Research Article

Influence of Parasite Infestation and Water Quality Deterioration During Mass Fish Mortality Event in Manzala Lake and its Corresponding Fish Farms

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Abstract | The present study estimated the relation between the prevalence of fish parasitic infestation and water quality deterioration thus evaluated their role in mass fish mortality event in Manzala Lake and its corresponding fish farms. Fish samples together with water samples were collected from 3 affected farms and 6 locations along Manzala Lake. Field observations and physicochemical analysis of water revealed deterioration of water quality indicating marked pollution. Parasitological examination of fish samples showed high total prevalence of 68.18% with 10 different ectoparasite species (4 Crustaceans, 4Monogeneans and2 Protozoans) of which the crustaceans recorded the highest prevalence (63.67%). In the present study, Manzala Lake and its corresponding fish farms considered new localities for the detected crustacean and monogenean species. Significant positive correlations between the prevalence of parasitic infestation and water quality parameters were reported. Pathological finding of the affected fish tissues revealed deleterious responses especially in gill tissues. Result analysis concluded that the associated impacts of ectoparasitic infestation together with the water quality deterioration played a significant role in mass mortalities event . The study recommended periodic monitoring of the lake water .Also, the proper sanitary and regular disposal of the waste remnants of clearing and dredging processes that were taking place by the government in the lake. Additionally, the bio security strategies should be applied in fish farms, also the dependency of farmers on fish seeds for farm stocking from natural open water instead of the hatcheries should be avoided to prevent the entrance of parasites and other undesirable organisms from open seas to the lake and corresponding fish farms. This study is the first to investigate the prevalence of parasite infestation in relation to water deterioration and their role in the mass mortality of fish during 2019 in Manzala Lake and corresponding fish farms.

Keywords | Fish, Parasites, Water deterioration, Mortality.

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INTRODUCTION

Fish parasitic infestation and aquatic pollution are considered of the greatest problems that threaten fish population and consequently human health worldwide. Ectoparasitic species of fish reported to have deleterious impact on their host tissues and thus assist the secondary pathogen invasion such as bacteria and viruses (Nofal and

Abdel –Latif, 2017). In Egypt, the lakes are economically important fishing source as they represent about 20% of the total areas of fish catch in Egypt (GAFRD, 2017). Furthermore, they provide vital habitat diversity for aquatic birds. Manzala Lake is the largest of the coastal northern lake in Egypt located between 31˚00´ - 31˚30´ N latitude and 31˚45´ - 32˚30´ E longitude. It is bordered from the west by Nile – Damietta branch, from east by Suez Canal (connected with the lake through El-Qabuty canal) and from the north by Mediterranean Sea. The lake is a brackish shallow lagoon has a depth of 0.5 – 1.0 m (Elmorsi et al., 2017). El – Boghdady, El – Gamil and Ashtom El – Gamil straits are the inlets permit water and biota exchange between Manzala Lake and Mediterranean Sea (El-mezayen et al., 2018). The northern part of Manzala Lake characterized by high salinity due to the connection with the Mediterranean Sea while the southern characterized by low salinity because of the received high amount of drainage water through many drains. In the north of the lake, the highly productive marine fish farms were clustered in Shata and Al Dieba zone. Manzala Lake is subjected to different aspects of pollution as huge amount of domestic, agriculture and industrial wastewater discharged through Hados, El Sarw, Bahr El Bakar and Farskor drainin addition to the Bioprocessing Plant and El Anania purification station. (Wally, 2016).

Mass fish mortality events were reported worldwide and mostly attributed to environmental problems (Lasheen et al., 2012). Pathogen invasion also reported as primary cause of fish mortalities including viral (Hedrick et al., 2000), bacterial (Nofal and Abdel-Latif, 2017) and parasitic infestation (Salama and Yousef, 2020). In Egypt, repeated cases of fish mass mortalities were recorded in different aquatic systems such as River Nile branches (Mahmoud et al., 2014a) as well as freshwater and marine fish farms (Ismael, 2012; Abou El – Gheit et al., 2012; Nofal and Abdel-Latif, 2017). A disaster of mass fish mortality was erupted during May and June, 2019 among farmed as well as wild fish populations in Manzala Lake, Egypt resulted in great economic and social problems. This case necessitated the conduct of the current study to investigate the prevalence of parasitic infestation in relation to the water quality parameter and evaluated the role of their associated impacts in this mortality event, thus we can put recommendations to prevent the recurrence of such disaster.

MATERIALS AND METHODS

Field Visits

Visits were conducted for recording the history of event, clinical finding and behavioural changes among the affected fishes. Also, fish and water samples were obtained for laboratory examination.

Fish And Water Samples

A total of 220 moribund and freshly dead fish samples together with water samples were collected from 3 affected marine fish farms and from the free fishing areas of Manzala Lake (Figure 1). The sampled fish species were *Dicentrarchus labrax, Sparus aurata, Argyrosomus regius, Mugil spp.* and *Tilapia spp*. Samples were transported to the laboratory of parasitology, Faculty of Veterinary Medicine, Cairo University for further examination. Water samples were collected in one litre sterile bottles labeled and transferred to the laboratory in ice box with ice gel bags. Temperature and salinity of water (by using portable optical TDS salinometer/refractometer), dissolved oxygen (by digital oxygen meter, HANNA-HI9142), EC (by digital conductivity meter AD-30: EC/TDF) and PH value (using PH meter, HANNA- mod. HI8314) were measured in situ during sampling and reconfirmed through laboratory analysis.

Figure 1: Manzala Lake Map showed the sites of sampling: (1-3) Marine fish farms in the northern area in Manzala Lake, (4-9) Free fishing areas of Manzala Lake, (5) Berket Shata, (6) El-Saiala, (7) El-Zarka, (8) Al-Homra. (9) El-Hosayneya. Map was carried out using ArcGIS (ArcMap) version 10.1 Software.

Parasitological Examination

Fish samples were grossly and microscopically examined for external parasites on skin, fins, eyes, gills, mouth and branchial cavities. The isolated parasites were prepared, and permanently mounted according to (Prichard & Kruse, 1982). Digital microphotographs and measurements were taken on fixed parasites by using light microscope (Olympus BX-43, Basler Germany) with digital camera (Olympus DP-27, Basler Germany). Prevalence of recorded parasites was calculated according to the guidelines of (Bush et al., 1997). The detected crustacean and monogenean species were identified according to (Antonelli et al., 2010; Abou Zaid et al., 2018; Morsy et al., 2018; Mahmoud et al., 2014b; 2019; WA Tadros et al., 2020). Identification of the recorded protozoan species was done according to the description reported by (Lom & Dyková, 1992; El-Mansy, 2005; Abu El Wafa et al, 2011).

Physical And Chemical Examination Of Water

Water samples were analyzed physically and chemically as described by the standard provided by the American Public Health Association (APHA, 2005) to evaluate the water quality. The physical analysis included specific gravity, pH, electrical conductance (EC), salinity, turbidity, dissolved oxygen (DO), and total dissolved solids (TDS). The investigated chemical parameters were the total alkalinity, hardness, carbonate (CO_3^{2}(-, bicarbonate (HCO_3 (-, ammonia (NH_3), nitrate (NO^-_2), nitrite (NO^-_3), phosphate ($PO_4$3(-), sulphate (SO_4^{2}-(, fluoride (F -), chloride (Cl (-, Sodium (Na+), potassium (K+), magnesium (Mg2+), Calcium (Ca2+), bromide (Br+), and lithium (Li+). All measurements were implemented following the American Public Health Association's protocols (APHA, 2005; Baird et al., 2017). Analysis of water samples was conducted in the department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University.

Pathological Examination

Fish samples were subjected to necropsy according to the technique mentioned by Weber & Govett, 2009 and grossly examined for any lesions. For histopathological examination, tissue specimens of gills, skin, intestine, liver and spleen of the freshly dead fish samples were fixed in 10% neutral buffered formalin. Tissues were then processed for paraffin wax, embeded and sectioned at 4 µm thickness using microtome. The tissue sections were routinely stained with hematoxylin and eosin (H &E) (Bancroft et al., 1990), examined under light microscope (Olympus BX-43, Basler Germany) and captured using digital camera (Olympus DP-27, Basler Germany).

Statistical Analysis

Independent sample t-test was used to compare means of water parameter from various locations. Bivariate Pearson correlation coefficient (*r*) was used to assess the association between physical and chemical water quality variables across study sites. Positive or negative *r* values ranged from 0.50 to 1.00 indicated strong correlations. Chi-square test for independence was used to test the relationship between parasitic infestation rates and different locations, as well as fish species. A $p \leq 0.05$ was considered significant. PASW Statistics Version 18.0 software (SPSS, Chicago, IL, USA) was used for analysis. Map was carried out using ArcGIS (ArcMap) version 10.1 Software.

RESULTS

Field Visits

Field visits showed that most of the marine fish farms corresponding to Lake Manzala Lake were affected. Thousands of dead and dying farmed *Dicentrarchus labrax, Sparus aurata, Argyrosomus regius and Mugil species* (150-250 g) were noticed in ponds and along their sides (Figure 2e-g). In addition, groups of small sized fishes (50-100 g) were aggregated on the water surface with signs of rapid operculum movement and gasping. In the free fishing zone of Manzala water, scattered groups of affected wild *D. labrax* and *Tilapia species* were spotted. Greenish to brownish putrefied algae were noticed covering huge areas of water surface under which numbers of dead small wild fishes were observed (Figure 2a). Dredgers, drilling and suction machines were showed working in several localities within the lake (Figure 2c) and large quantities of moldy and smelly waste products of the dredging and purification process were noticed piled up on both sides of the lake (Figure 2b). The lake water color varied from whitish to reddish brown in different locations (Figure 2d). Field data revealed that, the fish farmers depended mainly on fish fry collected from the natural sources (open seas) for farm stocking and also denoted that, the fish mortality coincided with the opening of the lake water entrances to relay the affected farm ponds.

Figure 2: Field visits findings: (a) Greenish to brownish putrified algae copvering large areas of water surface under which small dead fishes were detected, (b) Large quantities of moldy waste products were picked up on both sides of the lake, (c) Dreger and suction machines worked in the lake during the mass mortality, (d) Variations of the lake water color in different locations. (e) Mass mortality of different species of fish scattered in farm ponds and along their bridges as well as in Manzala water; f: *A. regius*, g: *D. labtax.*, h: *Sparus aurata*.

Parasitological Finding

Of the examined 220 fish samples 68.18% were infested with 10 ectoparasitic species; 4 crustaceans, 4 Monogeneans and 2 protozoan species. The collected fishes from the free fishing zone of Manzala Lake demonstrated a significant greater rate of infestation (70.45%) (p = 0.761) compared to that from the affected fish farms (65.74%) (p = 0.428). Concerning fish species, *D. labrax* recorded the highest total rate of infestation (83.09%) while *A.regius* recorded the lowest rate (42.85%).The recorded prevalence

Table 1: Prevalence of parasitic infestation

		Affected farms	Manzala lake	
Fish Spp.(family)	Total Infested/Examined (%)	Infested/Examined (%)	Infested/Examined (%)	p
***D. labrax* (Moronidae)**	59/71 (83.09)	21/26 (80.77)[b]	38/45 (84.44)[a]	0.044*
***S. aurata* (Sparidae)**	17/24 (70.83)	17/24 (70.83)	-	
***A. regius* (Sciaenidae)**	15/35 (42.86)	15/35 (42.86)	-	
***Mugil spp.* (Mugilidae)**	37/50 (74.00)	18/23 (78.26)	19/27 (70.37)	0.256
***Tilapia spp.* (Cichlidae)**	22/40 (55.00)	-	22/40 (55.00)	
Total	150/220 (68.18)	71/108 (65.74)[b]	79/112 (70.54)[a]	0.001*
P	0.122	0.428	0.761	

Table 2: Host, site of infestation and prevalence of isolated parasitic types.

Parasites	Host	Site of Infection
Monogenea (Total prevalence 30.87%)		
Family Diplectanidae:		
Diplectanum aequans	*D.labrax*	Gills
Furnestinia echeneis	*S.aurata*	Gills
Family Diclidophoridae:		
Diclidophora merlangi.	*S.aurata*	Gills
Family Axinidae:		
Loxuroides spp.	*S.aurata*	Gills
Protozoa (Total prevalence 10.74%)		
Family Trichodinidae:		
Trichodina truttae.	*Tilapia spp.*	Gills and skin
Family Myxobolidae:		
Myxopolus tilapae	*Tilapia spp.*	Gills
Crustacea (Total prevalence 63.67%)		
FamiliyCymothoidae:		
Livoneca redmanii	*Mugil spp.* *Tilapia spp.* *D. labrax* *A. regius*	Branchial cavities
Family Lernanthropidae:		
Lernanthropus kroyeri	*D. labrax*	Gills
Family Caligidae:		
Caligus elongates	*D. labrax*	Gills and skin
Caligus mugilis	*Mugil spp.*	Gills

of parasitic infestation revealed no significant dependence on the species of fish while displayed a significant relation with the geographic location of samples (p = 0.001) (Table 1).The crustacean species (Figure 3: a-d) recorded the highest infestation rate among the examined fishes (63.67%). The identified isopod *Livoneca redmani* was the most prevalent crustaceans infested all the examined fish species while *Diplectanum aequans* and *Myxopolus tilapae* were of the highest rate among the detected monogenean (Figure 3: e-h) and protozoan species respectively (Figure 3: i and j) (Table 2).

Water Analysis Finding

Results of physicochemical analysis of water samples (Table 3) showed significant differences in water quality parameters of the affected fish farms and the open Manzala Lake. Water pH was significantly more alkaline in fish farms (8.53±0.21) than in Manzala Lake (7.74±0.12) (p = 0.028). Also, significant higher water salinity (39.57±6.84 g/l) and more abundant TDS (36579.67±5473.06 mg/l)

Table 3: Physiochemical parameters of water sampled from the affected localities.

		Northern Affected farms			Manzala Lake			
	Permissible limits	**Min.**	**Max.**	**Mean±SE**	**Min.**	**Max.**	**Mean±SE**	**p**
Physical analysis:								
Specific gravity (sp.gr.) (g/ml)		0.995	1.106	1.04±0.03	0.986	0.995	0.99±0.00	0.307
pH	6.5 – 8.5	8.12	8.76	8.53±0.21[a]	7.62	7.97	7.74±0.12[b]	0.028*
Conductance (µS/cm)		40700.00	67300.00	56435.33± 8055.69	16500.00	291180.00	111706.67± 89792.22	0.601
Salinity (g/l)		25.90	46.90	39.57±6.84[a]	9.70	17.80	14.77±2.55[b]	0.027*
Turbidity (NTU)	1.0	16.00	25.00	19.00±3.00[b]	34.00	45.00	38.33±3.38[a]	0.013*
Dissolved oxygen (D.O)(mg/l)	5.0	4.60	6.26	5.36±0.48[a]	2.78	4.18	3.27±0.46[b]	0.035*
Total dissolved solid (TDS) (mg/l)	1000	25641.00	42399.00	36579.67± 5473.06[a]	10396.00	18230.00	15308.67± 2470.96[b]	0.024*
Chemical analysis:								
Fluoride (F) (mg/l)	0.8	0.00	9.82	5.87±2.99	3.03	4.35	3.50±0.42	0.513
Chloride (Cl) (mg/l)	250	12755.40	22349.20	18481.53± 2921.23[a]	3965.22	7459.40	6125.86± 1090.17[b]	0.017*
Ammonia (NH_3) (mg/l)	0.5	0.20	0.30	0.25±0.05	0.34	0.80	0.51±0.10	0. 012*
Bromide (Br) (mg/l)	-	32.20	58.99	48.04±8.11[a]	7.87	20.81	16.39±4.26[b]	0.026*
Nitrite (NO_2) (mg/l)	0.2	0.00	22.23	11.12± 11.12	0.00	0.00	0.00±0.00	0.500
Nitrate (NO_3) (mg/l)	45	35.05	370.00	147.38± 111.31	22.90	56.89	34.79±11.06	0.418
Phosphate (PO_4) (mg/l)	-	0.00	121.59	40.53±40.53	0.00	0.00	0.00±0.00	0.423
Sulphate (SO_4) (mg/l)	250	2033.30	3299.50	2877.13± 421.92[a]	786.22	1351.40	1123.98± 172.23[b]	0.018*
Lithium (Li) (mg/l)	-	0.28	0.47	0.39±0.06	0.47	1.18	0.72±0.23	0.287
Sodium (Na) (mg/l)	200	4377.07	12034.30	8124.29± 2211.95	2580.98	4782.69	3961.94± 694.56	0.147
Potassium (K) (mg/l)	-	214.04	464.70	374.66± 80.51	121.91	239.72	173.10±34.87	0.083
Magnesium (Mg) (mg/l)	-	555.21	1471.44	1033.82± 265.29	371.05	636.69	523.77±79.22	0.139
Calcium (Ca) (mg/l)	200	271.79	723.80	543.68± 138.32	269.43	328.31	307.74±19.17	0.229
Total alkalinity (mg/l)		56.00	138.00	84.00±27.01	88.00	280.00	153.67±63.18	0.368
Carbonate (CO_3) (mg/l)		0.00	19.00	11.67±5.90	0.00	8.00	4.67±2.40	0.333
Bicarbonate (HCO_3) (mg/l)		42.00	138.00	74.00±32.00	80.00	280.00	149.00±65.53	0.362
Hardness(mg $CaCO_3$/l)		2854.90	7736.60	5034.27± 1433.28	1851.70	3299.30	2718.03± 441.53	0.197

SE: Standard error; NTU: Nephelometer turbidity unit; EC: Electric conductance; DO: Dissolved oxygen; TDS: Total dissolved solids.

[a,b] Different superscripts within the same row indicate significance at $p < 0.05$.

Table 4: Bivariate correlations (*r*) between water chemical parameters.

		Sp.gr	pH	EC	Salinity	Turbidity	DO	TDS	F	Cl	NO_2	Br	NO_3	PO_4	SO_4	Li	Na	K	Mg	Ca	Alkalinity	CO_3	HCO_3	Hardness
Sp.gr	r	1.00	0.67	-0.16	0.74	-0.65	0.77	0.70	0.78	0.65	0.04	0.62	-0.19	-0.19	0.71	-0.22	-0.08	-0.06	-0.11	-0.26	-0.37	0.72	-0.39	**-0.08**
	p		0.144	0.755	0.095	0.159	0.074	0.118	0.069	0.159	0.948	0.190	0.717	0.719	0.112	0.680	0.888	0.917	0.839	0.622	0.466	0.105	0.442	0.887
pH	r		1.00	0.02	0.98	-0.83	0.81	0.98	0.70	0.98	0.12	0.97	0.01	-0.01	0.98	-0.50	0.64	0.50	0.62	0.33	-0.66	0.83	-0.68	**0.65**
	p			0.964	0.001	0.043	0.049	0.001	0.125	0.001	0.848	0.001	0.982	0.978	0.001	0.312	0.172	0.316	0.193	0.528	0.158	0.042	0.140	0.165
Electrical conductance (EC)	r			1.00	-0.11	0.54	-0.40	-0.10	-0.09	-0.13	-0.23	-0.12	-0.12	-0.21	-0.11	-0.20	-0.05	-0.29	-0.04	-0.32	-0.28	0.13	-0.28	**0.00**
	p				0.838	0.268	0.431	0.853	0.872	0.807	0.706	0.827	0.818	0.696	0.835	0.704	0.926	0.582	0.936	0.537	0.593	0.800	0.596	0.997
Salinity	r				1.00	-0.87	0.81	1.00	0.76	0.99	0.11	0.93	-0.02	-0.04	1.00	-0.54	0.61	0.51	0.58	0.30	-0.50	0.79	-0.53	**0.62**
	p					0.023	0.049	0.000	0.081	0.000	0.863	0.007	0.963	0.941	0.000	0.265	0.195	0.301	0.227	0.565	0.311	0.062	0.283	0.191
Turbidity	r					1.00	-0.92	-0.87	-0.60	-0.89	-0.31	-0.88	-0.13	-0.15	-0.88	0.31	-0.56	-0.59	-0.54	-0.48	0.41	-0.60	0.43	**-0.54**
	p						0.009	0.025	0.212	0.018	0.608	0.020	0.807	0.771	0.020	0.550	0.246	0.215	0.265	0.336	0.416	0.205	0.396	0.274
DO	r						1.00	0.79	0.60	0.80	0.35	0.89	0.09	0.10	0.82	-0.12	0.32	0.34	0.31	0.27	-0.65	0.72	-0.65	**0.29**
	p							0.059	0.208	0.055	0.565	0.018	0.866	0.846	0.047	0.828	0.538	0.511	0.549	0.599	0.166	0.107	0.159	0.571
TDS	r							1.00	0.73	1.00	0.13	0.93	0.00	-0.01	1.00	-0.58	0.65	0.55	0.62	0.34	-0.49	0.77	-0.51	**0.65**
	p								0.098	0.000	0.835	0.007	0.993	0.983	0.000	0.232	0.162	0.259	0.190	0.513	0.327	0.076	0.299	0.159
F	r								1.00	0.69	-0.73	0.66	-0.65	-0.65	0.72	-0.09	0.15	-0.09	0.08	-0.31	-0.38	0.88	-0.42	**0.23**
	p									0.126	0.164	0.154	0.167	0.166	0.108	0.862	0.772	0.871	0.879	0.553	0.462	0.020	0.412	0.667
Cl	r									1.00	0.16	0.95	0.04	0.03	1.00	-0.56	0.70	0.60	0.67	0.41	-0.50	0.74	-0.53	**0.70**
	p										0.799	0.004	0.936	0.956	0.000	0.244	0.120	0.206	0.142	0.420	0.311	0.090	0.284	0.120
NO_2	r										1.00	0.12	1.00	1.00	0.17	-0.45	0.24	0.62	0.32	0.72	0.04	-0.51	0.08	**0.11**
	p											0.848	0.000	0.000	0.788	0.442	0.698	0.261	0.604	0.170	0.947	0.385	0.904	0.864
Br	r											1.00	0.01	0.00	0.94	-0.32	0.64	0.50	0.62	0.39	-0.73	0.83	-0.75	**0.64**
	p												0.980	0.999	0.005	0.537	0.175	0.317	0.192	0.446	0.097	0.042	0.085	0.170
NO_3	r												1.00	1.00	0.03	-0.45	0.29	0.64	0.37	0.72	0.07	-0.48	0.11	**0.17**
	p													0.000	0.955	0.365	0.573	0.174	0.470	0.106	0.890	0.337	0.840	0.748
PO_4	r													1.00	0.01	-0.42	0.28	0.64	0.35	0.73	0.11	-0.50	0.14	**0.15**
	p														0.978	0.409	0.597	0.172	0.494	0.100	0.834	0.310	0.785	0.777
SO_4	r														1.00	-0.56	0.64	0.55	0.61	0.35	-0.51	0.77	-0.53	**0.64**
	p															0.244	0.169	0.256	0.194	0.497	0.303	0.076	0.277	0.170
Li	r															1.00	-0.60	-0.68	-0.60	-0.45	-0.14	-0.04	-0.13	**-0.56**
	p																0.207	0.135	0.204	0.365	0.792	0.942	0.805	0.253
Na	r																1.00	0.89	1.00	0.81	-0.24	0.25	-0.25	**0.99**
	p																	0.018	0.000	0.050	0.653	0.639	0.634	0.000
K	r																	1.00	0.91	0.94	-0.02	-0.04	-0.02	**0.82**
	p																		0.012	0.005	0.976	0.939	0.976	0.045
Mg	r																		1.00	0.85	-0.24	0.20	-0.25	**0.98**
	p																			0.032	0.643	0.707	0.631	0.001
Ca	r																			1.00	-0.07	-0.18	-0.06	**0.74**
	p																				0.890	0.739	0.907	0.095
Alkalinity	r																				1.00	-0.76	1.00	**-0.26**
	p																					0.076	0.000	0.617
CO_3)	r																					1.00	-0.79	**0.31**
	p																						0.060	0.550
HCO_3	r																						1.00	**-0.28**
	p																							0.593
Hardness	r																							1.00
	p																							

Sp.gr: Specific gravity; EC: Electric conductance; DO: Dissolved oxygen; TDS: Total dissolved solids; F: Fluoride; Cl: Chloride; NO_2: Nitrite; Br: Bromide; NO_3: Nitrate; SO_4: Sulphate; Li: Lithium; Na: Sodium; K: Potassium; Mg: Magnesium; Ca: Calcium; CO_3: Carbonate; HCO_3: Bicarbonate.
r: Pearson correlation coefficient (Bold); *p*: Significance was set at *p*< 0.05.

were recorded in the fish farms than in the lake. Water chemical analysis revealed that the levels of F^-, SO_4^{2-}, Na+ and Ca^2+ were over the permissible limits in both the affected farms and open Manzala Lake while NO_3^- and NO_2^- (in the fish farms) as well as NH_3 concentration (in Manzala Lake) exceeded the permissible limits. In comparison, significant greater concentrations of Cl^-, Br+, PO_4^{3-}, SO_4^{2-}, NO_3^- and NO_2^- and non significant higher values of Mg2+ , Ca2+ and Hardness were spotted in fish farm water than in Manzala' concentrations. On the other hand, Manzala Lake water exhibited significantly higher turbidity level(38.33±3.38 NTU) and higher concentration of ammonia (0.51±0.10 mg/l) .Significant reduced DO levels were reported in Manzala Lake water (3.27±0.46 mg/l) comparing to that in the affected farms (5.36±0.48 mg/l).The water temperature was measured during sampling ranged between 29° - 32°.5.

Regarding the correlation between various water parameters (Table 4), significant positive correlation was reported between pH and salinity .On the contrary, pH displayed significant negative correlation with turbidity level. A significant negative correlation was also observed between DO levels and turbidity degree of water. Significantly, strong positive correlations were reported between nitrite, nitrate, and phosphate concentrations in water.

The correlations between the recorded prevalence of fish

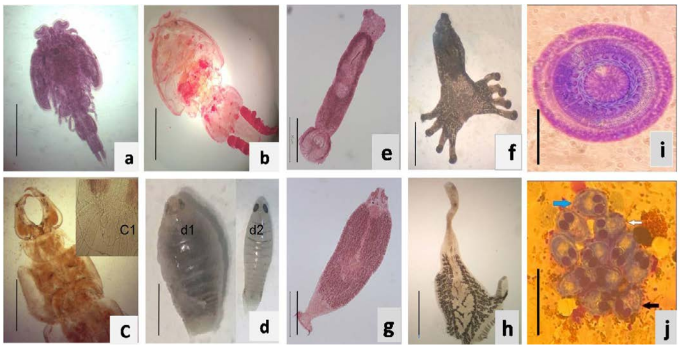

Figure 3: The isolated ectoparasite species: Crustacean species; (a) *Caligus elongatus* male, (b) *Caligus species female* (c) *Learnanthropus kroyeri* anterior end, (c1) *Learnanthropus kroyeri* posterior end, scale bar=0.4mm.(d) *Livoneca redmani.(d1)Adult female.* (d2*) Juvenile stage,* scale bar=0.7mm. Monogenean species; (e) *Furnistinia echeneis. (f) Diclidophora merlangi, (g) Diplectanum aequanus, (*h*) Loxuroides spp. Scale bar=0.2 mm in a &c.* Scale bar=0.5mm in b &d. Protozoan species : (Giemsa stain); (i) *Trichodina truttae*, scale bar=40 µm (b) *Myxobolus tilapae* stained spore, scale bar=20 µm. in a and b.

parasite infestation and water physicochemical properties were listed in Table 3 and showed significant positive correlation of the infestation prevalence with increased turbidity content of lake water (r = 0.94, p = 0.005). Furthermore, infestation rates exhibited significant negative correlation with increased DO (r = - 0.84, p = 0.035), and sulphate (r = - 0.81, p = 0.049) contents of water.

Pathology Finding

Gross examination of the affected fish revealed congestion of gills with excessive mucus. Marked elevation of the branchial cavities due to the presence of large isopod parasites in some cases was observed. Cases with skin lesion and paleness of gills and internal organs were also noticed (Figure 4).

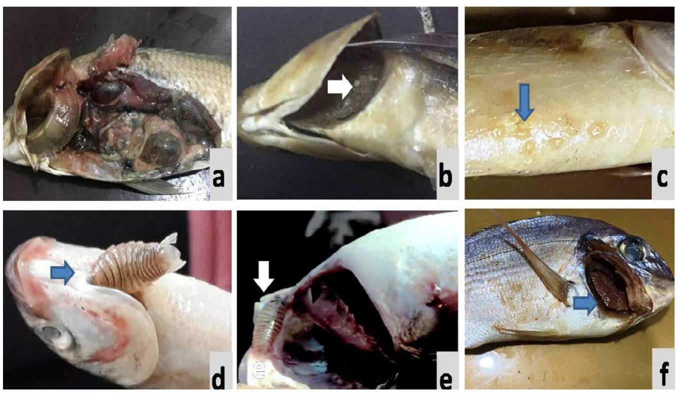

Figure 4: Macroscopic and Postmortem lesions: (a) affected *Tilaplia species* showed paleness of gills and internal organs, (b) Gills of *Mugil spp.* infested with *Caligus species*, (c) Skin of *Mugil spp.* infested with *Caligus elongatus*, (d) Mugil specie showed adult isopod *Livoneca redmani*in branchial cavity, (e) *D. labrax* showed isopods attached to the operculum, (f) *S. aurata* showed congestion of gills.

Examination of gill tissues of the affected fish exhibited variable histopathological alterations. The base of gills showed marked edema and congestion (Figure 5: a). Lamellar telangiectasia (Figure 5: b) was detected characterized by widening of the blood vessels of secondary gill lamellae which also showed heavy infiltration with eosinophilic granular cells (Figure 5: c). Some examined cases revealed hyperplasia of gill lamellae accompanied by aggregations of bacterial clusters (Figure 5: d). Severe cases showed massive destruction and necrosis of secondary gill lamellae admixed with sloughed gill elements (Figure 5: e-g). Additionally, parasitic infestation was recorded in variable numbers of examined fish samples that illustrated by attached parasites to the gills with subsequent fusion and destruction of secondary gill lamellae and intense inflammatory reaction (Figure 5:h and i).

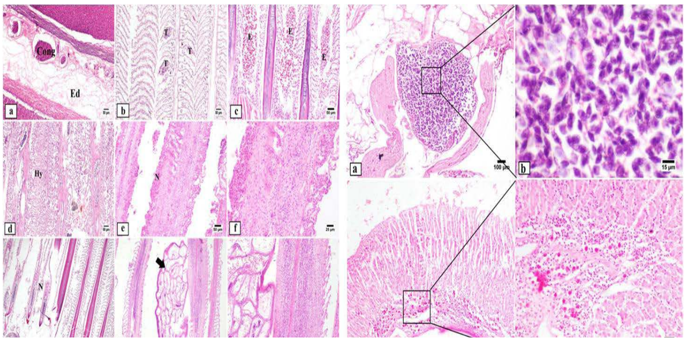

Figure 5: Photomicrograph of gills**:** Marked diffuse edema and severely congested blood vessels are seen in gill arch (**a**). Multifocal lamellar telangiectasia is noticed in affected gills (**b**). Heavy eosinophilic granular cells infiltration with variable number of inflammatory cells (**c**). Hyperplasia of the secondary gill lamellae admixed with necrosis of adjacent gill elements (**d**). Sever destruction and necrosis in the secondary gill lamellae with inflammatory cells infiltration (**e-g**). Attached isopod spp. (arrow) to the surface of gills with intense inflammatory reaction (**h-i**). Edema (**Ed)**, congestion (**Cong)**, telangiectasia (**T)**, necrosis (**N)**, hyperplasia (**Hy)**, eosinophilic granular cells (**E**). Photomicrograph of subcutaneous and intestine of fish**:** Subcutaneous tissue showing Myxosporidian cysts with higher magnification showing numerous myxobolus spores (**j-k).** Intestine of fish showing edema of mucosa associated with intense inflammatory cells infiltration in the sub epithelial layer (**l**). Intestine of fish showing higher power of inflammatory cells infiltration with numerous numbers of eosinophilic granular cells (**m**).

Some of the examined Tilapia spp. showed subcutaneous Myxosporidian cysts that contained numerous *Myxobolus* spores (Figure 5: j and k). The intestine of most of the examined fish samples showed necrosis of mucosal layer associated with edema and inflammatory cells infiltration. The submucosal layer exhibited marked inflammation that revealed numerous inflammatory cells and eosinophilic granular cells (Figure 5: l and m). No pathological changes or lesions were observed in the spleen or liver tissues of the examined fish samples.

DISCUSSION

Researching stressors on fish health requires investigating the emergent issues associated with the environmental factors and pathogens. As such, the role of parasites on fish health can't be overlooked. In the present study, ectoparasite infestation and water quality were investigated during event of fish mass mortalities among fishes in Manzala Lake and the corresponding marine fish farms during May and June 2019. Result showed that the examined moribund and freshly dead fish samples were highly infested with different ectoparasite species (68.18%). The detected crustaceans were the most prevalent among the isolated ectoparasites and *Dicentrarchus labrax* was the fish species showed the highest infestation rate, similar finding was reported by (El Deen et al., 2020; Eissa et al., 2020). The rate of infestation among the examined farm fish was lower than in Manzala Lake (65.74% and 70.54% respectively) ,this might be attributed to the species variation responses to the environmental factors in terms of water pollution, temperature, host parasite relationship and other biological factors of the host (Mortuza and Al-Misned, 2015). The recorded parasitic isopod species as well as the Sea lice Caligus species known to cause severe impacts on fish health , losses in fish populations and incriminated in fish mass mortalities (Rameshkumar and Ravichandran 2014; Vollset, 2019; Mahmoud et al., 2020). In the current study, the detected parasite species were previously recorded from the Mediterranean Sea, Red Sea and Suez Canal (Isbert et al., 2018; Eissa et al., 2012; 2020) and they might transmit to the investigated locations through the connected inlets or with the fish seeds obtained from the seas for farm stocking. So, in the present investigation, Manzala Lake and its corresponding marine fish farms considered new localities for the isolated parasite species except the protozoan *Trichodina truttae* and *Myxopolus tilapae* which were identified from Manzala Lake by Abu El Wafa et al, 2011 . Field observations, as well as results of water analyses, indicated a high rate of pollution in lake water and consequently affected its corresponding fish farms especially during low Mediterranean Sea tide when the lake was the main source of farm water. The recorded water pH values were found higher than the optimal mentioned by (Svobodová, 1993) (6.5-8.2) for the aquatic organism, the result which indicated domestic and agricultural wastes (El-Kafrawy, 2004; Nassar et al., 2014; Deyab et al., 2019). In addition, the recorded significant positive correlations between PH and other parameters as water salinity, TDS, chloride, bromide, and sulphate were came in agreement with that reported by (El-Zeiny et al., 2019). The detected high-water turbidity might be due to the suspended sediment, clay, waste, and other particulate materials resulted from the dredging process that was taking place in the lake at the time of fish mortality. Similarly, (El-Zeiny et al., 2019) attributed the levels of turbidity in lake's water to the levels of suspended agricultural and domestic particles from drains. The recorded high turbidity could be resulted in the pathological impacts detected in gills of the examined fish, the finding which agreed with (Parra et al., 2018; Hess et al., 2015) who denoted that, high water turbidity causes clogging and damage of gills manifested by excessive mucous discharge and growth of protective cell layers. Also, many pollutants resulted in serious injuries to gills micro structure that depends on the concentration and duration of exposure to the toxic agent (Reddy and Rawat, 2013). The recorded DO value in the lake water (2.78 mg/L) was less than the suitable value for support the aquatic animals which could be above 5 mg/L according to the report of (Baleta and Bolaños, 2016). This reduction in the DO concentration could be due to the high amount of waste waters dumped through many southern drains especially Bahr El Bakar drain which receive huge amount of agriculture and domestic waste from many sources in Delta region. Low DO concentration may also due to the recorded high turbidity and high temperature which reported to increase the oxygen requirements of fish (Svobodová, 1993). The detected values of the non-ionized ammonia (NH_3) were potentially toxic to fish as recorded by (Svobodová, 1993). The findings of water analysis indicated marked regional variations in the properties of the studied areas. These variations were attributed to the effects of the location and the seawater inflows from the Mediterranean Sea and different pollution sources. Meanwhile, water of fish farms were comparatively less polluted, but higher in salinity as compared to the lake water especially in the southern sites, as the northern locations were more influenced by the seawater inflows (Elmorsi et al., 2017). The recorded clinical and gross finding were found in agreement with (Svobodová, 1993)_who noticed that fish exposed to oxygen deficient and deteriorated water collected near the water surface, gasping for air, lose the ability to reflex and ultimately die. He also denoted that, pale skin colour, congestion of gills and small haemorrhagic lesion in skin were the major alterations.

On the other hand, the recorded correlations between the prevalence of parasitic infestation (68.18%) and the physical and chemical properties of water came in agreement with (Ojwala et al., 2018) and could be attributed to the fact that water pollution affect the immune status of fish, and thus became more susceptible to infestation (Hoole, 1997). Additionally, and according to the date of field visits, dependency of farmers on the fish seeds obtained from the natural sources (open seas) for stocking could play an important role in the entry of parasitic species as well as other undesirable aquatic organisms. Similar case was documented by (Mahmoud et al., 2019) about the role of Mediterranean Sea Mugiliid fry in transmitting the isopod *Livoneca redmanii* to Lake Qarun causing destruction of its fish stock.

Table 5: Correlation between parasite infestation rate and water physical and chemical properties.

	Correlation with Parasite infestation rate (*r*)	p
Sp.gr	-0.74	0.096
pH	-0.71	0.115
EC	0.61	0.195
Salinity	-0.81	0.050*
Turbidity	0.94	0.005*
DO	-0.84	0.035*
TDS	-0.8	0.054
F	-0.56	0.248
Cl	-0.8	0.053
NO_2	-0.22	0.679
Br	-0.72	0.107
NO_3	-0.18	0.726
PO_4	-0.22	0.679
SO_4	-0.81	0.049*
Li	0.41	0.420
Na	-0.4	0.428
K	-0.54	0.266
Mg	-0.38	0.451
Ca	-0.38	0.458
Alkalinity	0.16	0.758
CO_3	-0.45	0.367
HCO_3	0.18	0.734
Hardness	-0.36	0.486

Gills are considered multifunctional organs that show wide exposed surface area of the fish that trigger it highly sensitive to chemicals pollution in water. Gills are considered the respiratory system of fish that have thin epithelial covering for gases exchange, regulation of ionic acid base balance and elimination of nitrogenous wastes (Javed et al., 2017). Therefore gill parasites in association with the exposure to deteriorated water resulted in serious injuries to gills micro structure depending on the parasitic species as well as the concentration and duration of exposure (Reddy and Rawat, 2013).

The observed severe lesions in the gills such as lamellar fusion, hyperplasia, necrosis of filament cells like chloride and pavement cells are important tissue response against toxicants and might be related to interaction with ion transport proteins and affect their mechanism ,the result which were supported by that reported by (Butchiram et al., 2013). The detected lamellar epithelium hypertrophy and hyperplasia are considered a defence mechanism for decreasing the absorption of toxic agents; however, these changes decrease the oxygen uptake and markedly affect the gas exchange (Fernandes et al., 2007). In addition , the observed lamellar aneurysms and congested blood vessels may be occurred as sequence of intense inflammatory reaction that occurred after exposure to severe environmental stresses such as pollution (Rosety-Rodríguez et al., 2002) and parasitic infestation (Mahmoud et al., 2014b).

The examined gills of fish exposed to polluted water and found infested with parasites showed more severe lesions and marked changes in their structure from normal gills to which the increased mortality could be regarded to. As the Intestine is the first organ come into contact with food borne contaminants (Braunbeck et al., 1999), thus the pathological lesions demonstrated in the intestine of the examined fish samples might cause disturbance of the intestinal absorption that might led to deleterious impact on the fish health and survival as concluded by (Cengiz et al., 2001). Absence of pathological changes in liver and spleen tissues of the examined fish samples indicated per acute mortality as fish death occurred shortly after sudden exposure to highly polluted water especially with the impact of parasitic infestation, the conclusion which was supported by the clinical observation as well as the pathological finding of the affected fish gills. Unquestionably, the histopathological alterations in the respiratory system altered the host survival ability. The recorded pathological changes may lead to fish mortality through functional disorders in the gills due to parasitic infestation together with the extensive water deterioration.

CONCLUSION AND RECOMMENDATIONS

The study concluded that the impacts of the parasitic infestation in association with water quality deterioration played a considerable role in fish mass mortality emerged in Manzala Lake and its corresponding fish farms during 2019. The climatic condition, particularly the high temperature might be in consideration. The study recommended continuous monitoring of Manzala Lake water. The proper sanitary and regular disposal of the waste remnants of clearing and dredging processes that were taking place by the government in the lake. Additionally, the biosecurity strategies should be applied in the fish farms and the dependency of farmers on fish fry from natural open water instead of the hatcheries should be avoided to prevent the entrance of fish parasites from the open seas to the lake and consequently to the fish farms.

NOVELTY STATEMENT

This research is the first to study the prevalence of fish parasite infestation in relation to water deterioration and their role in the mass mortality of fish during 2019 in Lake Manzala and the corresponding marine fish farms. Additionally, the investigated areas considered a new locality records for the detected crustacean and monogenean species.

AUTHOR'S CONTRIBUTIONS

M.M.F.: supervised the present study, N.E.M.: designed and coordinated the study, A.M.M and M.R.M.: performed histopathological examination, M.M.F., N.E.M. and A.M.M.: performed clinical, postmortem and parasitological investigation, E. I. and M.M.Z.: performed water analysis and statistical analysis, all authors performed the field visit study. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL CONSIDERATIONS

All the standard international and national ethical guidelines concerning fish sampling handling and procedures were adopted by the investigator team (authors).

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