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Ultrastructural observation on the parasitic copepod, *Lamproglena monodi* Capart, 1944, infesting the gills of *Sarotherodon galilaeus* caught from the River Nile and Lake Manzala, Egypt, using the parasite as a bioindicator for pollution

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ABSTRACT
The parasite *Lamproglena monodi* Capart, 1944 is a crustacean copepod infecting various Cichlid fish in Egypt. The parasites were collected between December 2022 and April 2023, from the gills of *Sarotherodon galilaeus* Linnaeus, 1758 caught from three different locations: Beni-Suif, El-Mansoura, and Lake Manzala. The prevalence values of *L. monodi* on *S. galilaeus* varied from site to site. The values of mean intensity of the parasite showed a narrow range of variations among the different study sites ranging from 1.7 at Lake Manzala to 2.3 at Beni-Suif. The highest values of prevalence (60%) and the mean intensity (2.3%) were recorded at Beni-Suif while the lowest ones, 1.3% and 1.7 respectively, were recorded at Lake Manzala. The parasite's distinctive morphological characteristics were illustrated using Scanning Electron Microscopy. Moreover, some heavy metals (Pb, Cd, Zn, Cu, and Fe) in water and parasites in different study sites were analyzed and the results showed that mean concentrations of Cd and Pb in collected water samples exceed the permissible limits according to The Egyptian drinking water standards and WHO in all study sites. Additionally, the findings revealed that all heavy metals were accumulated in parasite tissue more than water in all study sites. Also, this study investigated parasite dynamics as a bio-indicator of heavy metals pollution. The present results showed that Fe was the highest accumulated heavy metal in the parasite whereas Cu and Pb exhibited the lowest concentrations. Additionally, the findings revealed that the greatest values of heavy metal in the parasite were found in Beni-Suif and the lowest concentrations were in El-Mansoura. The present study suggests the use of *L. monodi* as bio-indicators responding well to changes in the environment's heavy metals

INTRODUCTION

Copepods are a common ectoparasite which accumulated in fish of all species from all habitats **(Boxshall and Halsey, 2004)**. They make up the third greatest group of parasites in freshwater hosts and the second greatest group in marine fish **(Luque and**

Tavares, 2007). In pond ecosystems, copepods act as fish parasites, meal for small fish, vectors of human diseases, and micropredators of fish **(Piasecki *et al*., 2004).**

The important family of copepods is Lernaeidae. The parasites of family Lernaeidae affect freshwater fish **(Ho and Kim, 1997)**. There are ten genera and subgenera belonging to the Lernaeidae which comprising two greatest genera containing 77% of the genus *Lamproglena* and genus *Lernaea* **(Ho, 1984)**.

Lamproglena von Nordmann, 1832 is typically found on gills, and results in fish losses **(Eissa, 2002)**. More than forty species are belonged to *Lamproglena* **(Piasecki, 1993)**. Numerous studies have documented their existence in Asia, Africa and Europe **(Kumari *et al*., 1989; Marx and Avenant-Oldewage, 1996; Yambot and Lopez, 1997; Ibraheem and Izawa, 2000; Galli *et al*., 2001)**. Only Lernaea genera and the adult females of the *Lamproglena* are fish gill parasites **(Lester and Hayward, 2006)**.

The gills of *Tilapia rendalli, Serranochromis codringtonii,* and *S. macrocephalus*, were parasitized by *Lamproglena monodi* **(Douellou and Erlwanger, 1994).** According to **Azevedo *et al*. (2012)**, *L. monodi* was connected to the gills of *Astronotus ocellatus* and *Cichla ocellaris* in Brazil in addition two invasive species *Oreochromis niloticus* and *Tilapia rendalli.*

Fish macroinvertebrates, and planktons are examples of free-living biota that have historically been employed as bioindicator organisms to demonstrate the assessment of water quality **(Tweedley *et al*., 2014; Keke *et al*., 2017).** Due to the various ways in which parasites react to different environmental pollutants, they are currently used as accumulation and effect indicators **(Vidal-Martnez *et al*., 2014; Al-Hasawi, 2019; Mehana *et al*., 2020)**.

Heavy metals are the most harmful contaminants due to their environmental persistence and propensity to accumulate in aquatic environment **(Akpor and Muchie, 2011)**. As a possible bioindicator of the effects of metal, *L. clariae* has been identified in the study of **Pretorius and Avenant-Oldewage (2022)**. Then survival of *L. clariae* affected by increasing aluminium levels in a controlled environment and found that *L. clariae* may possibly extinct in regions where aluminium pollution happens **(Pretorius and Avenant-Oldewage, 2023)**. According to biological classification, there are two types of heavy metals: those that are biologically essential for fish metabolic processes (such as Zn, Cu, Ni, and Fe) and those that are toxic even at trace concentrations (such as Cd, Pb, and Hg) **(Mehana *et al*., 2020)**. Lead, zinc, copper, iron, and manganese are the heavy metals in relation to fish that contribute to water pollution **(Afshan *et al*., 2014)**. The food web, the metabolic cycling of elements, and other aspects of aquatic ecosystems are all significantly influenced by zooplankton **(Sures, 2005)**. Copepods are one of the zooplankton's most predominant constituents and are crucial to the construction and operation of marine planktonic food webs **(Vidal-Martnez *et al*., 2010)**. Copepods are zooplanktonic organisms and exposure to contaminants in the aqueous stage of the water column and through consumption of contaminated food are likely to have an impact on them. Some benefits of utilizing parasites as bioindicators are as follows: their taxonomic attributions and life-history are well known, and they lack richness of complex species, especially in benthic freshwater ecosystems **(Vidal-Martnez and Wunderlich, 2017)**. In comparing to free-living organisms, aquatic parasites are frequently able to absorb substances (such as heavy metals) at significantly higher quantities **(Sures, 2003, 2004; Nachev and Sures, 2016)**. Furthermore, because fish parasite species are unable to produce necessary fatty acids, they must instead collect nutrients from their hosts' tissues and bioconcentrate heavy metal pollution, even in minute amounts **(Mondal *et al*., 2016)**. Additionally, several parasitic helminths exhibit considerable tolerance to extremely high pollution loads. As a result, they can serve as sentinels for ecologically damaged environments **(Sures *et al*., 2017)**.

The present study aims to study the prevalence and mean intensity of the crustacean gill parasites *Lamproglena monodi* infesting *Sarotherodon galilaeus* which inhabit Beni-Suif (River Nile stream), El-Mansoura (Damietta branch of River Nile) and Lake Manzala in Egypt. Morphological features are studied by Scanning Electron Microscopy (SEM). Finally, the possible use of parasitic copepod *Lamproglena monodi* in *S. galilaeus* as bioindicator of heavy metal pollution was also evaluated.

MATERIALS AND METHODS

1. Host collection:

A total of 300 specimens of the cichlid fish, *Sarotherodon galilaeus* were collected from three sites between December 2022 to April 2023, from the River Nile at Beni-Suif and El-Mansoura cities and south of Lake Manzala were selected for the host collection of samples.

2. Prevalence and mean intensity of *L. monodi*:

Each fish and site's population of the copepode *L. monodi* was counted. According to **Margolis *et al*. (1982)**, calculations for prevalence (%) and mean intensity were conducted.

3. Microscopic examination:

For scanning electron microscopy examinations, *L. monodi* were removed from the fish and preserved in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at (4°C). Prior to the post-fixation in the same buffer with 1% osmium tetroxide, at 4°C for an hour, they were rinsed in the buffer. The samples were then critical-point dried after being dehydrated in a graded acetone series (30%, 50%, 75%, 90%, and 100%). After that, gold palladium was sputter-coated upon them. Scanning electron microscopy images were recorded in the Electronic Microscopy Unit, Faculty of Agriculture, Mansoura University, using a Jeol electron microscope at an accelerating voltage of 30 kV.

4. Analysis of heavy metals in water samples

Filter sheets made by Whatman were used for filtering the water samples. Until the heavy metals were determined by the Buck Scientific Accusys 211 Atomic Absorption Spectrophotometer, the filtrate was kept at 4^{o}C. According to **Allen *et al*. (1974)**, five heavy metals, including iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb), were assessed in the freshwater samples.

5. Analysis of heavy metals in the parasitic copepod

A total sample weighing 50 mg of parasitic copepods were homogenized and digested using 5 ml of acid digestion mixture (3 ml HNO3: 2 ml HCIO4) **(Tsoumbaris, 1990).** Heavy metal concentrations in the digested samples were determined by atomic absorption spectrophotometer (Buck scientific 210VGP).

6. Statistical analysis

The results are expressed as mean ± SE. The data were analysed using a one-way Anova followed by Tukey tests to compare the significant differences among heavy metal concentrations of the parasite from the study sites at $P < 0.05$. SPSS software (version 20.0) was used to conduct the statistical analysis.

RESULTS

1. The prevalence (%) and mean intensity values of crustacean (copepod) *Lamproglena monodi* (Capart, 1944) infesting cichlid fish host; *Sarotherodon galilaeus* Linnaeus, 1758

Table (1) showed the prevalence and mean intensity values of crustacean, *Lamproglena monodi* in *S. galilaeus* in three study sites, Beni-Suif, Lake Manzala, El-Mansoura. It has been found that, the prevalence values of *L. monodi* in *S. galilaeus* were varied significantly from site to site where it showed the highest value (60%) of prevalence in Beni-Suif and as compared with Lake Manzala and El-Mansoura sites. It has been also found that as shown in Table (1), the mean intensity values of the crustaceans showed generally a narrow range of variations among study sites. It varied from mean value of 1.7% in Lake Manzala to 2.3% in Beni-Suif.

Table 1. The prevalence values (%) and mean intensity of crustacean *L. monodi* infesting cichlid fish host *Sarotherodon galilaeus.*

Ecological Parameter	Study Sites		
	Beni-Suif	**El-Mansoura**	**Lake Manzala**
Prevalence values (%)	60.0^{a}	3.7^{b}	1.3^{b}
Mean Intensity	2.3^{a}	1.9^{a}	1.7^{a}

2. Morphological features with scanning electron microscopy of female *L. monodi*

Morphological features for female *L. monodi* were observed: the body parts showing Cephalothorax, Thorax and Abdomen (Fig. 1A, B). The antennae, which are much smaller than the antennules and are located behind them, are two-segmented, with a basal segment and a distal segment. They are located laterally on the anterior of the cephalothorax (Fig. 1C, D). Observation under SEM microscope showed that adult *L. monodi* used their prehensile antennae, maxilla and maxillipeds to attach to gill filaments (Fig. 1C, D, E, F).

Attachment of the antenna with the gills was also observed (Fig. 1E). Each maxilla terminated with chitinized claw. Maxillipeds have three terminal chitinized recurved claws (Fig. 1F). Abdomen consists of three segments, sub-cylindrical, tapering and ended posteriorly with caudal rami which are terminally bifurcated and have one long sharp process (Fig. 1G). The attachment of *L. monodi* in the host fish, *S. galilaeus* was associated with microscopic lesions marked by a sub-acute inflammatory response. The epidermis at the margins of the ulcers showed moderately hyperplasia, degeneration, necrosis and massive loss of filament tissues (Fig. 1B, D, E). At the site of attachment between two adjacent secondary gill lamellae, the parasite was seen penetrating deeply with its opithohaptor to the primary gill lamella causing damage, degeneration of epithelial cells and a cup-shaped depression (Fig. 1B). The haptor surrounded with proliferated and necrotic tissues (Fig. 1E). *L. monodi* causes epithelial hyperplasia with disorganization of epithelial layers, degeneration, necrosis and massive loss of filament tissues in gill filaments (Fig. 1B, C, D, E).

3. Levels heavy metals accumulated in water and parasitic Copepod *L. monodi* from the study sites.

Results in Tables 2 and 3 and Figures 2-4 showed detected heavy metals (Pb, Cd, Zn, Cu and Fe) in water and parasites in different study sites. According to water analyses, results showed that, the concentration of Cd and Pb in collected water samples exceeds the permissible limits according to World Health Organization (WHO, 2022) and The Egyptian drinking water standards (EWQS, 2007) in all study sites. Beni-Suif recorded 0.01 and 1.01 mg/L, El-Mansoura recorded 0.21 and 0.53 mg/L and Manzala Lake recorded 0.08 and 0.33 mg/L for Cu and Zn water concentration values, respectively. Also, There is a significant difference of mean Fe and Cd water concentration values between Ben-Suif (1.18 and 0.11 mg/L) and other study sites (El-Mansoura (0.82 and 0.04 mg/L) and Manzala Lake (0.93 and 0.03 mg/L)), respectively ($P \leq 0.05$). Additionally, a significant difference of mean Pb water concentration values between El-Mansoura (0.08 mg/L) and other study sites (Ben-Suif (0.60 mg/L) and Manzala Lake (0.59 mg/L)) ($P \leq 0.05$).

Results showed that all heavy metals were deposited in parasite tissues more than water in all study sites. Fe was the highest accumulated heavy metal in the water and parasite and recorded its highest concentration in Beni-Suif study site as 1.18 mg/L in water and 8.53 μg/g dry weight in parasite. Zn followed Fe in concentration in water and parasite tissues recording its highest concentrations in Beni-Suif as 1.01 mg/L in water and 7.36 μg/g dry weight in parasite as shown in Tables (2 and 3). Results showed that the greatest values of heavy metal in the parasite were found in Beni-Suif followed by Lake Manzala then El-Mansoura study site. The order of heavy metal accumulation in water was Fe > Zn > Pb > Cu > Cd. However the accumulation pattern in the parasite was Fe > Zn > Cd > Pb > Cu.

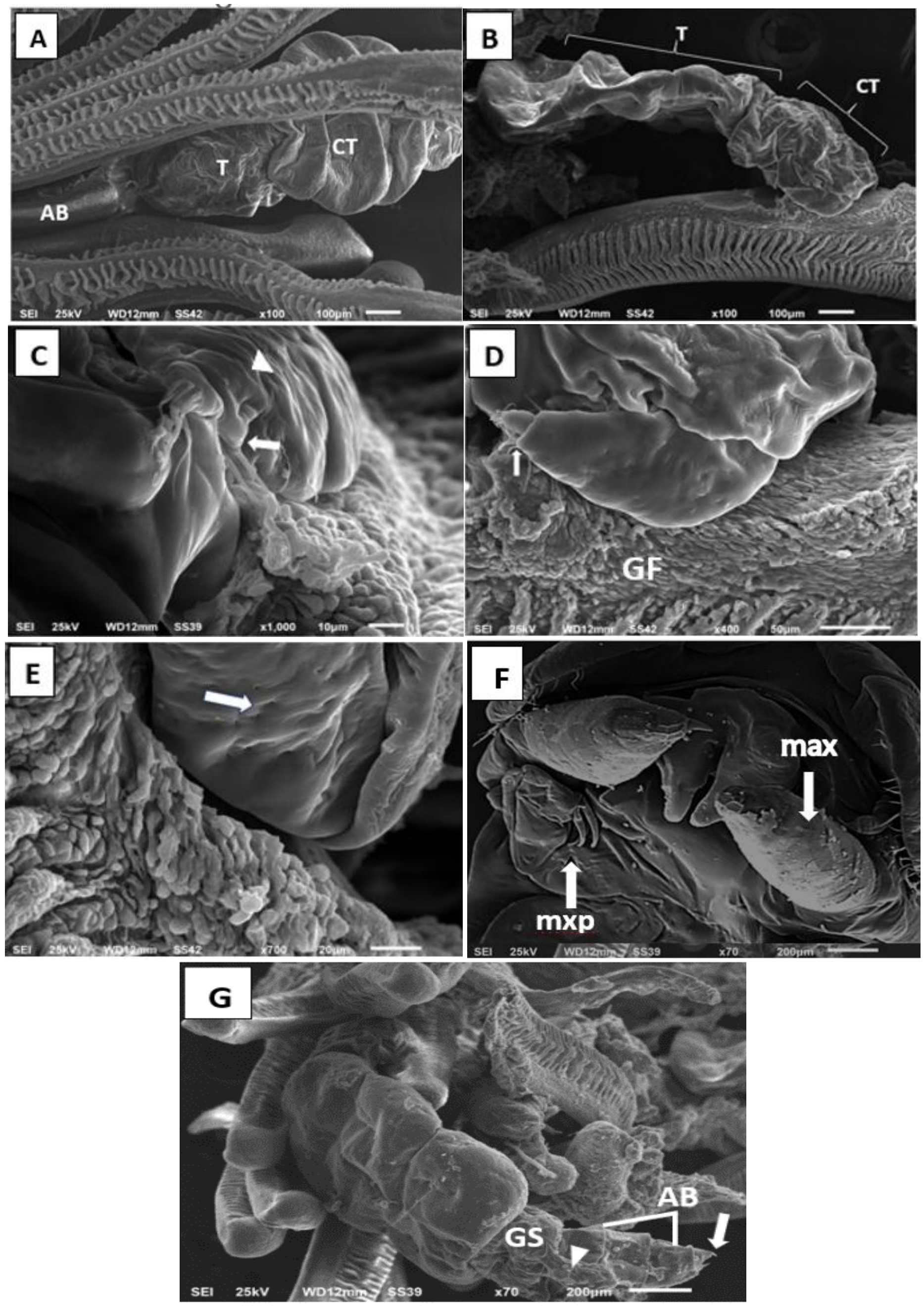

Figure 1. Scanning electron micrographs on female *Lamproglena monodi* Capart, 1944. **A&B)** The body parts showing Cephalothorax (CT), Thorax (T) and Abdomen (AB). Scale bar =100 µm. **C)** Cephalothorax showing antenna (arrow) and antennule (head arrow). Scale bar =10 µm. **D)** Antenna attached to the gills (arrow). **E)** Antennules with protuberances on dorsal surface. Scale bar =50 µm. **F)** Cephalothorax showing Maxillipeds (mxp) and maxilla (max). Scale bar =200 µm. **G)** Showing genital segment (GS), the ovigerous sac (head arrow) and abdomen terminated with Furcal rami (arrow). Scale bar =200 µm.

Table 2. Concentration of Fe, Cu, Zn, Cd and Pb (mg/L) in water from different study sites and their permissible limits according to World Health Organization (WHO, 2022) and The Egyptian drinking water standards (EWQS, 2007).

Site \ Metal	Fe	Cu	Zn	Cd	Pb
Beni-Suif	1.18 ± 0.07[a]	0.01 ± 0.01[c]	1.01 ± 0.23[a]	0.11 ± 0.04[a]	0.60 ± 0.02[a]
El-Mansoura	0.82 ± 0.04[b]	0.21 ± 0.10[a]	0.52 ± 0.12[b]	0.04 ± 0.02[b]	0.08 ± 0.02[b]
Lake Manzala	0.93 ± 0.06[b]	0.08 ± 0.04[b]	0.33 ± 0.08[c]	0.03 ± 0.01[b]	0.59 ± 0.03[a]
(WHO, 2022)	N*	2.00	N*	0.003	0.01
(EWQS, 2007)	3.00	2.00	3.00	0.003	0.01

N*: Not of health concern at levels found in drinking‐ water according to WHO (2022).

Data are represented as means ± SE (n=25) –Values with the different superscript on the same column are significantly different at P < 0.05. –Values with the same superscript are not statistically significant at P > 0.05.

Table 3. Concentration of Fe, Cu, Zn, Cd and Pb (μg/g dry weight) in parasitic Copepod *L. monodi* from the study sites.

Site \ Metal	Fe	Cu	Zn	Cd	Pb
Beni-Suif	8.53±1.22[b]	1.83±0.07[b]	7.36±1.02[bc]	4.4±0.3[ab]	2±.0.19[b]
El-Mansoura	5.97±0.61[a]	1.1±0.05[a]	4.67±0.17[a]	3.57±0.18[a]	1.12±0.0.09[a]
Lake Manzala	6.47±0.24[ab]	1.29±0.05[a]	6.23±0.33[b]	3.27±0.31[a]	1.53±0.11[ab]

Data are represented as means ± SE (n=25) –Values with the different superscript on the same column are significantly different at P < 0.05. –Values with the same superscript are not statistically significant at P > 0.05.

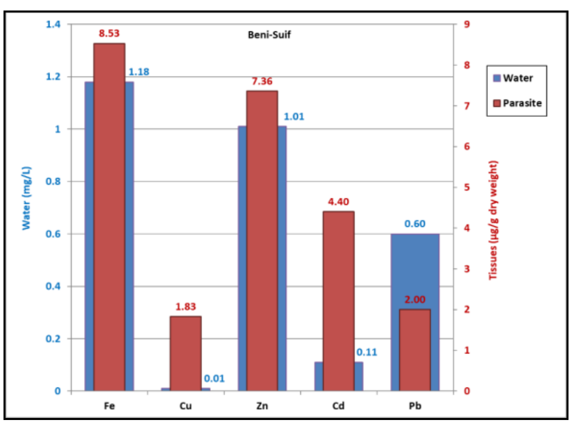

Figure 2. Concentrations of Fe, Cu, Zn, Cd and Pb deposited in water and parasitic Copepod *L. monodi* from Beni-Suif study site.

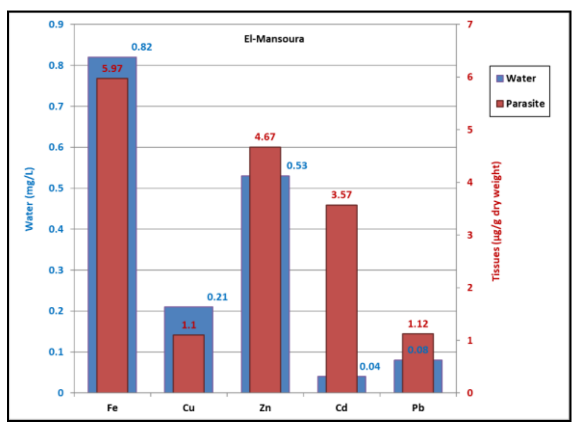

Figure 3. Concentrations of Fe, Cu, Zn, Cd and Pb deposited in water and parasitic Copepod *L. monodi* from El-Mansoura study site.

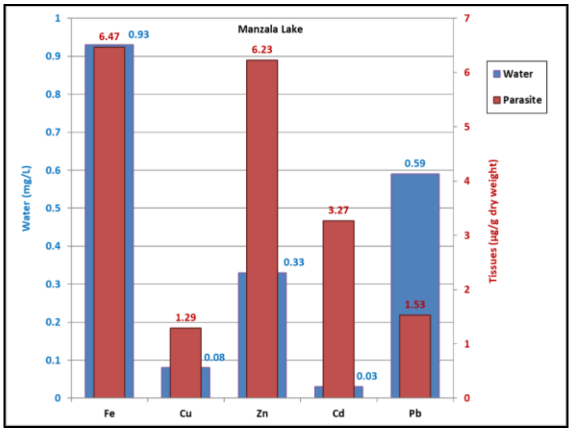

Figure 4. Concentrations of Fe, Cu, Zn, Cd and Pb deposited in water and parasitic Copepod *L. monodi* from Manzala Lake study site.

DISCUSSION

The second most significant copepod parasite of freshwater fish is *Lamproglena* von Nordmann, 1832 **(Piasecki *et al.*, 2004)**. According to **Fryer (1959)** *L. monodi* is a typical parasite found in fishes. In this study, *Sarotherodon galilaeus* fish from the study locations of El-Mansoura, Lake Manzala, and Beni-Suif had *L. monodi* adhered to their gills. In this study, the described species was recognized as *L. monodi* as features reported by **Capart (1944)** and the re-characterization of the parasite **(Ibraheem and Izawa, 2000)**. It has been observed also that the values of prevalence and mean intensity of majority of the parasite species in *S. galilaeus* showed relatively high values in Beni-Suif. It was clearly also obvious that, the values of prevalence and mean intensity were higher in River Nile locations than in Lake Manzala during the investigated period. These variations may be attributed to the time and/or locality-induced parasite community structure differences (**El-Naggar and Khidr, 1988; Poulin and Dick, 2007)**. In the present study, the prevalence value of *L. monodi* in *S. galilaeus* was 60% in Beni-Suif. In this context, **(Ghiraldelli *et al.*, 2006)** stated that 90% more commonly found of *Lamproglena* sp. However, a higher prevalence 90% of *Lamproglena* sp. Additionally, **Ibraheem and Izawa (2000)** found that *O. niloticus, S. galilaeus*, and *Tilapia zillii* infection rates at El-Minia, in the Nile River system, were 20%, 16%, and 20%, respectively. Consequently, this study showed a prevalence rate higher than previously observed, and this could attract attention to the spreading infestation over time. In Beni-Suif, El-Mansoura, and Lake Manzala study sites, the present study found mean intensity of 2.3, 1.9, and 1.7 parasites adhering to the gills, respectively. According to **(Ibraheem and Izawa, 2000)**, there was no description of the *L. monodi* mean intensity. However, *Lamproglena* sp.'s mean intensity in Brazil in cultured *O. niloticus* was 3.1% **(Ghiraldelli *et al.*, 2006)**. Also, Infestations of *Lamproglena* sp. were found in just 1% of the tilapia examined **(Azevedo *et al.*, 2006)**. Furthermore, **(Eissa, 2002)** reported that invasion intensities ranged widely. Fascinatingly, there is no relationship between the total lengths of the fish collected and many parasites were discovered on their gills. **(Austin and Avenant-Oldewage, 2009; Rindoria *et al*., 2022)**.

In the present study, the parasite copepod *L. monodi*'s attachment and histological consequences on the gills of *S. galilaeus* have been thoroughly investigated. In this investigation, the parasite was connected to the gill just below their tips. The similar finding was made about other parasite species, where *L. hoi* favourited to be found on both sides of the second gill and favourited the median location on the gill arches **(Austin and Avenant-Oldewage, 2009)**. Though, **Tsotetsi *et al*. (2004)** revealed that *L. clariae* was more common on the fourth gill. The optimal opportunity for attachment is thought to be provided by the water flow in these regions. Additionally, this will offer a benefit for the distribution of progeny **(Austin and Avenant-Oldewage, 2009).** As a result, **Tsotetsi *et al*. (2004)** hypothesised that the protection or decreased rough air currents in the gill area may provide the cause of connection position.

The antenna demonstrated by **Capart (1944)** had four segments, however the specimens used in this study only had three segments. The number of segments is not mentioned in the re-characterization by **Ibraheem and Izawa (2000)**. The caudal rami also had three papillae in the current specimens but **Ibrahim and Izawa (2000)** reported that caudal rami had four papillae.

Teloest fish infected with copepod parasites were noticed in the current investigation to exhibit clumping and fusion of lamellae. According to **Kabata (1985)**, any epithelial thickening will significantly impede diffusion across it, disrupt or prevent normal ionic exchanges, and serve as an ideal substrate for bacteria and fungus (secondary infections). Additionally, the damage and degeneration noted in this study's findings could lead to the loss of respiratory surface and expose underlying tissue to secondary infections. These findings were in agreement with those made by **Abu Samak (2005)** for the parasitic copepod, *Lernanthropus kroyeri*.

Heavy metals are among the most hazardous pollutants in aquatic environments because they are persistent, bioaccumulative, and infrequently biodegradable **(Keke *et al*., 2020)**. These pollutants may have an impact on the survival and performance of parasites **(Akinsanya *et al*., 2020)**. Results showed that, the mean concentration of Cd and Pb in collected water samples exceeds the permissible limits according to World Health Organization **(WHO, 2022)** and The Egyptian drinking water standards **(EWQS, 2007)** in all study sites.

The United States Environmental Protection Agency (USEPA) has classified lead (Pb) as potentially dangerous to the majority of life forms. In addition, according to **Moore (1973)**, two-thirds of the African countries have lead levels that are greater than the permitted global median amount. High levels of home, commercial, and industrial wastewater dumped into these waterways may be the cause of high lead levels. The lead contents in water of Lake Manzala, according to **Zyadah (1995)**, were (0.343-1.185 mg/L), greater than those found in the current study. Cadmium (Cd) is one of the most poisonous heavy metals and is regarded as non-essential for living things **(Woodbury, 1998)**. Due of its high mobility in the fish and soil environments, cadmium is regarded as the heavy metal of greatest concern **(Wilson and Bell, 1996)**.

Parasitic species may be used as biological markers of environmental pollution **(Mashaly *et al*., 2020)**. Consequently, parasites might be viewed as helpful tools for learning more about the host environment. *L. monodi* worms of *S. galilaeus* accumulate heavy metals at high rates, suggesting that they potentially be utilised as heavy metal pollution biomonitors. Digeneans, acanthocephalans, and nematodes all showed similar results **(Sures, 2001; Al-Hasawi, 2019)**. According to **Sures (2003)**, fish parasites can effectively detect contamination with heavy metals in aquatic habitats. Researchers examined how nine heavy metals built up in the water, gills, intestines in digenean parasites of the *Bagrus bajad.* **Mashaly *et al*. (2021)** discovered that digenean parasites accumulated greater amounts of all studied heavy metals than gills, intestine, and water samples with the exception of chromium, which recorded its highest concentration in the gills. The present study showed that the highest concentrations of all studied heavy metals were noticed in parasitic copepods collected from Beni-Suif and Manzala Lake may be due to industrial and agricultural wastes. Many researches have stated that many parasites accumulate heavy metals in their bodies. **(Baruš et al. 2007; Akinsanya *et al*., 2020).** The present study showed that Fe was maximally accumulated in the parasite whereas Cu and Pb were the least concentrations. Consequently, Parasite could be used as bioindicators of heavy metal toxicity of water. It is apparent that fish and parasites are utilized as biological indicators to evaluate the environment they inhabit **(Omar *et al*., 2021)**.

CONCLUSION

The parasite's distinctive morphological characteristics were illustrated using Scanning Electron Microscopy. The ecological results showed that the prevalence values of *L. monodi* in *S. galilaeus* highest value was detected in Beni-Suif. Moreover, mean concentrations of Cd and Pb in collected water samples exceeds the permissible limits according to The Egyptian drinking water standards and WHO in all study sites. Additionally, the findings revealed that all heavy metals were accumulated in parasite tissue more than water in all study sites and the result showed that the potential use of *L. monodi* as bioindicator of heavy metals by accumulating them. The parasite responds well to changes in the environment's pollution.

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