *A. hydrophilia* increased (**Xu *et al*., 2012c**). In Nile tilapia, similar impacts were demonstrated in the co-infections between *I. multifiliis* and *S. iniae* (**Xu *et al*., 2009**). *Trichodina* sp. co-infections with bacterial fish diseases were found in channel catfish, whereas, their susceptibility to *S. iniae* or *S. agalactiae* was greatly elevated following co-infection with *Trichdina* sp. and mass mortalities were found (**Evans *et al*., 2007**).

The infestation with crustacean parasites caused damage the fish skin which assists the secondary bacterial invasion. It was reviewed that, the experimental infestation of the Atlantic Salmon with Sea loose improved the co-infection with *Piscirickettsia salmonis* (**Lhorente *et al*., 2014**). Also, the infestation with fish lice (*Argulus* sp) elevated the responsiveness of rainbow trout to *F. columnare* co-infection (**Bandilla *et al*., 2006**). Therefore, the study was focused mainly on the determination of the parasitic and bacterial co-infections isolated from diseased cultured fishes from Manzala farms and their relationship with water quality parameters.

2. Material and Methods

Fish species and sampling area: -

During summer 2016, heavy mortalities occurred in pond cultured fishes at Al-Manzala fish farm related to General Authority of Fish Resources and Development (GAFRD), Dakahlia province, Egypt. Fifty fish were sampled from diseased Nile tilapia (*Oreochromis niloticus*), Common carp (*Cyprinus carpio* L), Silver carp (*Hypophthalmicth molitrix*), and African catfish (*Clarias garipenus*), and examined clinically, bacteriologically and parasitologically, to find out the direct mortality causes.

Clinical examination of the surveyed fishes: -

Clinical signs of the diseased sampled fishes was done to determine any clinical signs (**Amlacher, 1970**), while the postmorteum (PM) examination was done on the freshly dead fishes for a demonstration of different tissue lesions (**Conroy and Herman, 1981** and **Schäperclaus *et al.*, 1992 a, b)**.

Bacteriological examination (Isolation methods and biochemical identification): -

Surface sterilization of fish skin by swapping with 70% ethanol and inocula were sampled from the fish liver and kidney with full aseptic sampling to be inoculated into a tryptic soya broth and incubated at 20°C for 24 - 48 hrs then sub-cultured on Thiosulphate Citrate Bile salts sucrose (TCBS) agar medium. Other inocula were cultured onto R.S medium (**Shotts and Rimler, 1973,** and **Sağlam *et al*., 2006**) at 37°C for 24 hours. After the colony purification, pure culture was preserved onto a nutrient agar slope (for detection of the bacterial pigmentation) or semisolid agar medium (to be served as a stock culture for further biochemical identification). The preserved isolates were subsequently stored for a long preservation period in glycerol 15% and kept at – 80 °C. The identification of the preserved bacteria was determined (**Baumann and Baumann, 1981** and **Austin and Austin, 2007**).

Biochemical identification was demonstrated using traditional biochemical tests including catalase, cytochrome oxidase (oxidase strips), motility, citrate utilization using Simmons's citrate (Remel), sugar utilization using triple sugar iron (TSI), oxidation and fermentation of glucose (BD Biosciences), and esculin hydrolysis using bile esculin agar (Remel). The enrichment and identification were done (**Buller, 2004**).

Parasitological examination: -

Skin and gill smears were taken for ectoparasites examination (**Lucky, 1977**), and parasites were identified according to the parasitological identification keys (**Hoffman, 1967, 1999; Lucky, 1977; Woo, 1995; Paperna, 1996;** and **Zhokhov, 2010**). Large parasites can be seen by naked eye.

Water sampling: -

Water samples were taken during the survey period, in dark brown bottles at a depth of half a meter from the water surface. The samples were acidified with nitric acid and chilled on ice box for transport to the laboratory for heavy metals determination.

Determination of physicochemical water properties: -

For demonstration of the physicochemical properties of water, a Dissolved Oxygen (DO) meter, Salinometer, pH meter and Kits for measuring the levels of unionized ammonia and Sulphate in the water (USA, Virginia Company) were used.

Spectrophotometric method for detecting the levels of heavy metals: -

For detection of the heavy metal content in water, Atomic Absorption Spectrophotometry (Model Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK,) instrument was used (**APHA, 1995**). The concentrations of heavy metals were expressed as mg / L for water.

Statistical Analysis: -

The frequencies of ectoparasites and bacterial isolates retrieved from the surveyed fish species were statistically analyzed by Chi-square test (**SAS, 2002**).

3. Results: -

Results of clinical examination of diseased cultured fishes: -

The examined diseased fishes were off food, lethargic, exhibit sluggish movement just before death. They have shown generalized septicemic signs (Plates-1, 2, 3 & 4), such as opercular and ocular hemorrhages and hemorrhages at the base of all fins

with ulcerations over the skin and back and around the anus. Internally, congestion and hemorrhages over all the internal organs with hemorrhagic ascitic fluid.

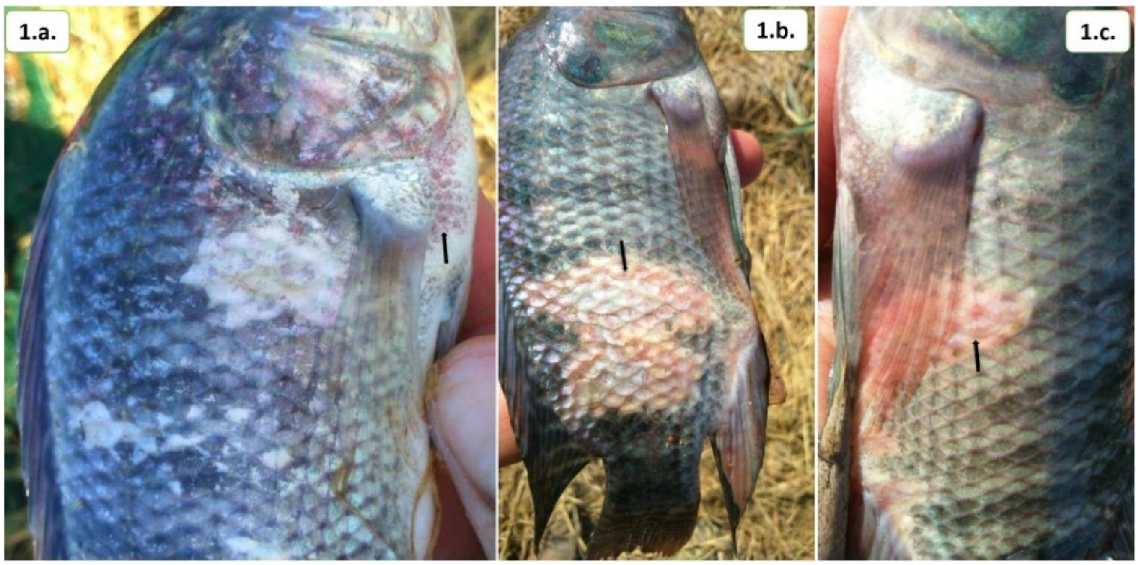

Plate-1: Diseased cultured Nile tilapia (*Oreochromis niloticus*) showed generalized septicemic signs as haemorrhages over the operculum and at the base of pectorals (arrow) and (**photo 1.a.**), large haemorrhagic ulcerations at the back especially at the caudal peduncle (arrow) (**photo 1.b.**) and at the belly (arrow) (**photo 1.c.**).

Plate-2: Diseased cultured Common carp (*Cyprinus carpio L.*) showed huge hemorrhagic ulcer over the dorsum (arrow) and (**photo 2.a.**), bloody exudate in the abdominal cavity (arrow) (**photo 2.b.**) with congested internal organs and hemorrhages over the liver (arrow) (**photo 2.c.**) and swim bladder.

Plate-3: Diseased cultured Silver carp (*Hypophthalmichthys molitrix*) showed ulcer over the dorsum (arrow) (**photo 3.a.**), hemorrhages at the base of pectoral fin (arrow) with ocular hemorrhage (arrow) (**photo 3.b.**).

Plate-4: Diseased cultured African catfish (*Clarias garipenus*) showed ulcerations below the dorsal fin (arrow) (**photo 4.a.**), at the back musculature (arrow) (**photo 4.b.**), with deep opened ulcers and hemorrhages over the anus (arrow) (**photo 3.c.**).

Results of bacteriological examination of the diseased fishes: -

Table (1): Morpho-biochemical characteristics of bacterial isolates retrieved from diseased cultured fishes during summer mortalities from Al-Manzala farms.

Items	*Ed. tarda* (51 isolates)	*A. hydrophilia* (72 isolates)	*V. harveyi* (63 isolates)	*V. alginolyticus* (54 isolates)	*E. faecalis* (32 isolates)
Gram stain reaction	G-ve	G-ve	G-ve	G-ve	G-ve
Cell morphology	rods	rods	Rods	Rod-shaped bacilli	Cocci
Motility	+	+	+	+	-
Catalase test	+	+	+	+	-
Cytochrome oxidase test	-	+	+	+	-
Vogus Proskauer test	-	+	-	+	+
Growth on TSA+8% NaCl	-	-	-	+	-
Growth on TCBS	-	-	+	+	-
Growth on RS medium	-	Small, smooth, and yellow colonies	-	-	-
Hemolysis of RBCs	Non-hemolytic	hemolytic	hemolytic	Non-hemolytic	Non-hemolytic
Sensitivity to 0/129 disc	-	-	+	+	-
Production of: -					
H2S production	+	-	-	-	-
Indole (peptone H2O)	+	+	-	+	-
Urease	-	-	-	-	-
Utilization of: -					
Simmon's Citrate	-	+	+	-	-
D-Mannitol	-	+	+	+	+
Glucose	+	+	-	+	+
Lactose	-	-	-	-	+
L-Arabinose	-	+	-	+	-
Maltose	-	+	-	+	+
Sorbitol	-	+	-	+	+
Sucrose	-	+	-	+	+

(+): positive, (-) negative

Five main bacterial fish pathogens were retrieved from cultured fishes from Al-Manzala farms during last summer mortalities were *V. alginolyticus, A. hydrophilia, E. faecalis, E. tarda,* and *V. harveyii*, and their prevalence % were 19.85, 26.47, 11.76, 18.75 and 23.16 respectively (**Table 2**). It was found that *V. harveyii* was the main predominant isolate.

Table (2): Prevalence % of the bacterial isolates retrieved from summer mortalities of diseased cultured fishes at Al-Manzala Farms.

	No. of isolates	Prevalence %
V. alginolyticus	54	19.85
A. hydrophilia	72	26.47
E. faecalis	32	11.76
Ed. tarda	51	18.75
V. harveyii	63	23.16
Total No. of isolates	272	

The prevalence % of the bacterial fish pathogens identified from diseased cultured fishes from Al-Manzala farms (**Table 3**, and **Fig. 1**) showed that *V. alginolyticus* was a predominant bacterial isolate from Common Carp (36.36 %), *A. hydrophilia* from Nile tilapia (33.33%), *E. faecalis*, *Ed. tarda* and *V. harveyii* from African catfish (15.38%), (32.05), and (25.64) respectively.

Table (3): Prevalence % of the bacterial isolates among the examined cultured fishes at Al-Manzala Farms.

Fish species / Isolates	Common carp		Nile tilapia		African catfish		Silver carp		Chi-square value	P value
	No.	Prevalence %	No.	Prevalence %	No.	Prevalence %	No.	Prevalence %		
V. alginolyticus	20	36.36	11	16.67	8	10.26	15	20.55	14.38	(P<0.01)
A. hydrophila	14	25.45	22	33.33	13	16.67	23	31.51	6.42	(P>0.05)
E. faecalis	5	9.09	5	7.58	12	15.38	10	13.7	2.74	(P>0.05)
Ed. tarda	3	5.45	15	22.73	25	32.05	8	10.96	19.03	(P<0.001)
V. harveyii	13	23.64	13	19.7	20	25.64	17	23.29	0.72	(P>0.05)
Total No. of isolates	55		66		78		73			

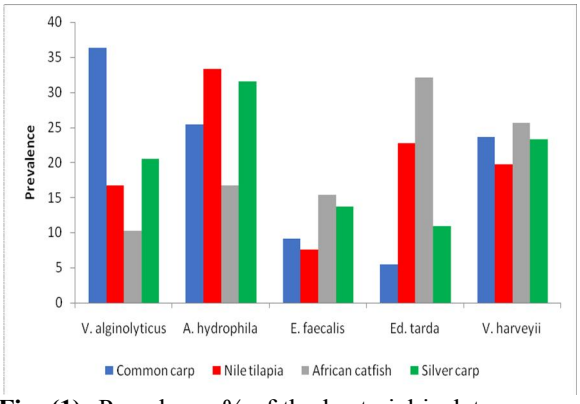

Fig. (1): Prevalence % of the bacterial isolates among the examined cultured fishes at Al-Manzala Farms.

Results of parasitological examination of diseased fishes: -

Results showed that the infestation percentages (%) in African catfish, Nile tilapia, Common carp and Silver carp were 60, 76, 58 and 20 % respectively. The identified parasites were; Monogenetic fluke (Gyrodactylus sp), ciliated protozoan parasites (*I. multifiliis* and *Trichodina* sp) and ectoparasitic crustacean parasites (*L. cyprinacea,* and *Argulus* spp).

Concerning the identified ectoparasites from diseased cultured African catfish (**Table 4**, and **Fig. 2**) and Nile tilapia (**Table 5**, and **Fig. 3**), the prevalence % was elevated *Trichodina* sp, *I. multifiliis* and *Gyrodactylus* sp (P<0.0001), with no crustacean parasites infestation.

Table (4): Infestation and prevalence percentages (%) of ectoparasites isolated from cultured African catfish from Al-Manzala farms.

	No. of examined fish	No. of infested fish	% of infested fish	No. of the identified parasites	Prevalence %
I. multifiliis	50	30	60	16	26.67
L. cyprinacea				0	0.00
Argulus spp				0	0.00
Trichodina spp				25	41.67
Gyrodactylus spp				19	31.67
Total No. of identified parasites =				60	54.38 (P<0.0001)

Table (5): Infestation and prevalence percentages (%) of ectoparasites isolated from cultured Nile tilapia from Al-Manzala.

	No. of examined fish	No. of infested fish	% of infested fish	No. of the identified parasites	Prevalence %
I. multifiliis	50	38	76	12	22.22
L. cyprinacea				0	0.00
Argulus spp				0	0.00
Trichodina spp				19	35.19
Gyrodactylus spp				23	42.59
Total No. of identified parasites =				54	52.18 (P<0.0001)

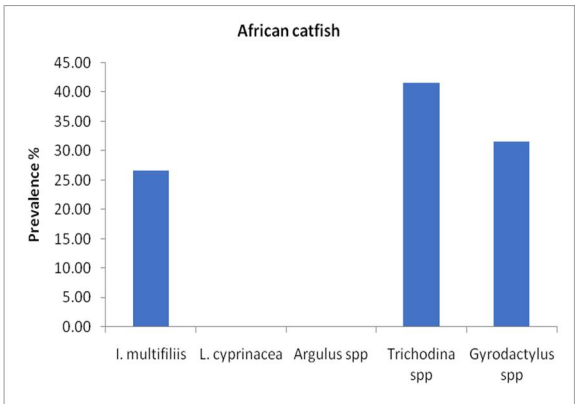

Fig. (2): Infestation and prevalence percentages (%) of ectoparasites isolated from cultured African catfish from Al-Manzala.

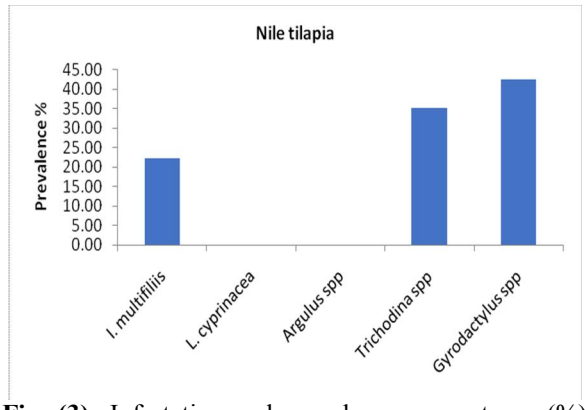

Fig. (3): Infestation and prevalence percentages (%) of ectoparasites isolated from cultured Nile tilapia from Al-Manzala farms.

Table (6): Infestation and prevalence percentages (%) of ectoparasites isolated from cultured Common carp from Al-Manzala farms.

	No. of examined fish	No. of infested fish	% of infested fish	No. of the identified parasites	Prevalence %
I. multifiliis	50	29	58	11	12.64
L. cyprinacea				21	24.14
Argulus spp				7	8.05
Trichodina spp				31	35.63
Gyrodactylus spp				17	19.54
Total No. of identified parasites =				87	24.94 (P<0.0001)

Table (7): Infestation and prevalence percentages (%) of ectoparasites isolated from cultured Silver carp from Al-Manzala farms.

	No. of examined fish	No. of infested fish	% of infested fish	No. of the identified parasites	Prevalence %
I. multifiliis	50	10	20	12	19.35
L. cyprinacea				8	12.90
Argulus spp				7	11.29
Trichodina spp				18	29.03
Gyrodactylus spp				17	27.42
Total No. of identified parasites =				62	10.2 (P<0.05)

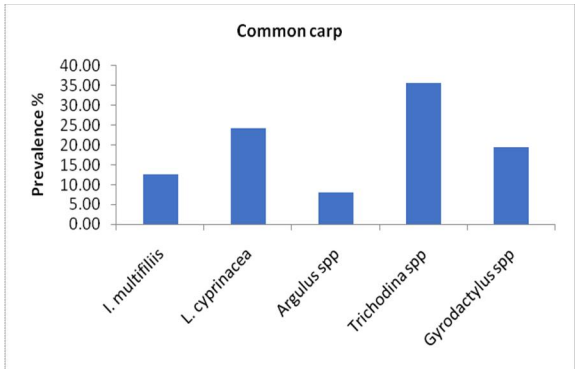

Fig. (4): Infestation and prevalence percentages (%) of ectoparasites isolated from cultured Common carp from Al-Manzala farms.

Concerning the identified ectoparasites from diseased cultured Common carp (**Table 6**, and **Fig. 4**) (P<0.0001) and Silver carp (**Table 7**, and **Fig. 5**) (P<0.05), the prevalence % was elevated *L. cyprinacea*, *I. multifiliis,* and *Trichodina* spp.

Concerning the prevalence % of the identified parasites among the examined fish species (**Fig. 6**), it was found that there was a higher infestation of *I. multifiliis, Gyrodactylus* sp, and *Trichodina* sp were found in African catfish and Nile tilapia. Furthermore, the crustacean parasites (*L. cyprinacae* and *Argulus* sp) were only identified from Common carp and Silver carp.

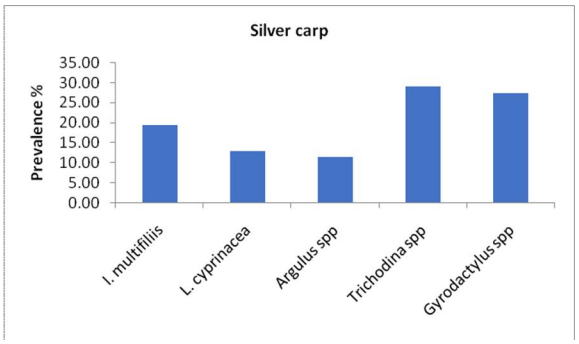

Fig. (5): Infestation and prevalence percentages (%) of ectoparasites isolated from cultured Silver carp from Al-Manzala farms.

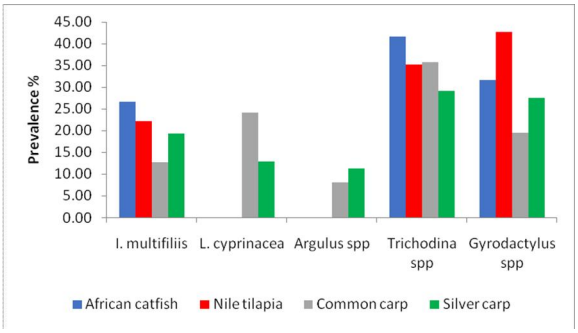

Fig. (6): The prevalence % of the identified parasites among the examined cultured fish species from Al-Manzala farms.

Results of water quality parameters of Al-Manzala farms: -

Physicochemical properties of water of Al-Manzala farms (**Table 8**), showed elevated nitrite, Un-ionized ammonia, hydrogen sulfide levels and organic matter content. Additionally, there were higher levels of Iron (Fe), Copper (Cu) and Nickel (Ni) over the permissible levels.

Table (8): Physicochemical properties of water of Al-Manzala farms.

Water parameters	Reading	Permissible limits (PL) (WHO, 1989)
Dissolved oxygen (mg / L)	5.5	5-6
Salinity (PPT)	30 - 34	-----
Temperature (^{o}C)	30	-----
Nitrite (NO_2) (mg / L)	0.04	0.01
Unionized ammonia (NH_3) (mg / L)	0.90	0.01
Organic matter (mg / L)	3.79	2-3
Hydrogen sulpide (mg / L)	153.1	70-120
pH	8.4	8.0 - 8.5
Iron (mg / L)	2.45	1.00
Copper (mg / L)	1.55	0.2
Nickel (mg / L)	1.15	0.001
Zinc (mg / L)	0.5	2.0

4. Discussion

Fish diseases are stress related and have multifactorial nature (**Austin and Austin, 1993**). Parasitic and bacterial co-infections isolated from diseased cultured fishes at Al-Manzala during hot weather in summer were numerous and they caused high mortalities. In this study, the bacterial isolates retrieved were *V. alginolyticus*, *V. harveyii*, *A. hydrophilia*, *E. faecalis*, and *Ed. tarda*. Also, the identified parasites were; Monogenetic fluke (*Gyrodactylus* sp), ciliated protozoan parasites (*Icthyophithirius multifiliis* and *Trichodina* sp), and ectoparasitic crustacean parasites (*Lernaea cyprinacea,* and *Argulus* spp). It was found that the parasitic and bacterial fish pathogens identified from cultured fishes at Al-Manzala have documented by several authors; whereas **El-Refaey (2013)** demonstrated that *A. hydrophilia*, *Pseudomonas fluorescence*, *Flexibacter columnaris*, *Streptococcus faecalis*, *E. coli*, *Y. ruckeri*, *Citrobacter* sp and *Ed. tarda* have been isolated from diseased *Oreochromis niloticus*, *Clarias gariepinus* and Grey mullets obtained from waters of Lake Manzala. Also, **Zaky and Ibrahim (2017)** has screened the bacterial and fungal infections of Nile tilapia in Lake Manzala and they isolated enteropathogenic bacterial pathogens, *Klebsiella pneumoniae*, *E. coli*, *Proteus* sp and *Citrobacter freundii*. Additionally, **Awadin *et al.* (2012)** surveyed the parasites infesting cultured African catfish, *Clarias garipinus* from Al-Manzala fish farm and they demonstrated *Quadriacanthus clariadis*, *Orientocreadium* sp, and

Polyonchobothrium sp.

Regarding the clinical signs of the surveyed diseased cultured fishes at Al-Manzala, it was found that generalized septicemic signs were observed especially hemorrhage in the skin, at the base of fins, superficial and deep ulcerations of the skin, dorsal musculature. Also, there was congestion of the gills, liver, kidney with an accumulation of bloody tinged exudates in the abdominal cavity. These findings were in concordance to that reported by **El-Bouhy (1995)**, **El-Gamal (1995)**, **Eissa *et al.* (1996), El-Ashram (2002),** and **Gamal *et al.* (2002).**

The lesions observed may be occurred due to various factors, 1) potent bacterial proteases (proteolytic enzymes) (**Toranzo *et al.*, 2005)**, and 2) The synergistic parasitic and bacteria interactions which increase the disease occurrence (**Kotob *et al.*, 2016**).

The ectoparasites identified as *Gyrodactylus* sp, *I. multifiliis, Trichodina* sp, *L. cyprinacea,* and *Argulus* sp favoured the invasion of bacterial pathogens of the diseased cultured fishes; a) they can act as a portal of entry of secondary bacterial pathogens, b) caused a damage to the host fish, and c) they can transmit bacteria to their host when feeding on host tissues (**Bowers *et al.*, 2000**).

It has been observed that *G. niloticus* served as a mechanical vehicle to *Strep. iniae in O. niloticus* **(Xu *et al.*, 2007**) and *D. intermedius*, enhanced the *Flavobacterium columnare* invasion to the gold fish (**Zhang *et al.*, 2015**). This may be caused by the mechanical damage of the fish epithelium caused by monogenetic flukes. Additionally, they caused down-regulation of the gills and kidneys immune genes (TGF-β and complement 3) (**Zhang *et al.*, 2015)**, and this improved the bacterial invasion.

Also, Ciliated protozoans have increased bacterial invasion to host fishes by damaging the epithelium of the gills and skin (**Matthews, 2005**). It was found that *I. multifiliis* co-infection with *Ed. tarda* and with *A. hydrophilia* can increase the mortalities of Channel catfish (**Shoemaker *et al.*, 2012** and **Xu *et al.*, 2012 a, b, c**). Also, high mortalities were demonstrated in Nile tilapia, where the co-infections between *I. multifiliis* and *S. iniae* occurred (**Xu *et al.*, 2009**). Additionally, *Trichodina* sp. increased the susceptibility to *S. iniae* or *S. agalactiae* (**Evans *et al.*, 2007**). Furthermore, it was reviewed that, the infestation with fish lice (*Argulus* sp) elevated the responsiveness of rainbow trout to *F. columnare* co-infection (**Bandilla *et al.*, 2006**).

In this study, the physicochemical water quality parameters of Al-Manzala farms as organic matter, and the un-ionized ammonia were elevated over the permissible limits. It was demonstrated that the continuous unhygienic disposal of agricultural, chemical and sewage pollutants in domestic water supply usually lead to deterioration of the water quality parameters (**Abbassy *et al.*, 2003**), which caused environmental stresses on cultured fishes. It was found that the deterioration of water quality and environmental stresses can predispose for fish bacterial infections (**Plumb *et al.*, 1976, Abdel-Latif, 2013,** and **El-Far *et al.*, 2015**). Various bacterial isolates were identified from the polluted water of Al-Manzala like the fecal coliforms (*E. coli*, *Enterococci,* and *Clostridium perfringes)*, and some pathogenic species such as, *A. hydrophilia* and *A. sobria*, *Ps. aeruginosa*, *Ps. fluorescence* and *V. anguillarum*, were isolated from the gills, intestines, and musculature of the fish specimens **(El-Sarangawy, 1990)**.

Elevated Iron (Fe), Copper (Cu) and Nickel (Ni) levels were associated with the elevated Vibrios identified from diseased cultured fishes from Al-Manzala. This may be attributed to the properties of Iron as an excellent oxygen transporter, iron tends to stimulate the growth of common bacteria (**Kutsky, 1982**), the in vivo acquisition of iron by bacterial pathogens by chelation to host iron-binding protein (transferrin and lactoferrin) (**Bullen, 1981**), and different pathogens adopt numerous strategies to overcome iron restriction (**Weinberg, 1995**) such as siderophores (**Halle and Meyer 1992**, and **Anderson *et al.*, 1994**).

Also, Copper can increase the infection with Vibriosis especially *V. Anguillarum* infection (**Rodsaether *et al.*, 1977**). This may be because the exposure to copper toxicity resulted in coagulation of the mucus layer of the gills, which inhibited oxygen transport and caused respiratory stress or reduced the number of lymphocytes and granulocytes in the blood, leading to reduced phagocytosis (**Mushiake *et al.*, 1985**).

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