

**Research Article****Influence of Water Quality Parameters on the Prevalence of *Livoneca redmanii* (Isopoda; Cymothoidae) Infestation of Mediterranean Sea Fishes, Egypt**NE Mahmoud¹, MM Fahmy¹, MM Abuowarda^{1*}, MM Zaki², E Ismael² and EM Ismail²¹Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt; ²Department of Veterinary Hygiene and Management Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt

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The quality of water in the aquatic ecosystem is a very sensitive issue and is controlled by many physical and chemical factors. The deterioration of water quality has variable effect on parasitic population and their rate of infestation and consequently the negative impact can impede fish viability and productivity. The current study aimed to: i) Surveying the parasitic isopod infesting some of the edible fish species inhabit the Egyptian Mediterranean Sea water ii) Assess the seasonal variations in water quality parameters of Mediterranean coastal water of Egypt. iii) Investigating the effect of water quality parameters on the rate of parasitic isopod infestation among the examined fishes. Water samples during each season were analyzed for physico-chemical parameters using standard methods. The selected parameters namely: temperature, pH, salinity, oxidizable organic matter (OOM), ammonia, nitrite, nitrate and some heavy metals (Lead, Copper, Arsenic and Mercury). A total of 400 Mediterranean Sea fish of *Tilapia zilli*, *Solea spp*, *Mugil capito* and *Sardinella species* were examined for isopod parasites. Parasites were preserved and identified. The results revealed isolation of the isopod species *Livoneca redmanii*, with an infestation rate of 19% among the examined fish species with the highest rate among *Mugil capito* (36%) and reached its total maximum value during summer (32%). Correlation analysis revealed that infestation rates were highly correlated (positively) with certain water quality parameters, such as temperature, oxidizable organic matter (OOM) and nitrite. High water temperatures during summer and spring seasons, and high nitrite concentrations were significantly associated with high infestation rates in *Tilapia zilli* ($R^2=0.91$, $P=0.046$ and $R^2 = 0.97$, $P=0.015$). The findings suggested that deterioration of water quality with varying seasons was stressful to fish, and consequently increased the incidences of the parasitic Isopod (*Livoneca redmanii*) so considered as a predisposing agent to parasitism. The study recommended periodical monitoring of water quality parameters in fish water resources and the need to take all measures by the responsible authorities to prevent pollution of these resources to minimized and control the prevalence of parasite infestations particularly of isopods.

Key words: Isopods, *Livoneca redmanii*, Infestation rate, Mediterranean Sea, Physico-chemical parameters**INTRODUCTION**

Egypt has a long coast on Mediterranean and Red sea water and was ranked as the eighth among the top fish producing countries in the world in 2014 (FAO, 2016). Egypt possessed about 70.5% of the total fish production of Africa (Sadek, 2011; Kleih *et al.*, 2013; Samy-Kamal, 2015). The quality of water in the aquatic ecosystem is a very sensitive issue and it is controlled by many physical and chemical factors (Sargaonkar and Deshpande, 2003; Basavaraja *et al.*, 2011). Mediterranean Sea is considered as a semi-closed sea, so it has limited capacity of water interchange with the surrounding seas

and Atlantic Ocean. Many northern Egyptian lakes including lake Manzala, lake Maryout, lake Borolous and also lake Edku are connected to Mediterranean seas through different outlets. This geographical location made the Mediterranean a forum for many types of pollutants of serious dramatic impacts. About 80% of the fish disease causative agents are parasitic type (Shaheen, 2013) and are implicated in high economic losses (Bunkley-Williams *et al.*, 2006; Toksen, 2007; Shaalan *et al.*, 2018). Crustaceans ectoparasites represented about 25% of total parasites infesting fish (Öktener and Sezgin, 2000; Tansel and Fatih, 2012). Isopods are the largest ectoparasites that infest fish worldwide and could infest various part of fish

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body such as body surface, fins, inside the mouth, the gills, nostrils, or occasionally burrowing in special tunnels in the musculature of their hosts, leading to high fish mortalities, stunting growth and sever tissue damage (Hoffman, 1998; Rhode, 2005). Moreover, isopods could act as a vector that transmits other pathogenic organisms (Horton and Okamura, 2001). Recent studies revealed that the host-parasite relationship was found to be principally ecological as these parasites infest the suitable hosts that live within a certain biotope (Ahmad *et al.*, 2016). Water physico-chemical parameters and their impact on the healthy state of fish are the factors controlling parasitism and parasite community and consequently the incidence and rate of parasite infestation were associated with these parameters. (Rameshkumar and Ravichandran, 2010; Ojwala *et al.*, 2018). Physical and chemical parameters reported to influence the aquatic environment, included dissolved oxygen, pH, salinity, ammonia level and temperature variations, total suspended and dissolved solids, total alkalinity and acidity and heavy metal contaminants, which were increased due to various anthropogenic activities (El-Nemaki *et al.*, 2008; El-Amier *et al.*, 2015). The Mediterranean has been proven to be the main source of isopod infestation problems in Egypt especially for Lake Qaroun through the transfer of its infected mugiliid fry and also the infestation of farms and lakes corresponding to it (Mahmoud *et al.*, 2019). The deterioration of water quality recorded to have variable effects on the incidence of parasitic infestation and consequently impede fish productivity (Ojwala *et al.*, 2018). Therefore, the present study was initiated to estimate the parasitic isopod infestation among fishes inhabit the Egyptian Mediterranean Sea and evaluate the impact of water quality on the prevalence of such infestation.

MATERIALS AND METHODS

The study area and sampling

During 2017, water as well as fish samples were seasonally collected from different sites along the Egyptian Mediterranean coast at Bughaz Al-Meadeyya. It is one of the most important fisheries in Egypt connects between Lake Edku and Mediterranean Sea at Abu Qir Bay estuary. Abu Qir Bay receives different pollutants contributing to various waste source categories through three main drains and openings including El-Tabia pumping station, the outlet of Lake Edku (Boughaz Al Meadeyya) and Rosetta mouth of the Nile River. Moreover, various industrial wastes for about 22 factories were discharged into the bay (Hassan, 1999).

Fish sampling and parasitological examination of fish for isopod parasites

A total of 400 fish samples of *Tilapia zilli*, *Solea spp*, *Mugil capito* and *Sardinella spp*. (100 of each species) were seasonally collected from the investigated area and transported to the laboratory of Parasitology Department, Faculty of Veterinary Medicine, Cairo University. Fish samples were identified and measured to the nearest centimeter (cm) of body length then macroscopically examined for any gross lesions and /or isopod parasites on the body surface, buccal cavity, branchial cavity and fins. The observed isopods were

photographed using digital camera of 12 mega pixel. The parasites were dislodged from the fish host tissues, washed in normal saline then kept in 70% ethyl alcohol according to Pritchard and Kruse, 1982).

Identification of the detected isopod species

Identification of isopod were done according to the description given by Brusca, 1981 and Mahmoud *et al.*, 2017.

Water sampling

Water samples were collected in clean sterilized plastic bottles and transported to the laboratory of Veterinary Hygiene and Management Department, Faculty of Veterinary Medicine, Cairo University to be stored for major chemical water quality parameters according to Standard Methods described by APHA (American Public Health Association, 2005).



Fig. 1: Illustrated Map of the investigated area along the Mediterranean coast of Egypt (Al-Meadeyya).

Measuring of Physico-chemical parameters:

Hydrographical conditions (water temperature, pH, salinity, oxidizable organic matter, and total hardness) and eutrophication parameters (ammonium, nitrite, and nitrate) were measured in the collected water samples. All measurements were carried out according to the international standards. Water temperature, pH and salinity were measured in situ using digital thermometer (HANNA®, range 0–100°C), pH meter (Jenway® Model: 550) and salinometer (Portable optical TDS salinometer/refractometer®). The method used for the determination of oxidizable organic matter (OOM) was that described by Food and Agriculture Organization (FAO) (1976). Total hardness was measured using the EDTA titration method (APHA, 2005). Ammonium, nitrite, and nitrate were analyzed using specific kits (HANNA® instruments) according to SMWW, 1985.

Determination of heavy metals

Atomic Absorption Spectrophotometry (ICP Optim 2000 DV- Perkin Elmer) instrument was used for determination of the heavy metals content in water. Concentrations of Lead “Pb”, Copper “Cu”, Arsenic “As” and Mercury “Hg” were determined and expressed as ppb (µg/L) (AOAC, 2012).

Data analysis

Association between Infestation rates and water parameters were analyzed using Pearson’s correlation coefficient and linear regression. Data analysis was performed using and PASW Statistics, Version 18.0 software (SPSS Inc., Chicago, IL, USA). A *P*-value <0.05 was considered statistically significant. Graphs were made using Microsoft Excel 365.

RESULTS

Prevalence and seasonal variation of isopod infestation

Out of 400 examined fish specimens, 76 were found infested with Cymothoid isopods with an infestation rate of 19%. The highest infestation rate was observed in summer (32%) followed by spring (23%) while the lowest rate was observed in winter (7%). Regarding fish species, *Mugil capito* recorded the highest rate of isopod infestation while no record of infestation among the examined *Sardinella spp.* Out of the examined *Solea spp.* 10 samples of the infested fish (83.3%) were found infested with juvenile stages on their body surface (mainly on the base of pectoral fin) while only 2 fish samples were infested with the adult stages of isopod. In the present investigation, the recovered isopod species was morphologically identified as *Levonicia redmanii* (Leach,

1818). It was noticed that 11 specimens (14.4%) of the infested fish (9 *Mugil capito* and 2 *Tilapia zilli*) were harbored both male and female *L. redmanii* in their branchial cavities (Table 1& Figure 2).

The infestation rates of the examined Mediterranean Sea fish samples showed strong positive correlations with water temperature, OOM, ammonia and nitrite ($r > 0.50$), while negative correlations were observed with water salinity ($r = -0.69$) (Table 3). There were significant strong positive correlations between infestation% in *Tilapia zilli* and levels of both water temperature and nitrite ($r = 0.95$, $P = 0.046$; $r = 0.99$, $P = 0.015$; respectively). Linear regression analysis revealed strong association between high infestation rates and high-water temperature, OOM and nitrite ($R^2 > 0.80$) (Figure 4).

Average heavy metal concentration

The mean concentrations of the tested heavy metals showed that levels of copper (36 µg/L) and mercury (1.902 µg/L) were extremely higher than the maximum permissible limits (10 µg/L and 0.4 µg/L; respectively). Also, the recorded mean concentration of Lead (7.138 µg/L) was found higher than the average acceptable limit (5 µg/L), but was within the maximum permissible limit (20 µg/L). On the other hand, the recorded mean concentration of arsenic (2.5 µg/L), was lower than the average acceptable limit (36 µg/L).



Fig. 2: A: *L. redmanii* adult male and female in the branchial cavity (upper and lower respectively) of *Mugil capito*, B: adult *L. redmani* attached to the gill cover of *Tilapia zilli*, C: Juvenile of *L. redmanii* attached to the base of pectoral fins of *Solea species*.

Table 1: Infestation rate of *Livoneca redmanii* in Mediterranean Sea fishes, Egypt.

Season	Total No. of examined host	Winter		Spring		Summer		Autumn		Total No. of infest. (%)
		No. of infested	%							
Mediterranean Sea fishes(L)										
<i>Tilapia zilli</i> (8-10)	100	3	12	12	48	10	40	3	12	28 (28)
<i>Solea spp.</i> (9-12)	100	0	0	4	16	6	24	2	8	12 (12)
<i>Mugil capito</i> (15-20)	100	4	16	7	28	16	64	9	36	36 (36)
<i>Sardinella spp</i> (11-14)	100	0	0	0	0	0	0	0	0	0 (0)
Total No. of infest. (%)	400	7	7	23	23	32	32	14	14	76 (19%)

No.: Number, infest: infestation L: Length of the examined fish species in cm.

Table 2: Seasonal Physic-chemical and Eutrophication Parameters of the investigated water sampled

Parameters	Winter	Spring	Summer	Autumn	Acceptable/Permissible limit
Temperature (°C)	24.8	25.3	25.4	24.8	20.27 – 25.7 °C
PH	7.8	7.85	7.26	7.8	Average 7.56 – 8.12*
Salinity	41.85	30.65	35.05	36.15	-----
oxidizable organic matter (OOM) (mg O2/L)	4.4	15	20	3	1–3 mg O2/L
Ammonia (NH4) mg/L	2	2.5	2.5	1	Average 0.003 – 0.017 mg/L*
Nitrite mg/L	0	0.1	0.1	0	PL = 0.5 - 2.4 mg/L
Nitrate mg/L	0	0	0	0	Average 0.003 – 0.019 mg/L*

* Average levels of Egyptian coastal Mediterranean Sea water, 2016; by EIMP/EEAA, (2017a).

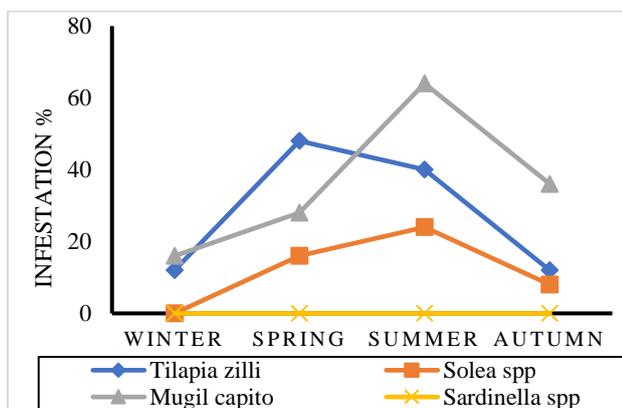


Fig. 3: Seasonal infestation rate of *Livoneca redmanii* in Mediterranean Sea fishes, Egypt.

Table 3: Water parameters Vs infestation rates in different Mediterranean Sea fish spp.

	Pearson's Correlation (r)	P - value
Temp. Vs infestation rates in		
<i>Tilapia zilli</i>	0.95	0.046*
<i>Solea spp</i>	0.93	0.073
<i>Mugil capito</i>	0.65	0.347
Total	0.94	0.065
Salinity Vs infestation rates in		
<i>Tilapia zilli</i>	-0.83	0.174
<i>Solea spp</i>	-0.73	0.275
<i>Mugil capito</i>	-0.36	0.643
Total	-0.69	0.308
OOM Vs infestation rates in		
<i>Tilapia zilli</i>	0.91	0.091
<i>Solea spp</i>	0.92	0.079
<i>Mugil capito</i>	0.70	0.302
Total	0.94	0.065
Ammonia Vs infestation rates in		
<i>Tilapia zilli</i>	0.80	0.196
<i>Solea spp</i>	0.55	0.452
<i>Mugil capito</i>	0.23	0.769
Total	0.58	0.423
Nitrite Vs infestation rates in		
<i>Tilapia zilli</i>	0.99	0.015*
<i>Solea spp</i>	0.89	0.106
<i>Mugil capito</i>	0.57	0.434
Total	0.90	0.102

* Significance at P<0.05.

Table 4: Heavy Metals concentrations in water sampled on the Egyptian coastal waters of the Mediteranean Sea (Al-Meadeyya), year 2017

Parameters	Minimum	Maximum	Mean	Acceptable/ Permissible limit
Lead (Pb)	5.179	9.097	7.138	Average 5 µg/L Maximum 20 µg/L
Copper (Cu)	31	41	36	Average 5 µg/L Maximum 10 µg/L
Arsenic (As)	2	3	2.5	Average 36 µg/L Maximum 69 µg/L
Mercury (Hg)	1.454	2.350	1.902	Average 0.16 µg/L Maximum 0.4 µg/L

DISCUSSION

Fish health and aquatic environment are strongly interrelated in which aquatic environment receive array of chemicals with harmful health impacts on the habituated

fish that threaten their live (Mackenzie *et al.*,1995). There is increasing responsiveness that parasitism should be investigated in the light of corresponding environmental conditions (Mackenzie *et al.*,1995; Lafferty *et al.*, 2004). Water pollution can effectively limit the occurrence of some species of fish parasites and affect their qualitative and quantitative composition through influencing their eggs, free living larval stages and their intermediate or final hosts (Popiolek, 2001).

In this study, the total infestation rate with the isopod *L. redmanii* (19%) of the examined Mediterranean Sea fishes was markedly higher than that reported by Samn *et al.*, 2014 (3.13%) for *Nerocila bivittata* infestating the Mediterranean Sea fish *Lithognathus mormyrus*. Also, it was higher than that revealed by Rania and Rehab (2015) for the isopod *Anilocra leptosome* (from *Argyrosomus regius*) and *Cymothoa indica* (from *Pagrus pagrus*) and by Hamdy and Dorgham, 2018 for *Cirolana bovina* (12.3%). On the other hand, the present rate was found lower than the rate reported by Ramadan *et al.*, 2011 for the *Cymothoa sp.* and *Aega sp.* infesting *Sardinella gibbosa* captured from the coastal waters of Mediterranean Sea in Port Said, Egypt. This variation of infestation rates might be regarded to the differences of the examined fish species, the isolated isopod species also the time and site of the sampling. The highest infestation rate of *L. redmanii* in Mediterranean Sea fishes was recorded in summer season, the result which came in agreement with that mentioned by Noor El-Deen *et al.* (2013), Samn *et al.*, 2014, and Mahmoud *et al.*, 2017. The isolated *L. redmani* in the current investigation was previously recorded to cause a catastrophic invasion state in Lake Qarun, Fayoum Governorate, Egypt by Mahmoud et al 2017 who practically proved that the juveniles of this isopod species were transported to the lake through transporting infesting mugiliid fry from the Mediterranean Sea (Mahmoud *et al.*, 2019). This isopod was also isolated by Helal and Yousef (2018) from Lake Qarun.

The current investigation illustrated that, the recorded temperature, pH as well as the values of nitrite and nitrate of the analyzed Mediterranean water samples fell within the normal and acceptable limits (EIMP/EEAA, 2017a). While the values of OOM and ammonia were higher than the acceptable limits.

Water temperature varied seasonally from a minimum of 24.8°C during winter to a maximum of 25.4°C during summer. The same pattern was recorded by Abdel-Halim and Aly-Eldeen, 2016. In this study, water temperature showed a significant positive correlation with the isopod infestation rate. Several studies suggested the possibility that Isopod prevalence vary seasonally, especially during warmer months (Garcia-Guerrero & Hendrickx, 2003).The pH recorded from this study ranged between 7.26–7.85, with the highest value was recorded in spring. The pH value varies according to the photosynthesis of algae and the amount of sewage pollution. Moreover, decreased pH-values correlate with the decrease of oxygen concentration due to high levels of organic pollutants as well as the brackish water drainage (Abdel-Halim and Aly-Eldeen, 2016). These results agreed with Marcogliese and Cone, 1996 who mentioned that water with alkaline PH has slight effect on the parasite abundance. Salinity indicates the rate of dilution

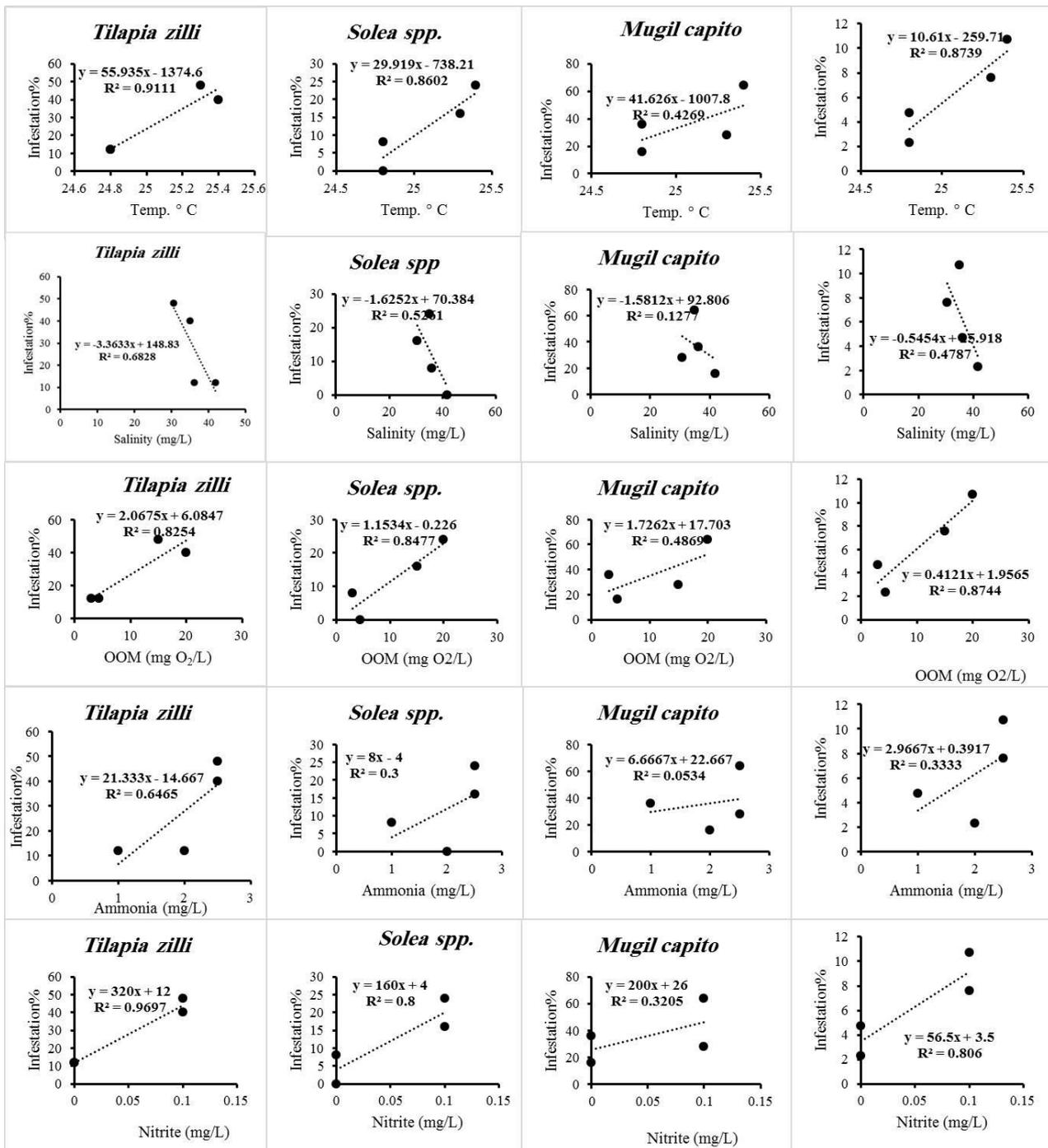


Fig. 4: Regression plots showing correlations of *L. redmanii* infestation seasonally in Mediterranean Sea fishes with water temperature; salinity; oxidizable organic matter (OOM) (mg O₂/L); Ammonia (NH₄) mg/L; Nitrite mg/L; Nitrate mg/L.

of seawater by land water discharges, so, it reveals the degree of contamination in aquatic environment (Zyadah *et al.*, 2004). Salinity in this study recorded the lowest average value of 35.05 mg/L during spring while winter represented the highest average value of 41.85 mg/L as Emam *et al.*, 2013 and Elmorsi *et al.*, 2017 who determined the higher salinity % in Egyptian coastal of Mediterranean Sea in winter. Oxidizable organic matter, (OOM) is considered one of the most important indicator of sewage pollution and organic load (Redfield, 1958). Levels of Oxidizable organic matter (OOM) were extremely high in spring and summer seasons (15 to 20 mg O₂/L; respectively). These results came in agreement

with that reported by Danovaro, 2003 who stated that, the parasites are became 2–3 times more efficient in the presence of high load of organic matter which provide food sources to the most of these parasites.

Similarly, Abdel-Mawla and El-Ekiaby (2012) stated that the parasitic infestation increased in the highly polluted water. On the other hand, Shafi *et al.*, 2015 claimed that anthropogenic pollution of water has not increased in the parasitic load. Ammonia is the second gas of significance in fish culture, following dissolved oxygen; its effect on aquaculture production is overwhelming. The ammonia levels recorded seasonally in this study ranged between 1 and 2.5 mg/L. The

recorded levels were higher than the averages that were recorded by EIMP/EEAA, (2017b) (0.003 –0.017 mg/L for ammonia). The desirable limit of ammonia is 0- 0.05 mg/l, with acceptable limit less than 0.5 mg/L (EIFAC 1970). This study found high ammonia levels that exceeded the tolerable level, especially during spring and summer seasons. Ammonia is produced from the bacterial decomposition of the organic matter containing nitrogen (Tadros *et al.*, 2005). Protein rich feed and fish excreta liberate toxic ammonia gas. A positive relation was observed between ammonia and isopod infestation rate, and this result agreed with the findings of Ahmad *et al.*, 2016. Nitrite levels recorded in this study showed a seasonal variation, the maximum nitrite concentrations were recorded during spring and summer periods (0.1 mg/L), as reported by (Abdel-Mawla and El-Ekiaby 2012). The permissible limit of nitrite is 0.5-2.4 mg/L. Nitrate was within the average levels (0.003–0.019 mg/L) recorded by EIMP/EEAA, (2017a). It is considered the most stable form of inorganic nitrogen in oxygenated water (Abdel-Mawla and El-Ekiaby, 2012).

Hazardous metals and organic pollutants

The levels of lead (Pb), copper (Cu), arsenic (As) and mercury (Hg) were determined, as they were classified as chemical hazardous metals (USFDA, 1993; EC, 2001; FDA, 2001). Results revealed that levels of Cu and Hg were extremely higher than the maximum acceptable limits. Elevated concentrations of these metals could be attributed to the presence of different pollution source categories pumped directly into both water surfaces, as; industrial, marine transportation, fisheries and human activities. Mercury (in particular; methyl mercury) is toxic to large number of aquatic organisms and accumulates in their tissues and food web. The high contamination by copper was due to wastewater mixed with waste effluents from oil refineries and power station. The concentration of Cu was above the maximum limit (Maximum 10 µg/L). Arsenic level was below the permissible limits. The minimum concentration of lead that has a negative impact on aquatic organisms is about 5 µg/L. Levels of lead in tested samples exceeded the average level recorded by EIMP/EEAA, (2017a).

This confirm the results of Eissa *et al.*, 2013 who claimed that prolonged exposure to water pollutants even in very low concentrations may increase the susceptibility of aquatic animals to various diseases by interfering with the normal functioning of their immune, reproductive and developmental processes. In addition, discharge of various treated and untreated liquid wastes to the water can introduce large quantities of heavy metals to the surface water. Similarly, Palanques, 1994 studied the distribution of heavy metal pollution associated with suspended particulate matter on Mediterranean area to evaluate the environmental impact of anthropogenic metals. He found that the main sources of heavy metal pollution on this area were the sewage sludge produced in certain wastewater treatment plant. The present research showed that there was strong relationship between parasitic infestation and water pollution. These results agreed with Valtonen *et al* (1997); Dzikowski (2003) and Mahmoud *et al.*, 2015 who mentioned that water pollution effect directly on monoxenous parasites (gill

parasites) which developed more intensely after the adaptation to the surrounding and also coincided with Nachev and Sures (2009); Chapman *et al.*, (2015) who reported a clear relationship with parasite abundance and the pollution gradient. In Mediterranean Sea, environmental variables strongly fluctuated with seasonal variations related to precipitation, temperature and currents (Afli *et al.*, 2009b). Therefore, only some invertebrate species were tolerant to the extreme salinity and temperature changes between winter and summer, and able to survive (Afli *et al.*, 2009a).

Conclusions

Three species (*Mugil capito*, *Tilapia zilli* and *Solea spp*) of the four examined Mediterranean Sea fishes were markedly infested with the isopod species *Livoneca redmanii*, with a rate of 19% and this rate was highly correlated with certain water quality parameters, such as temperature, oxidizable organic matter (OOM) and nitrite. The study also concluded that water parameters deterioration have noticeable impacts on the prevalence of isopod parasite and can confirm the assumption that parasite populations are a good indicator of environmental stress. Regular monitoring of water quality parameters in fish water resources in Egypt is recommended and the responsible authorities should take appropriate measures to prevent pollution of these sources to minimized and control the rate of parasite infestations.

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