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Surveillance of Catastrophic Fish Decline in Lake Qarun: Endurance of the Egyptian Sole (*S. aegyptiaca* Chabanaud, 1927) Against Isopod infestation

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ABSTRACT

Lake Qarun in Egypt has experienced a catastrophic decline in fish populations, with isopod infections identified as one of the major contributing factors. A ten-season surveillance study was conducted to investigate disease patterns, fish species composition, cymothoid species identification, and parasite behavior across the eastern, middle, and western regions of the lake. The study examined clinical signs of disease, prevalence and intensity indices, and the relationships between fish species and both free-swimming **(**FSCs**)** and permanent cymothoid stages. A total of 1,901 *Tilapia* spp., 2,056 *Solea aegyptiaca*, and 7,650 *Atherina* spp. were captured, along with 823 FSCs. The isopod species infesting Lake Qarun was morphologically identified as *Livoneca redmani*. The prevalence of isopod infestation among *Tilapia* spp., *S. aegyptiaca*, and *Atherina* spp. was 43.46, 12.59, and 5.27%, respectively. *Tilapia* spp. and *S. aegyptiaca* exhibited both gill and skin infestations, whereas *Atherina* spp. showed only skin infestations. Double-gill pouch infestations were observed exclusively in *Tilapia* spp., with a branchial infestation intensity index ranging from 1.2 to 2.0, compared to 1.0 in *S. aegyptiaca*. *Tilapia* spp. demonstrated a strong association with the parasite, while *S. aegyptiaca* and *Atherina* spp. exhibited weaker associations. The decline of *Tilapia* spp. was correlated with a reduction in FSC numbers. The study revealed that *Tilapia* spp. serve as permanent hosts, whereas *S. aegyptiaca* and *Atherina* spp. act as transitory hosts. Notably, *S. aegyptiaca* could become a permanent host in the absence of *Tilapia* spp. A stock improvement program for *S. aegyptiaca* could help mitigate the decline in Lake Qarun's fish populations by sustaining fish stocks capable of surviving *L. redmani* infestation, thus preserving economic value.

INTRODUCTION

Lake Qarun, one of the oldest Egyptian lakes, dates back to prehistoric times as a natural freshwater lake **(Ball, 1939)**. It is the remnants of the ancient "Moeris Lake" and occupies 214km^2 of Fayoum Governorate, 80 kilometers southwest of Cairo. The lake is unique in nature, as it is a habitat for numerous animals and birds, both resident and migratory. It was locally declared as a nature reserve since 1989, and internationally as a

Ramsar site since 2012 **(Fouda & Fishar, 2012, El-Sayed *et al*., 2015)**. From an archaeological perspective, the lake demonstrates evidence of a great civilization, including the oldest paved way in the world, in its northern zone **(Ibrahim & Abd El-Sattar, 2010)**. The lake has been receiving freshwater from the River Nile since ancient times. It served as a natural reservoir for the Nile floodwaters, storing excess water during the annual flood season from June to September **(Mohamed *et al*., 2005)**. However, in the last century, particularly after the construction of the High Dam in 1961, the flow of excess water ceased. Consequently, combined with various environmental, topographical, and anthropogenic factors, the lake's ecological conditions have progressively changed, gradually shifting from freshwater to brackish and ultimately towards marine-like conditions **(El-Shabrawy & Dumont, 2009; Baioumy *et al*., 2010)**. Although it receives 50 million cubic meters of freshwater annually from twelve different drains, this amount is not enough to decrease its salinization **(Abdel-Satar *et al*., 2010)**. Additionally, the shortage of rains in the Fayoum region has worsened the situation, with an average of only 2.9 rainy days annually **(Mehrim & Refaey, 2023; Seif *et al*., 2023)**. In addition, global warming and climatic changes contribute to the increased lake's evaporation rate **(Konsowa, 2006)**. Concerning the fresh water feeding system of the lake, El-Batts and El-Wadi drains are the major sources of freshwater, providing over two-thirds of the lake's incoming freshwater inflow **(Abd-El-Baky, 2022)**. There are also ten minor sources, eight of which drain by gravity and two are collected in a water body called "Dayer-El Barka" (Fig. 1). These sources are mainly consisted of agricultural, insufficiently treated industrial, sewage, and domestic effluents, which magnify the problem of eutrophication due to increased nutrients, especially ammonium (NH_4^+) and phosphate (PO_4^{3-}) ions **(AbuKhadra *et al*., 2020)**. The Lake Qarun ecosystem is facing multifactorial environmental challenges, including the climatic conditions, amount of discharged wastewater, seepage from the surrounding cultivated land, and geological aspects **(Abdel-Satar *et al*., 2010)**. Economically, it is considered as one of Egypt's largest lakes, it contributed 5% of the country's total lakes' fish production in 2014 **(GAFRD, 2021)**. However, its fish production is considered low due to the shift from freshwater fish to marine ones, leading to the disappearance of previously coexisting species **(Abdelmageed *et al*., 2022)**. To address this, new fish species, such as *S. aegyptiaca* and *Tilapia* spp., have been introduced **(El-Zarka, 1963; El-Zarka, 1986)**. Previously, *Solea vulgaris* was thought to be the ancestral species of *S. aegyptiaca*, but current research shows it is a distinct species with distinct morphological and molecular characteristics **(Ouanes *et al*., 2011; Khalifa & Abdelmageed, 2024)**. To increase the lake's production, governmental authorities annually transplant seedlings from northern lakes containing various fish species, including mullets (e.g., *Mugil cephlaus, Chelon saliens, Chelon ramada,* and *C. labrosus*), the gilthead seabream, *Sparus aurata*, and the European seabass *Dicentrarchus labrax.* Anchovy seedlings, particularly *Atherina* spp., were accidentally transplanted and successfully lived in harmony with the lake conditions, reproducing, and establishing themselves as native fish components of the lake **(El-Serafy *et al*., 2014)**. Although the total lake fish production increased to reach 4518 tons in 2014, unfortunately it dropped to 1124 tons in 2015 **(GAFRD, 2021)**. The dramatic scenario in fish production continued to remain critically low in 2018, including native species (e.g., *Tilapia* spp. and *S. aegyptiaca*) and exotic ones (e.g., mullet, seabass, etc.). Research has shown that isopod infestation is the major cause of the crisis that predominantly parasitize the gill chambers of fish, attaching within the branchial cavity between the operculum and the gills **(Mahmoud *et al*., 2017; Khalaf-Allah & Yousef, 2019; Mehanna, 2020; Shalloof *et al*., 2022; Mahmoud *et al*., 2023)**. Isopod species have been confirmed to belong to the Cymothoidae family and exhibit a complex life cycle, consisting of free-swimming stages that attach to suitable hosts and parasitic stages that attach permanently **(Fogelman, 2005)**. Throughout their life cycle, cymothoids interact with two types of hosts: temporary hosts for free-swimming stages to attach and feed, and permanent hosts for growth and reproduction **(Hadfileld *et al*., 2025)**.

The study aimed to understand the status of isopod infestation in Lake Qarun through quarterly monitoring over ten-seasons spanning from autumn 2021 to winter 2023. The morphological characterization of the detected isopod species and their attacking strategies were examined. The prevalence and intensity indices of isopod infestation among different fish species were recorded with a focus on the presence of free-swimming cymothoid stages as a sensitive biomarker for infestation risk and persistence of reproductive activity. Special attention was taken to identify fish species during the monitoring period to understand how both free-swimming and persistent cymothoid stages correlate with temporary and permanent fish hosts.

MATERIALS AND METHODS

1. Study area

Lake Qarun (Fig. 1) is an irregular closed basin located roughly 80 kilometers southwest of Cairo. It is situated between latitudes 29°24' and 29°33' N and longitudes 30°24' and 30°49'. It has an elongated shape with average dimensions of 47km long, 6km wide, and 4.2m deep. The lake depth varies gradually from 2 to 8m. The lake's water surface is at least 43 meters below mean sea level (BMSL) and reaches a maximum of 43.5 meters below BMSL **(Goher *et al*., 2018)**. The designated area of study included three regions (east, middle, and west) to facilitate the process of investigation and sampling collection.

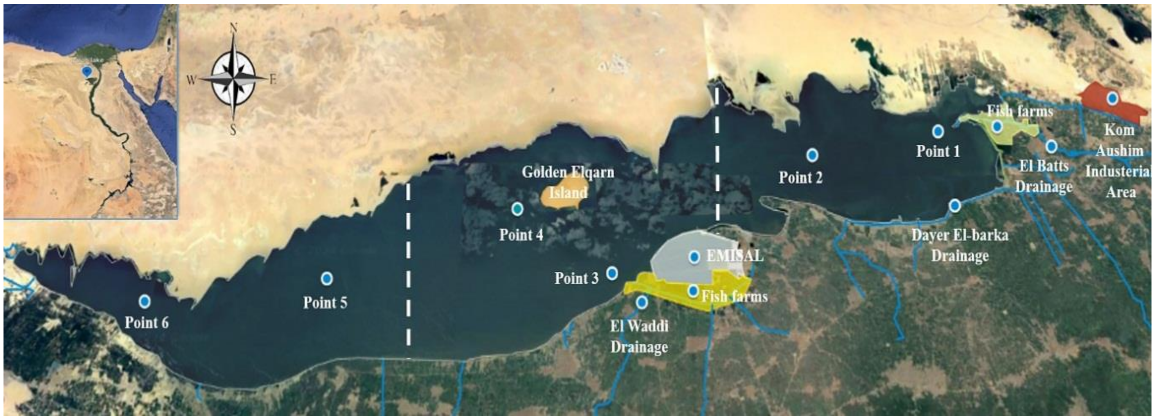

Fig. 1. A modified Google map shows the surveillance investigation's three regions and selected points of fish sample collection. These points included the east, middle, and west regions Qarun. El Batts and El Waddi, the primary drainage feeding effluents, are depicted by blue lines, which stand for the freshwater feeding sources. The map also displayed the lake territories and the golden-Elqarn Island centralized the lake a little bit toward the northern border

2. Fish sampling

Thirty fishing expeditions were carried out, with ten in every location that was chosen, and ten consecutive seasons starting in the autumn of 2021 and ending in the winter of 2023. These sampling expeditions were focused on capturing not just the various fish species present in the lake, but also on capturing and gathering the free-swimming cymothoidal stages (FSCs). Only non-motorized boats were permitted for the sample visits due to the Lake's status as a natural reserve. Table (1) shows all of the sampling equipment, which includes several kinds of fishing gear. Prior to starting the trip by day, all different net types, except beach seine, were set at the designed sites of investigation. Beside the different net types used, there was a traditional way used to collect samples, particularly the FSCs of the isopods, called fish aggregating system (FAS) or "*Heml*" by native language **(El-Serafy *et al*., 2014; Mehanna, 2020)**. This tool is designed to attract fish and other swimming creatures and is constructed of tree branches, tree roots, floating vegetation, and grasses. It used to be set up a week before the trip. Each trip began before sunrise, starting with heading to the Heml area, followed by the retrieval of trammel nets. The last stage of the sampling trip involved employing the beach seine at the investigated area **(**was conducted for about one hour**)** for at least an hour. All collected fish samples were subjected to preliminary inspection on the boat deck for isopod infestation, with special concern for the fish's external surface, gill pouch, and mouth. Special care was taken for the separation of the collected FSCs in a separate container during transportation. Sequentially, samples were collected in barrels (*Ca*. 200L) and transported to the wet laboratory of the Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Beni-Suef University, Egypt, for further investigation.

Table 1. Fishing gears used to catch various kinds of fish and the mancae during the study

Fishing gear type	Local name	Mesh size (mm)	Height (m)	Length (m)
Tilapia Trammel nets	*Ghazl Bolti*	Inner: 22-29.4 Outer: 62.5 – 90.5	2	1000
Solea Trammel nets	*Ghazl Moussa*	Inner: 20.8- 29.4 Outer: 62.5- 85.2	2.5	1500
Beach seine	*Goraphate Zardina*	8.3	3	300
Fish aggregating system(FAS)	*Heml*	25	4	8

3. Parasitological examination, isopod infestation prevalence, and clinical signs

On laboratory arrival, all species of the collected fish samples were categorized according to their species, size, weight, and/or length before being subjected to parasitological examination. All fish specimens were carefully examined grossly for clinical signs and deeply using a dissecting microscope (American Optical Corp., Buffalo®, NY, USA) for isopoda (any stage). The examination included the fish's external surface, operculum, gills, branchial cavities, and mouth cavities. The collected parasites were then described morphologically by employing the description previously outlined **(Brusca, 1981; Mahmoud *et al*., 2023)**. Selected infested fish and isopoda stage(s) was/were then photographed. Additionally, fish with infested opercula were subjected to opercular dissection, and close gill examinations were performed. On the basis of the collected data, the prevalence of isopod infestation among investigated fish species was calculated following the methodology described by **Margolis *et al.* (1982)** and **Bush *et al.* (1997)**, as follows:

$$\text{Prevalence }\% = \frac{\text{The number of infested fish}}{\text{The total number of examined fish}} \times 100$$

The branchial infestation intensity indexes were calculated and experienced based on the number of fish afflicted with branchial infections and the number of isopoda in both branchial cavities.

4. Clarifying cymothoid's attacking strategy

To further understand the attacking strategy of cymothoids, a preliminary experiment was conducted to elucidate the "playing opossum" and "klinokinesis" attacking behavioral patterns noted by **Thatcher (2000)** and **Fogelman and Grutter (2008)**. Briefly, three small glass aquaria (30 L × 10 W × 25 H cm, dimensions) were set at room temperature (26±0.5ºC), filled with filteredlake water, and supplied by air stones. To avoid attacking competition, one apparent healthy juvenile seabass, *Dicentrachus labrax*, (15 ± 5 *g*; 12 ± 1cm), and one FSCs were cohabitated together in each aquarium and were observed for two hours. Available attacking cymothoid's behaviors were recorded and photographed.

5. Statistical analysis

GraphPad Prism version 9.1.0.221 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com was utilized to assist the statistical analysis of total fish catch, total catch of *Tilapia* spp., *S. aegyptiaca*, *Atherina* spp., FSCs, and the prevalence were performed using two-way ANOVA and Tukey's multiple comparisons test. Moreover, the software was also employed to conduct statistical analysis via straightforward logistic regression test with a 95% confidence level to investigate the potential relationship between the caught fish and the FSCs. To validate the results of the linear regression coefficient and the strength (r value) of the relationship, Pearson's correlation test was utilized. The very strong strength, strong, moderate, weak, and very weak were experienced as r value = 0.8~1.00, 0.6~ 0.79, 0.4~0.59, 0.2~0.39, and 0.0~0,19, respectively. Differences are deemed significant at $P < 0.05$.

6. Ethics approval statement

The Egyptian Code of Practice and ethical guidelines for the treatment of animals used for studies and other scientific purposes were rigorously followed in the conduct of all "*in vivo*" procedures. The Beni-Suef University Institutional Animal Care and Use Committee (BSU-IACUC) has approved each experimental procedure and given it an approval number (022-516).

RESULTS

1. Fish sampling

A total of 1901, 2056, and 7650 individuuals of *Tilapia* spp., *S. aegyptiaca*, and *Atherina* spp., respectively, were captured by using different fishing gears labelled in Table (1). They weighed 137, 132, and 255kg, respectively. The total catch of each species varied according to both sampling sites and seasons throughout the study period (Table 2, Fig. 2). The western region recorded the highest catches of *Tilapia* spp. and *Solea aegyptiaca*, while the middle region had the highest catch of *Atherina* spp. In contrast, the eastern region consistently exhibited the lowest catches across all species. The autumn and winter of 2021 showed the highest total catches for the three fish species, compared to corresponding seasons in 2022 and 2023. Conversely, all seasons in 2023 recorded the lowest catches across the study period (Table 3).

Tilapia spp. experienced a sharp decline, reaching a minimum in winter 2023, and were notably absent from the eastern region during the final consecutive seasons of the study (Fig. 3A). In contrast, the catch of *S. aegyptiaca* showed marked fluctuations, with a significant drop in spring and summer 2022, followed by a partial recovery that, though reaching no initial levels (Fig. 4C). *Atherina* spp. catches remained relatively stable, with the exception of a sharp decline observed in spring 2023 (Fig. 4D).

Regarding the collection of free-swimming cymothoids (FSCs), a total of 823 parasites at various life stages were collected over the ten-season surveillance period (Table 3, Fig. 3B). The highest FSC count was recorded in autumn 2021, with 361 individuals collected. This number steadily declined, reaching just 4 individuals by winter 2023.

Table 2. The fish catch, infested fish, and isopod infestation prevalence analyses across various regions and seasons

Region/Season		Total fish catch			Total infested fish			Isopod infestation prevalence (%)			Prevalence mean (Mean±SE) %
		T	S	A	T	S	A	T	S	A	
Region	East	410	566	2190	226	92	157	55.12	16.25	7.17	26.18±14.71
	Middle	638	607	4140	309	87	201	48.43	14.33	4.86	22.43±13.23
	West	864	885	1941	296	80	78	34.26	9.04	4.02	15.77±9.36
Season	Autumn	782	583	2370	471	102	243	60.23	17.50	10.25	29.32±15.59
	Winter	483	978	3900	90	80	77	18.63	8.18	1.97	9.59±4.86
	Spring	460	242	720	154	23	31	33.48	9.50	4.31	15.76±8.98
	Summer	187	255	1281	116	54	85	62.03	21.18	6.64	29.95±16.58
Total		1912	2058	8271	831	259	436	**43.46**	**12.59**	**5.27**	

T, referred to *tilapia* spp; S, referred to *S. aegyptiaca* ; A, refer to *Atherina* spp. and * (mean±SE).

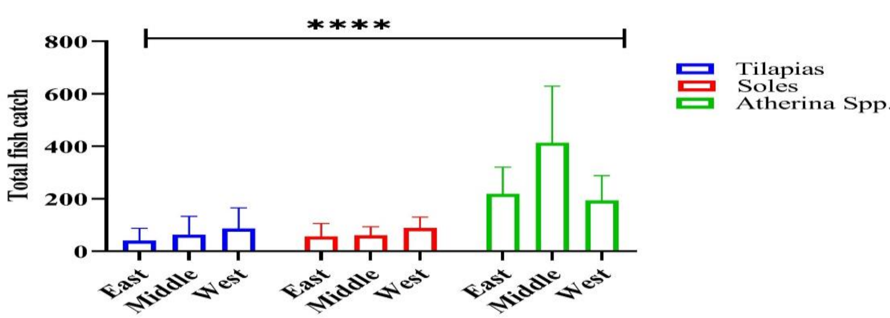

Fig. 2. Total fish catch of the three fish species caught during the thirty fishing expeditions carried throughout the ten-season surveillance study. Notice the high significance (*P<0.05*) differences between *Atherina* spp. and both *Tilapia* spp. and *S. aegyptiaca.*

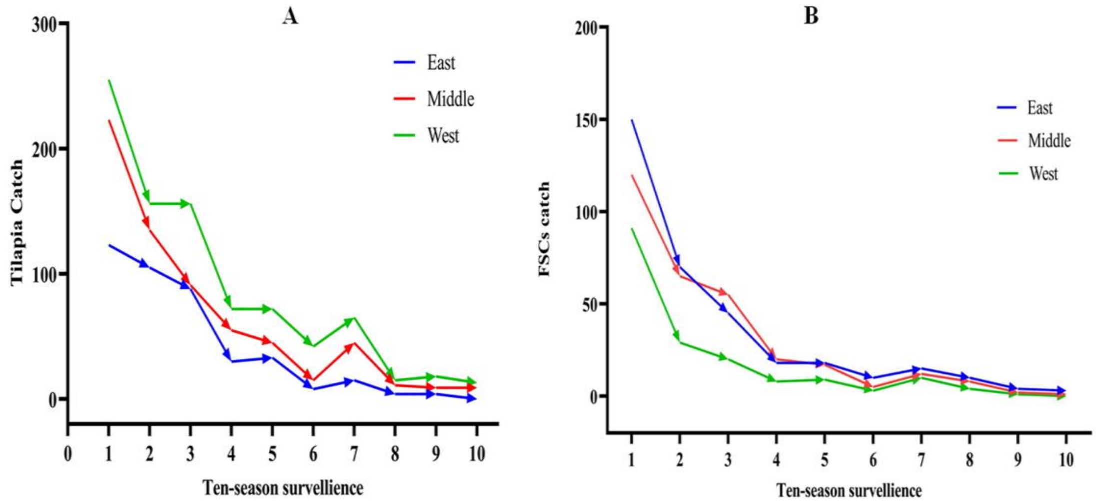

Fig. 3. Total catch of *Tilapia* spp. and free-swimming cymothoids **(**FSCs**)** caught during the ten-season surveillance study in accordance with each investigation region. **A**, showed a gradual decrease in *Tilapia* spp. catch, especially in the last three consecutive seasons. **B**, exhibited a gradual decrease in caught FSCs that seemed to be correlated with the gradual decrease in caught *Tilapia* spp.

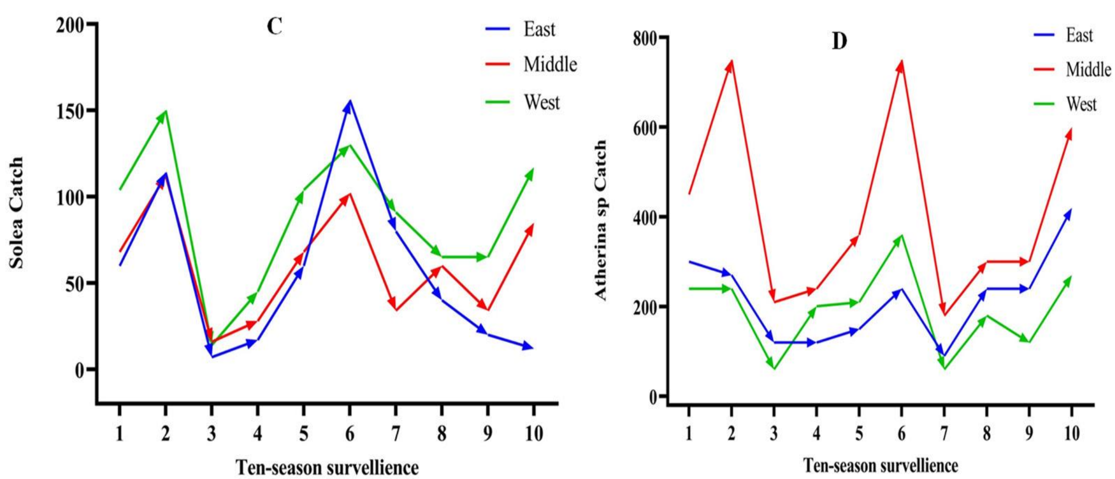

Fig. 4. Total catch of Egyptian *S. aegyptiaca* and *Atherina* spp. caught during the ten-season surveillance study in accordance with each investigation region. **C** and **D**, showed alterations in the total *S. aegyptiaca* and *Atherina* spp. catch that seemed at the same levels in different investigated regions

Table 3. The fish catch, infested fish, and isopod infestation prevalence in comparison with the (FSCs) catch

Season/Region		Total fish catch			Total infested fish			Prevalence %			FSCs catch
		T	S	A	T	S	A	T	S	A	
Autumn 2021	east	123	60	300	90	20	70	73.17	33.33	23.33	150
	middle	223	68	450	140	15	91	62.78	22.06	20.22	120
	west	255	104	240	142	18	28	55.69	17.31	11.67	91
Winter 2021	east	105	114	270	30	15	20	28.57	13.16	7.41	70
	middle	135	112	750	31	17	31	22.96	15.18	4.13	65
	west	156	150	240	21	18	10	13.46	12.00	4.17	29
Spring 2022	east	88	7	120	48	2	8	54.55	28.57	6.67	45
	middle	91	16	210	44	4	12	48.35	25.00	5.71	55
	west	156	14	60	32	3	2	20.51	21.43	3.33	20
Summer 2022	east	30	17	120	24	6	9	80.00	35.29	7.50	18
	middle	55	28	240	40	7	20	72.73	25.00	8.33	20
	west	72	45	201	39	6	14	54.17	13.33	6.97	8
Autumn 2022	east	33	60	150	25	16	19	75.76	26.67	12.67	18
	middle	45	68	360	30	12	15	66.67	17.65	4.17	17
	west	72	104	210	30	9	5	41.67	8.65	2.38	9
winter 2022	east	8	156	240	1	10	7	12.50	6.41	2.92	10
	middle	15	102	750	2	8	8	13.33	7.84	1.07	5
	west	42	130	360	3	6	0	7.14	4.62	0.00	3
Spring 2023	east	15	80	90	5	6	4	33.33	7.50	4.44	15
	middle	45	34	180	11	3	2	24.44	8.82	1.11	12
	west	65	91	60	14	5	3	21.54	5.49	5.00	10
Summer 2023	east	4	40	240	2	10	12	50.00	25.00	5.00	10
	middle	11	60	300	5	14	18	45.45	23.33	6.00	8
	west	15	65	180	6	11	12	40.00	16.92	6.67	4
Autumn 2023	east	4	20	240	1	6	8	25.00	30.00	3.33	4
	middle	9	34	300	5	5	3	55.56	14.71	1.00	2
	west	18	65	120	8	1	4	44.44	1.54	3.33	1
winter 2023	east	0	12	420	0	1	0	0.00	8.33	0.00	3
	middle	9	85	600	1	2	1	11.11	2.35	0.17	1
	west	13	117	270	1	3	0	7.69	2.56	0.00	0
Total		1912	2058	8271	831	259	436	43.46	12.59	5.27	823

Prevalence mean	*Tilapia* spp. (T)	*S. aegyptiaca* (S)	*Atherina* spp. (A)
	38.75±4.25*	**16.00±1.78**	**5.62±0.99**

2. Parasitological examination, isopod infestation prevalence, and clinical signs

2.1. Isopoda identification

Description of the isolated isopod species (Cymothoids) was performed on the basis of the morphological characteristics of adult ovigerous females following the descriptions and identification keys outlined by **Brusca (1981)**, **Mohammed-Geba *et al*. (2019)** and **Mahmoud *et al*. (2023)**.

The adult ovigerous female had an ovate, twisted to one side, and light brown body with dark chromatophores with an approximate average length and width of 16.2±2.34 and 6.5-11.5mm, respectively. The body is composed of three main parts, including the cephalon, pereon, and pleon. The cephalon is concave and pointed, with a folded anterior edge where the armed mouth with powerful, sharp canine-like incisors and the large compound eyes are occupied. Along with the compound eyes, on the tip of the cephalon, two pairs of antennae exist as the organs of sensation. The second main part of the isopods is the pereon. The pereon is the widest part of the parasite, which is composed of seven segments (pereonites) densely packed with chromatophores, especially at their posterior edges. Each segment is supported by a pair of modified legs (pereopods) terminated with a powerful claw (dactylus), providing a strong locomotor and fixing capability to the parasite. The pereopods usually show a sequential size increase towards the seventh one, with an opposite directional orientation for the first three pereopods and the last four ones. Pleon is the third main part of the parasite’s body and is composed of five segments (pleonites) that decrease gradually in width towards the posterior, resulting in a relatively narrower attribute than pereon. Each segment is equipped by a pair of pleopods, which are laminar-shaped with highly branchiated haired peduncles and enlarged lateral margins accommodating respiration fitness and swimming speediness. By the end of the pelon, a triangular protrusion forms a round posterior end of the parasite (pleotelson), which displays two pairs of unjointed pods (uropods) extending beyond the posterior margin. The uropod pairs include the exopod pair and endopod pair; the former is taller and thinner than the latter; however, both are bluntly rounded and possess plumose marginal hairs (setae). The gravid female forms the brood pouch, which is a plate-like expansion along the ventral surface (oostigites) carrying the young stages (pre-mancae). At all levels, morphological characteristics are illustrated in the Fig. (5) (gravid female). On the basis of these morphological characteristics, the investigated isopods were validated as *Livoneca redmani Leach*, 1818 (*L. redmani*).

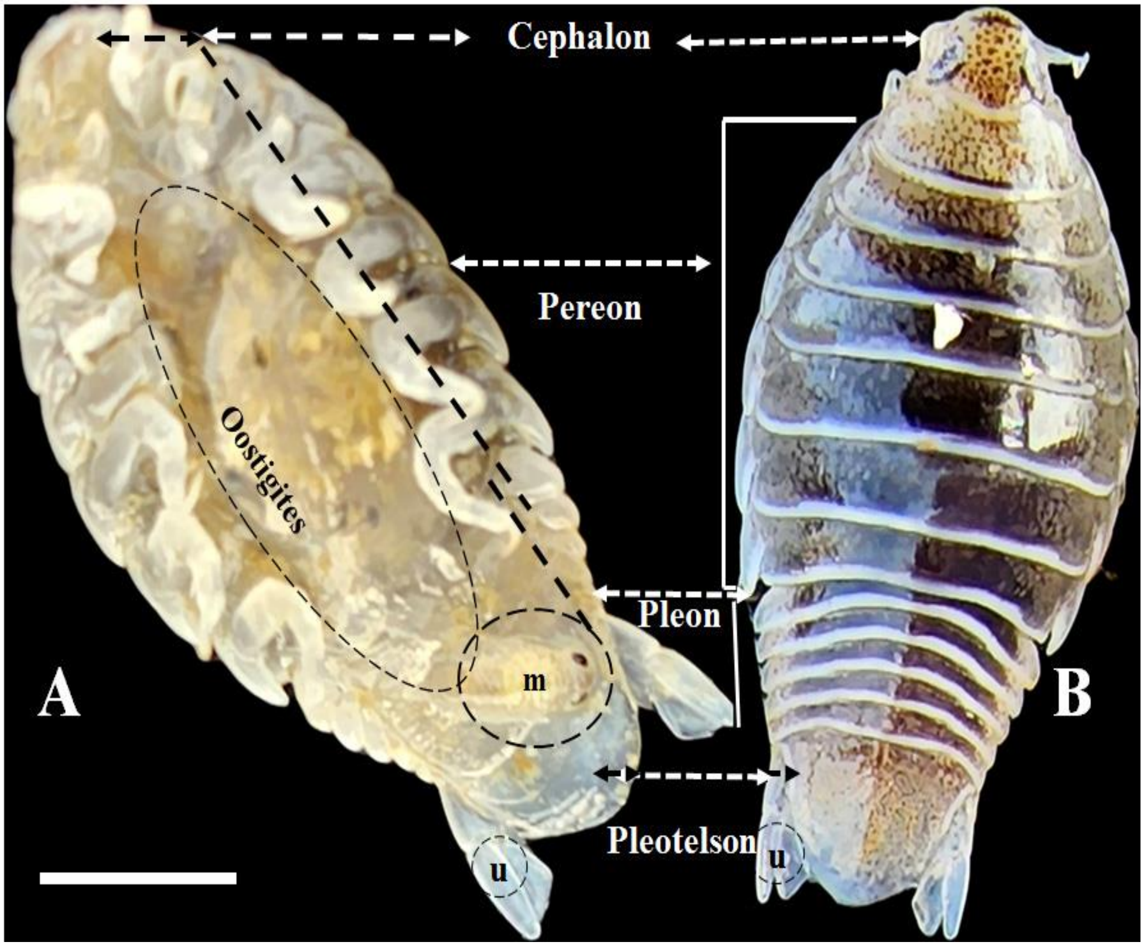

Fig. 5. Macrograph illustrated the gravid female *Levonica redmani* from its ventral **(A)** and dorsal **(B)** surfaces, showing their main body parts, the cephalon, pereon, and pleon. Pleotelson and two pairs of uoropods **(u)**. **A**, displayed distinct locomotor and fixation pods, comprising seven preopods, and five pleopods together with an abdominal plate featuring oostigite sacs containing freshly hatched baby manca **(m)**. **B**, illustrated large compound eyes anteriorly at the cephalon, the seven pereon segments, and the five pleon ones, along with light brown and dark chromatophores all over the body. *Scale bar* = 3mm.

2.2. Isopod infestation prevalence

The prevalence of the isopods was linked to several parameters, included, sites, seasons, water quality, fish species, and the fishermen activities in the lake. Concerning investigated sites, the eastern region exhibited a higher significant ($P< 0.05$) prevalence rate of isopod infestation and showed a prevalence mean of 26.18±14.71% (mean ± SE), followed by the middle at 22.43±13.23%, and then the west at 15.77±9.36%, which had the lowest one (Table 2 & Fig. 6). On the other hand, both summer and autumn seasons exhibited relatively high prevalence means of 29.95±16.58 and 29.32±15.59%, respectively, then spring season recorded 15.76±8.98%, while winter season was relatively the lowest and experienced 9.59±4.86% (Table 2). The surveillance results showed that a total of 831 *Tilapia* spp., 259 *S. aegyptiaca*, and 436 *Atherina* spp. got isopod infestation with various stages of the cymothoid parasite, with corresponding prevalence rate of 43.46, 12.59, and 5.27% and prevalence means of 38.75±4.25, 16.00±1.78, and 5.62±0.99%, respectively (Table 3). However, the prevalence rate of gill pouch infestation was pronounced in *Tilapia* spp. and low in *S. aegyptiaca*, representing 77.26 and 8.88%, and their prevalence means were 76.05±3.84 and 9.48±1.90% (Table 4), while no gill pouch infection was recorded in *Atherina* spp. Additionally, the double-gill pouch infestation was observed exclusively in *Tilapia* spp., with a branchial infestation intensity index ranging from 1.2 to 2.0 compared with 1.0 in *S. aegyptiaca* (Table 4). Generated and analyzed data, including the caught fish species (infested and not infested) and the FSCs during the thirty fishing expeditions carried out, confirmed significant variation in relationships among the three caught fish species and the FSCs. Ultimately, the results indicated a substantial relationship between FSCs and *Tilapia* spp., and very weak correlation with both *S. aegyptiaca*, and *Atherina* spp. experiencing R-values at 0.76, 0.04, and 0.14, respectively (Fig. 7).

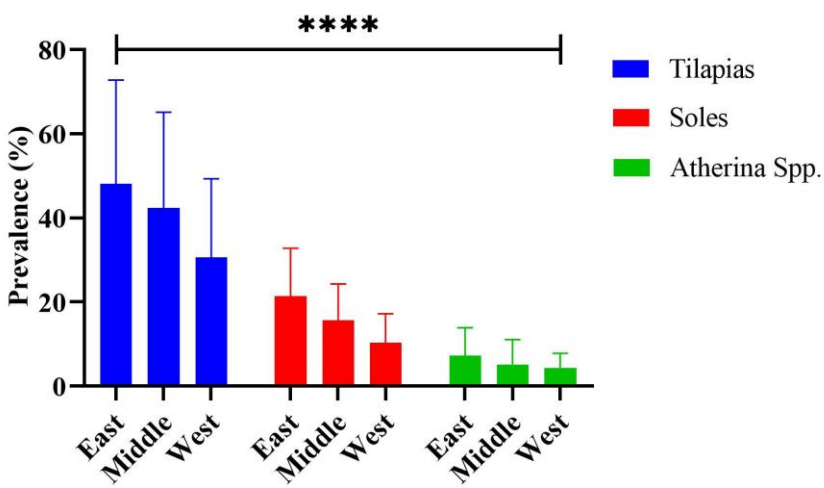

Fig. 6. The prevalence of isopod infestation among the three infested fish species in accordance with the three investigated regions of Lake Qarun. Notice the high significance (P<0.05) differences among infested *Tilapia* spp., *S. aegyptiaca* and *Atherina* sp.

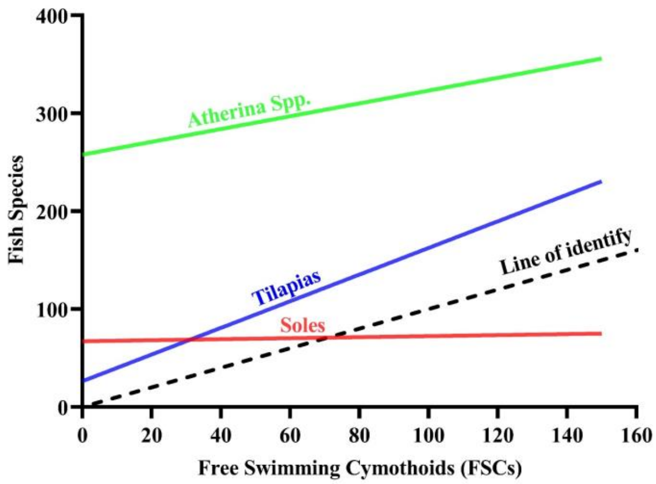

Fig. 7. Linear regression analysis followed by Pearson's correlation test showed variable relationships among the three caught fish species and the FSCs. Notice the strong relationship between FSCs and *Tilapia* spp., a weak relationship with *S. aegyptiaca*, and *Atherina* spp.

2.3. The clinical signs

Concerning the gill lesions, infested *Tilapia* spp. revealed considerably varied lesions. Specifically, fish with opercular infestation showed incompletely closed opercula, and the cymothoidal stages appeared to fix their ventral surfaces (cephalones) at the internal junction between the two gill pouches in a diagonal direction (Fig. 8B, C). Not only did the cymothiodal infections occur in one gill pouch, but both might have been impacted. Twisted opercula were seen in the infected *Tilapia* spp. in the directions that did not correspond to their original orientation (Fig. 9D). Infested *S. aegyptiaca*, as a benthic organism with diminutive opercula openings, existed with the parasite attacked primarily the dorsal gill pouch (Fig. 9B). No ventral gill pouch infestation was observed. Gill lesions were not observed in infested *Atherina* spp. The gills of infested fish showed paleness, corrosion, degrees of parasitic bites, sloughed gill filaments, and a few actual loss of the gill tissues. The severity of gill lesions was depended on the size of the parasite, the duration of the infestation, and the size of the infested fish. The skin lesions included scale loss, hemorrhages, and occasionally the parasite fingerprint (bite scars) upon exiting the skin site were observed in infested *Tilapia* spp. or *S. aegyptiaca* (Fig. (9A, C). Different clinical patterns were displayed by infested *Atherina* spp., primarily linked to varying degrees of skin inflammation, primarily excessive mucus secretion, and dispersed hemorrhages as reactions to the parasitic bites. This image was clear because the parasite could be half as big as the fish (Fig. 9E).

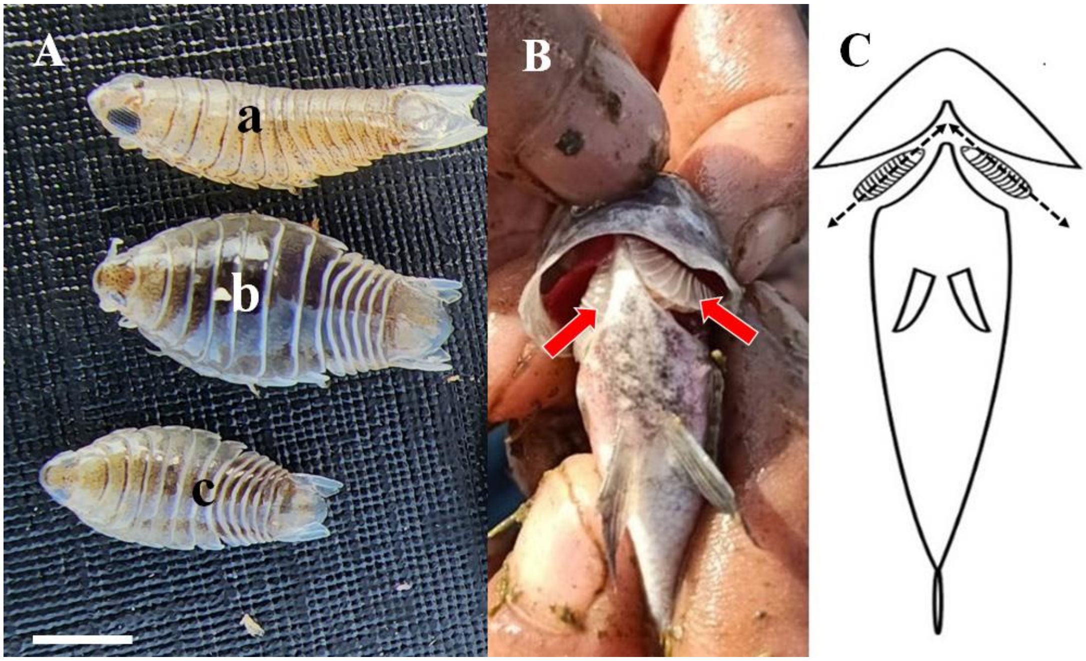

Fig. 8. (A), illustration of different cymothoidal stages of *L. redmani*: **a**, free swimming stage, **b**, adult gravid stage, and **c**, adult stage. *Scale bar* = 3 mm. **(B)**, showed a double-gill infestation of tilapia with *L. redmani* isopoda **(***Red arrows)*. **(C)**, a diagram illustrated the longitudinal positional orientation of *L. redmani* inside the gill pouch of the infested fish

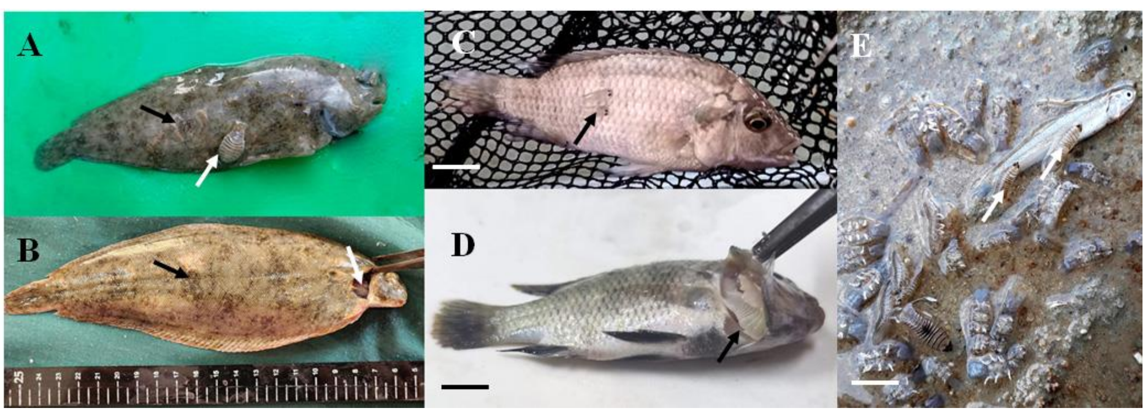

Fig. 9. Different clinical signs associated with isopod infestation caused by different cymothoidal stages of *L. redmani* infestation were displayed in diseased fish species caught during the ten-season surveillance study. A, *S. aegyptiaca* attacked by *L. redmani* sub-adult cymothoid **(***white arrow***)** together with a bite finger print of the skin surface **(***black arrow***)**. B, *S. aegyptiaca* infested with *L. redmani* displayed an old skin scar **(**black arrow**)** and an attacked dorsal gill pouch **(***white arrow***)**. C, Tilapia skin infestation displaying two distinct *L. redmani* free-swimming cymothoidal stages **(***arrows***)**. *Scale bar* = 2 cm. D, Tilapia displayed an infection of a single gill pouch containing *L. redmani* individual **(***arrow***)**. *Scale bar* = 2 cm. E, skin infestation of *Atherina* spp. with *L. redmani* in two different cymothoidal stages **(***white arrows***)**. Scale bar = 1 cm

3. Clarifying cymothoid attacking strategy

The observation results obtained from the conducted experiment elucidated clarity for the FSC attacking behavior strategy, particularly for "playing opossum" (Fig. 10). In this attacking technique, the FSC appeared to lie in calm at the bottom of the aquarium on their dorsal surface, subjecting their pereopods upward with complete cessation of movement. Once the fish came to the near distance in a trial to eat it, the FSC, in a quick jump, climbed upward to fix itself onto the ventral aspect of the lower jaw and/or the pectoral region. Then, in a slowly creeping movement, the FSC moved toward the fish's gill pouch as its main target. On the other hand, the "klinokinesis" attacking behavior by the FSC occurred as rapidly as could be photographed. In this attacking technique, the FSC was speedily swum in irregular, random directions around the fish. Once a chance was available, the FSC attacked the fish body surface.

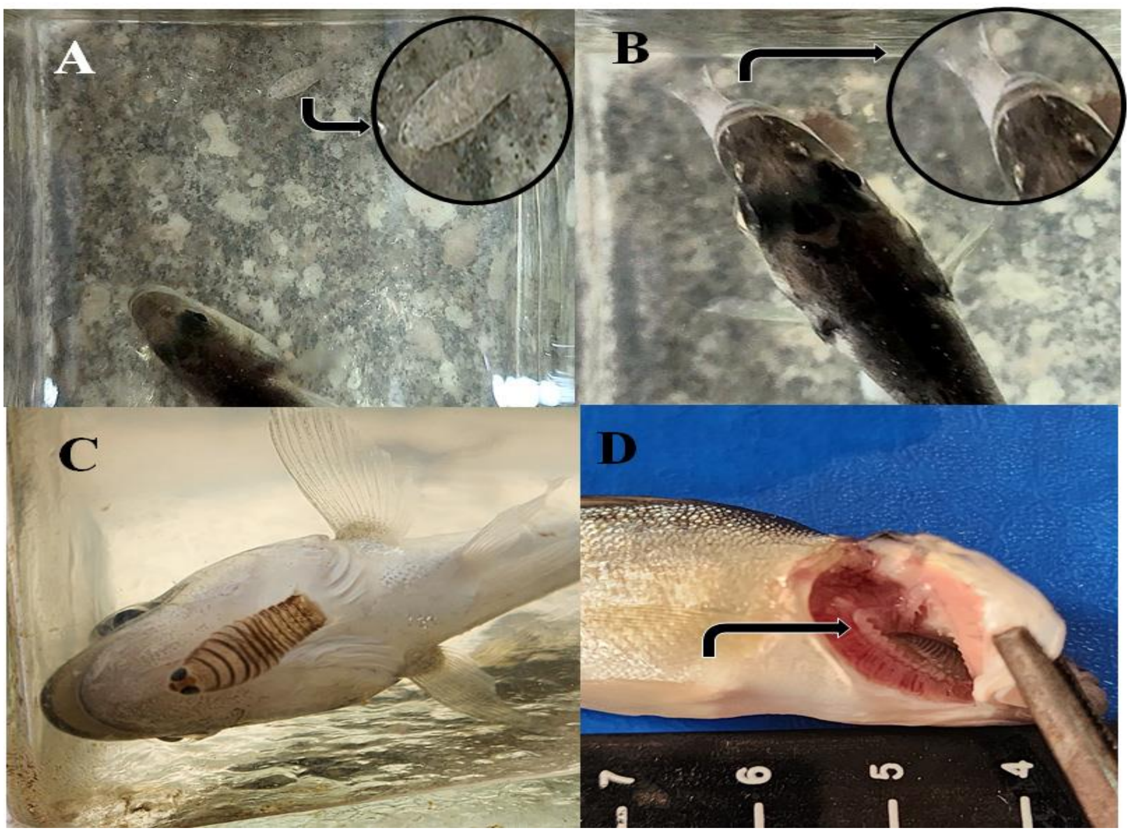

Fig. 10. Clarification of the *playing-opossum* assaulting tactic involving *L. redmani* free swimming cymothoids. **A**, the FSC appeared to lie in calm at the bottom of the aquarium on its dorsal surface. **B**, the FSC, climbed upward to fix itself onto the ventral aspect of the lower jaw and/or the pectoral region. **C**, the FSC shifted into position and advanced towards its primary objective, the fish's gill pouch. **D**, the cymothoidal isopoda fixes itself in the right diagonal orientation inside the gill pouch

DISCUSSION

Despite the fact that aquaculture contributes more than 70% of the total production of marine fish in Egypt, fisheries remain a significant contributor to fish supply in Egypt **(GAFRD, 2021)**. Egyptian natural lakes are considered a glorious source for fishing activities for various fish and crustacean species. Such activities are considered lifesaving to cope with population poverty. Recently, unknown cymothoid parasites appeared in Lake Qaroun, and enormous fish species started to disappear from almost all of the lake while fishing was performed **(Afifi, 2015)**. At this time, restocking the lake with juvenile fish obtained from the Mediterranean Sea were blamed for the transmission of the furious crustacean parasite in extremely high numbers **(Mohammed-Geba *et al*., 2019)**. Consequentially, several investigations were carried out to address the issue. Finally, the etiological parasite was morphologically and molecularly identified as *L. redmanii* **(Mohammed-Geba *et al*., 2019; Mahmoud *et al*., 2023)**.

Generally, the generated data from the presented surveillance study outlined the situation of isopod infestation and its role on cohabitated fish species of the lake. In the presented surveillance study, almost all of the fishing trips conducted using different catching fish tools could only collect three fish species, namely *Tilapia* spp., *S. aegyptiaca*, and *Atherina* spp. (Fig. 2). The primary cause of the absence of other exotic fish species (mullet, seabass, etc.) in the total fish catch is most likely isopod infestation, followed by illegal overfishing **(Mehanna, 2020)**, the incapacity to withstand the conditions of the lake, and the preventive decisions made by the component authority regarding the restocking of the lake with these species. These findings are consistent with those published by **Mehanna (2020)**, the author stated that the isopod epidemic in subsequent years was timed to correspond with the lake's catastrophic fish reduction that began in 2014. The obtained results revealed the persistence of isopod infestation in *Tilapia* spp., *S. aegyptiaca*, and *Atherina* spp. with total prevalence rates of 43.46, 12.59, and 5.27%, respectively (Table 3). Although these results are in accordance with those reported by **Mahmoud *et al*. (2017)**, the authors included four fish species, namely *Tilapia zilli, Mugil capito, Dicentrarchus labrax*, and *Solea vulgaris*. However, the same authors reported that the prevalence of isopod infestation in *S. aegyptiaca* decreased to only 8% in 2021 **(Mahmoud *et al*., 2023)**. Despite the fact that both studies didn’t include *Atherina* spp. in their reports, the species exists in the lake Qaroun since it was accidentally introduced when restocking the lake with different fish seeds and is now considered one of the lake inhabitants **(El-Serafy *et al*., 2014; Shalloof, 2020)**. Additionally, it seemed that such fish (*Atherina* spp.) may offer the micropredation transitory host for the FSCs until they find their definitive host **(Fogelman & Grutter, 2008)**. From a seasonal prevalence perspective, the results showed that the prevalence was nearly similar in both the summer and autumn seasons, followed by spring and winter (Table 2). The possible explanation for the high prevalence in autumn might be related to the persistence of high temperatures during the season as a result of lake topographical features and/or global warming climatic changes **(Mehrim and Refaey, 2023)**. Additionally, it was noted that female ovigourous *L. redmani* parasites from lake Qarun were observed twice a year in February and August **(Helal & Yousef, 2018)**. This might clarify the influences of topographical and/or climatic effects on the isopod infestation prevalence, which are evident when the obtained results are compared with those of **Abdalla and Hamouda (2022)**, who declared that isopod infestation prevalence in Mediterranean European seabass. *D. labrax* was high in the summer, followed by the spring, autumn, and winter.

The surveillance results showed that the eastern region of the lake was relatively deprived, to a little extent, of inhabitant fish individuals (the three caught species) and represented the lowest collected fish (caught); however, it was the highest in the isopod infestation prevalence as well as in the abundance of FSCs (Tables 2, 3). The prevalence percentages were 55.12, 16.25, and 7.17% for *Tilapia* spp., *S. aegyptiaca*, and *Atherina* spp., respectively. This region constitutes the main gate for the lake freshwater feeding source, originating from the "El-Bats" drainage effluent (Fig. 1), where the nearby rural, agricultural, aquaculture, and industrial drainage effluents are shared. As a result of surveillance, the feeding effluents are insufficiently treated, predisposing to alluviation with a huge amount of pollutants **(Abdelmageed *et al*., 2022)**. Such circumstances directly altered the fish composition in the region and facilitated the propagation of the isopods to produce more FSCs. Eventually, many research reports indicated the impact of pollution and water deterioration on fish immunity **(Uribe *et al*., 2011; Fahmy *et al*., 2022; Mokhtar *et al*., 2023)** and their relationships with disease incidence, particularly isopod infestation. Similarly, the pattern did not look different in the middle region and experienced prevalence rates of 48.43, 14.00, and 4.85% for *Tilapia* spp., *S. aegyptiaca*, and *Atherina* spp., respectively. On the other side, the collected (caught) *Atherina* spp. from the middle region was the highest among collected fish species (Fig. 2). From the perspective of surveillance observations, two noteworthy findings could account for the quantity of *Atherina* spp. that are caught. Firstly, the middle region's water column is the deepest in the lake, ranging from 6.5 to 8 meters, making it appropriate for the fish's scholarly swimming behavior **(Fouda & Fishar, 2012)**. The second finding was the mutualistic coexistence of aquatic birds (local residents and/or migratory), and fish in the area, especially at the golden-Elqarn island (Fig. 1). In the same manner, the feeding freshwater source represented in the "Al-Wadi" drain might play the same role in isopod infestation as that of the "El-Bats" drain. In contrast, the west region had the highest total amount of *Tilapia* spp., and *S. aegyptiaca* that were collected (caught) out off the three regions, but it also had the lowest isopod infestation prevalence rates for *Tilapia* spp., *S. aegyptiaca*, and *Atherina* sp., at 34.26, 9.04, and 4.02%, respectively.

The identification of cymothoid species is primarily based on the morphological features of the adult females **(van der Wal & Haug, 2020; Fujita & Okumura 2025)**, the adult ovigerous female cymothoid were confirmed as *L. redmanii* Leach. These results align with those previously reported by **Mohammed-Geba *et al*., (2019)**, **Mahmoud *et al*. (2023)** and **Zayed *et al*. (2024)**. In parallel with the surveillance findings, the authors also confirmatively identified the parasites as strictly gill parasites and clearly described them as protandrous hermaphrodites that exhibited five different stages during their life cycle. These protandorous life stages (manca, juvenile, male, transitional, and mature female) mainly exhibited biphasic phases including, free swimming and permanent ones **(Jones *et al*., 2008)**. Moreover, the existence of parasites linked to the branchial cavities of infested fish in characteristic positional orientation (Fig. 8B, C) acts as an important tool for confirmative identification of the cymothoid species **(Brusca, 1981)**. Furthermore, it was molecularly declared that the cymothoids of Qarun Lake is *L. redmani* and it was originated from Mediterranean Sea throughout restocking process either by infested fish and/or contaminated water with FSCs **(Mohammed-Geba, 2019)**.

According to the worthwhile surveillance observations, almost all cymothoidal stages that parasitized the gills of *Tilapia* spp. as permanent hosts experienced a total prevalence rate and intensity indices of 77.8% and 1.40, respectively (Table 5). Additionally, these results were statistically significant ($P < 0.05$) and confirmed the strong relationship (R-value 0.76) between the parasite (FSCs) and *Tilapia* spp. On the other hand, *S. aegyptiaca* and *Atherina* spp. seemed to act as transitory micro-predatory hosts for the FSCs rather than permanent hosts. These findings were evident as the former fish experienced gill infestation prevalence and intensity indices of 8.88 and 1.00%, respectively; however, the latter two fish experienced gill infestation prevalence and intensity indices of 0.0% and 0.0, respectively (Table 5). Moreover, the relationship between *S. aegyptiaca* and FSCs was very weak (R-value, 0.04) and that was the same situation for *Atherina* spp. and FSCs (R-value, 0.014) (Fig. 7). It's interesting to note that these findings showed that *Atherina* spp. were firmly acting as transitory hosts, and *S. aegyptiaca* typically behaves in a similar manner; however, *S. aegyptiaca* might act as a permanent host in the absence of *Tilapia* spp. It was documented that the majority of cymothoids are very site- and host-specific; nevertheless, a fascinating feature of cymothoids' interactions with their hosts is also their particular place of attachment, which appears to be both species- and occasionally genus-specific **(Smit *et al*., 2014)**. Besides, the parasite's requirements and the host's morphology and behaviors place restrictions on the site specificity **(Smit *et al*., 2014)**. Additionally, the data demonstrated that the parasite was primarily present in *Tilapia* spp. with both gill chamber infestations, whereas *S. aegyptiaca* had a uni-gill chamber infestation. *Athernia* spp. also solely showed skin infections (Fig. 9). These results may help to explain how fish with small gill pouches, such *S. aegyptiaca* and *Atherina* spp., are able to withstand attacks by *L. redmanii* and go on to survive and propagate. That was evident when the three species total catch were compared (Figs. 3, 4). Moreover, isopods feed and grow during each stage of their biphasic life, both as free-swimming (FSCs) and adult forms. There is an ontogenetic shift in size influenced by feeding strategy and enrichment levels, which vary across parasite stages and hosts. Each developmental stage may prefer different fish hosts **(Demopoulos & Sikkel, 2015)**. Supportively, it has been reported that *L. redmanii* can infest the Atlantic bumper (*Chloroscombrus chrysurus*) as a transitory host, while it permanently parasitizes the Spanish mackerel (*Scomberomorus regalis*). This host-switching behavior has been attributed to differences in host size **(Costa & Chellappa, 2010)**.

The ability of cymothoids to select permanent hosts appears to be influenced by three main variables. First is the availability of protection, which is essential throughout their lifecycle **(Sahadevan *et al.*, 2020)**. Second is the availability of appropriate nutritional resources, as the parasite is a high-potential intermittent feeder, consuming blood, mucus, and fine tissues **(Östlund-Nilsson *et al.*, 2005; Fogelman & Grutter, 2008)**. The third variable is fish size, particularly the dimensions of their opercular pouches. This may explain why *Atherina* spp. never act as permanent hosts (Fig. 9E).
These factors likely drive the biphasic molting process, in which the parasite sheds its exoskeleton in two stages: first, the posterior half (pleon and posterior pereonites), followed by the anterior half (cephalon and anterior pereonites). This molting sequence facilitates establishment of infestation **(Panakkool-Thamban & Kappalli, 2020; Sahadevan *et al.*, 2020)**. Thus, the findings suggest that *L. redmanii* exhibits host-size specificity depending on its developmental stage. This specificity ensures adequate space for growth while reducing predation risk, as observed in *Tilapia* spp., which possess wider gill cavities than *S. aegyptiaca*, while such cavities are absent in *Atherina* spp. Therefore, fish species with sufficiently large gill cavities can act as permanent hosts for *L. redmanii*, while others cannot.

Several studies on isopod infestation outbreaks in Lake Qarun reported infections in various fish species including *mullet* spp., *seabass*, *T. zillii*, *S. vulgaris*, and *S. aegyptiaca* **(Mahmoud *et al.*, 2017; Mehanna, 2020; GAFRD, 2021; Abdallah & Hamouda, 2022)**. However, *Atherina* spp. were not included.

Despite ecological stress, Lake Qarun supports rich benthic and planktonic food webs **(Napiórkowska-Krzebietke *et al.*, 2016; Hussian *et al.*, 2019)**, providing consistent nutritional resources for both *Solea aegyptiaca* and *Atherina* spp., even under parasitic pressure. Infested fish tend to eat more than uninfested individuals to meet increased metabolic demands from parasitic infection. This compensatory feeding aligns with host-parasite energy dynamics **(Barber *et al.*, 2000; Östlund-Nilsson *et al.*, 2005; Chodkowski & Bernot, 2017)**. The lake’s diverse prey—benthic invertebrates for *S. aegyptiaca* and plankton for *Atherina* spp.—supports their energy needs, aiding in their survival despite infestation.

Clinically, the signs of isopod infestation observed in the three caught fish species (illustrated in Fig. (9)) match previous reports **(Eissa *et al.*, 2012; Helal & Yousef, 2018; Abdallah & Hamouda, 2022; Mahmoud *et al.*, 2023)**.

This study also included a preliminary experiment using juvenile seabass to mimic isopod attack behavior. Observations confirmed the "Opossum behavior" of FSCs, whereas "Klinokinesis behavior" was not detected. A plausible explanation is that the seabass served as a potential permanent host, attracting the parasite through visual and chemical cues from the host's shadow and excretions **(Cook & Munguia, 2013)**. Thus, "Opossum behavior" may be the most efficient means for infestation within gill cavities (Fig. 10).

In contrast, the aggressive swimming behavior of FSCs associated with "Klinokinesis" enables them to briefly attach to a transitory host to feed and detach afterward (Fig. 9A, C, E). This short-term micro-predation prolongs FSC survival until a permanent host is found **(Fogelman & Grutter, 2008)**. This may be the only viable method to parasitize fish with small gill pouches, as seen in *Atherina* spp. and small *S. aegyptiaca*. These behaviors suggest that FSCs accurately select their hosts based on physical compatibility—either transitory or permanent. Uniquely, while "Opossum behavior" has been described in freshwater isopods **(Thatcher, 2000)**, this study reports it in *L. redmanii* within marine waters.

Immediate and comprehensive control strategies are necessary to prevent the collapse of Lake Qarun's fish populations due to isopod infestation. Notably, *S. aegyptiaca* has demonstrated resilience due to its economic value, environmental tolerance, and capacity to withstand infestation. The Egyptian government has supported this species through restocking efforts **(LFRBDA, 2022)**. However, to sustain its population, a stock improvement program is recommended based on the current findings.
Local environmental factors—such as surrounding terrain, microclimate, and especially freshwater input—will significantly affect lake salinity. Sites with lower salinity levels tend to show reduced isopod infestation. Effective mitigation requires understanding parasite threats, potential routes of entry (particularly via infested restocking fish), and the isopod life cycle.

Additionally, dredging the lake bed and suspending restocking efforts have been cited as effective in improving water quality and disrupting the isopod life cycle **(Abdelkhalek *et al.*, 2017)**. Increasing freshwater inflow may also help reduce salinity to levels conducive to both parasite control and fish health **(Abdelmageed *et al.*, 2022)**.

CONCLUSION

The study data output clearly articulates three main conclusions. The first conclusion is that, by employing an appropriate attack strategy, *L. redmani* infestation stages are capable of selecting their host fish, either permanently or temporarily. The second conclusion indicated that *S. aegypti*aca and *Atherina* spp. can survive and propagate while being attacked by *L. redmani*. Lastly, given that *S. aegyptiaca* is relatively resistant to *L. redmani* attacks, a comprehensive stock improvement effort for this species may be able to reverse the decline in Lake Qarun fish populations from an economic value perspective.

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