



Further studies on Cymothoid isopods of some fish species from lake Qarun, Egypt

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Abstract:

Cymothoids are obligate parasites of marine, brackish and freshwater fishes. They were considered to cause significant economic losses to fisheries through mortalities, stunting growth or damaging tissues of the infested fish. In the last 4 years, lake Qarun suffered from isopod infestation causing fish loss and marketing problems. In the present study out of 375 fish samples belonged to *Mugil capito* (125), *Liza carinata* (125) and *Tilapia zilli* (125) were collected from lake Qarun , El Fayoum governorate and seasonally examined for isopod infestation from the beginning of November 2015 till the end of October 2016. The study revealed the isolation of two species of isopods ; *Nerocila orbignyi* and *Levonica redmani* with total infestation rate of 32.53% (21.07% and 11.47% for *Nerocila orbignyi* and *Levonica redmani* respectively) with the highest rate recorded during summer. Histopathological study was conducted for estimating the impacts of *L. redmani* infestation on the affected tissues of *M. capito* and revealed complete sloughing of the gill lamellae with eosinophilic granular cells infiltration. The study recommended the need to develop a scientific strategy to control the parasitic isopods and prevent its spread among Egyptian water bodies.

Key words: Cymothoidae, Isopods, Lake Qarun, and Pathology.

INTRODUCTION

Parasitic infestation especially with crustacean species constitutes a major problem for production, development and sustainability of fish industry. Such infestation, act as a stress factor resulting in reduction of body weight, lowering of fish resistance and rendering fish to be susceptible to other etiological affections associated with sever impacts on the infested fish (Noga, 2010 and Eissa *et al.*, 2012).

In addition,severe pathological damage of fish skin and gills were recorded (Timi & Lanfranchi, 2006 and Osman *et al.*, 2014).

Cymothoid isopods are obligatory ectoparasite of marine, brackish and freshwater fishes (Trills, 1994, Heckmann, 2003 and Raja *et al.*, 2014).. Several species settle in the buccal cavity of fish, others live in the chamber or on the body surface including the fins (Lester and Hayward, 2006; Alas *et al.*, 2008 and

Samm et al., 2014). Moreover, they are considered to cause significant economic losses to fisheries, not only through mortalities, stunting growth or damaging tissues of the fish (**Bunkley-Williams et al., 2006 and Toksen, 2007**). They feed on blood and macerated tissues (**Woo, 2006**), causing anemia and reduction of fish quality. (**Bunkley-Williams et al., 2006 and Raja et al., 2014**).

Lake Qarun is one of the largest natural lakes in Egypt and it is located north of the city of Fayoum, about 27 km away. It is one of the deepest lakes, with places of depths of up to 14 meters. The area of the lake is currently 330 km², 40 km long and 7 km wide. Its area has been reduced by drought over the centuries **Gupta and Abd El- Hamid (2003)**. Nowadays, Lake Qarun considers from the heavily affected water bodies in Egypt with exposure to ecological variations where problem of isopod infestation among fishes is noticed causing fish loss **Mahmoud et al. (2016)**.

Therefore, the objective of the present study is recording the isopod infestation state of lake Qarun fishes referring to the total and seasonal incidence, location on the host and estimating the impacts of *L. redmani* infestation on the affected tissues of *M. capito* through histopathological investigation.

MATERIAL AND METHODS

1- Fish sampling:

Total of 375 Samples of *Mugil capito*, *Liza carinata* and *Tilapia zilli* were collected from Lake Qarun during the period from November 2015 to October 2016. Samples were brought alive in an ice box with their

original water to the laboratory of parasitology department, Faculty of Veterinary Medicine, Cairo University.

2- Parasitological examination

Fish samples were macroscopically examined for any gross lesions and for isopod infestation body surface including fins, branchial cavity and buccal cavity according to fish and isopod species. The detected isopods were collected and transferred into test tubes to be washed and cleaned by PBS. Isopods were fixed in 3% formalin and preserved in equal amount of 70% alcohol with 5% glycerin. The specimens were mounted in glycerin then mounted in glycerin- gelatin according to standard method mentioned by **Mahmoud et al. (2016)**.

3-Histopathological examination:

Specimens for histopathological techniques were freshly taken from infested organs and tissues of the infested *Mugil capito* with *Levonica redmani*. Samples were trimmed and fixed in 10 % phosphate buffered formalin. Then washed in running tap water for 24 hours then dehydrated in different concentration gradients of alcohol and cleared in xylol. Samples embedded in paraffin wax and sectioned into thin sections of 5 microns thickness. Sections were stained with Hematoxylin and Eosin (H&E) stain and examined microscopically according to (**Roberts, 2001**).

RESULTS

1- Macroscopical examination:

In cases of gill infestation , protrusion of the opercular cover of the affected fish(mostly bilateral) was noticed . isopods also

found attached to the skin in different localities .

2- Incidence of isopods infestation:

The total number of examined fish specimens were 375 fishes of *Mugil capito*, *Liza carinata* and *Tilapia zilli* (125 from each fish species). The total number of infested fish with cymothoid isopod species was 122 fishes with an infestation rate of 32.53%. Two species of cymothoids (Crustacea,:Cymothoidae) were detected which were *Nerocila orbignyi* and *Levonica*

redmani with an infestation rate of 21.07% and 11.47% respectively (Table1 and fig. 1). The current study revealed that the highest rate of infestation with isopods was recorded during summer (33.33%) while the lowest infestation rate was during autumn (25%) (Table 2 and fig.2). Dealing with the isopod infestation rate among different fish species, *Mugil capito* showed the highest rate of infestation (59.2%) while *Tilapia zilli* recorded the lowest rate (8%) (Table 3 and fig3)

Table1. The infestation rate of the detected isopod species among fish species.

Isopod species	<i>Mugil cap</i> No. Infested (%)	<i>Liza carinata</i> No. Infested (%)	<i>T.zilli</i> No. Infested (%)	Total No. of infested fish	% of infestation acc. to total No. of examined fish spp.
<i>Nerocila orbignyi</i>	44 (36.07%)	25 (20.49%)	10 (8.2%)	79	21.07%
<i>Levonica redmani</i>	30 (24.59%)	13 (10.66%)	0 (0%)	43	11.47%
Total No. of inf. fish(%)	74 (60.66)	38 (31.15%)	10 (8.2%)	122	32.53%

No. of inf. fish = Number of infested fish.,

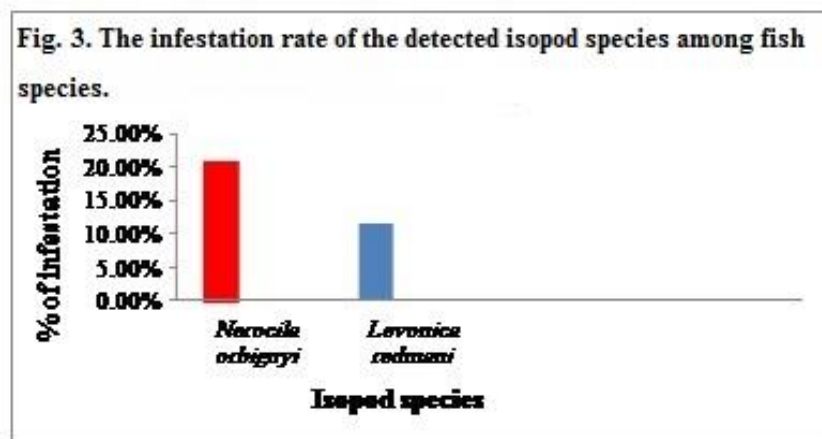
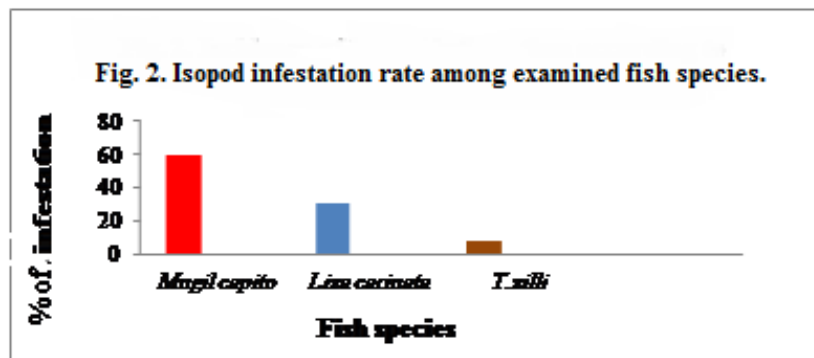
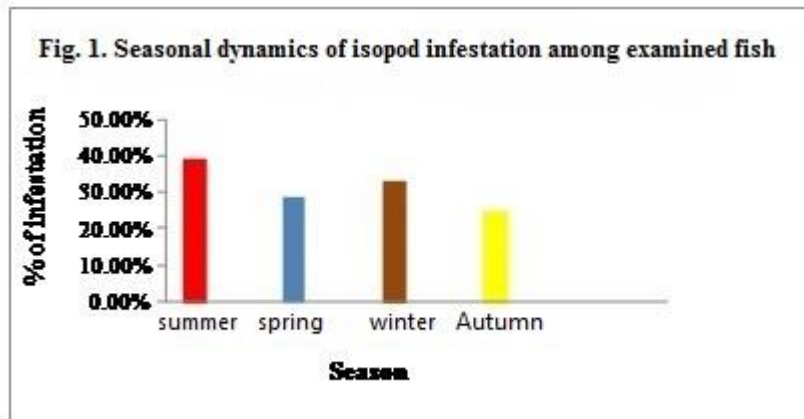
Table 2. Seasonal dynamics of isopod infestation among examined fish:

Season	No. of examined fish	No. of infested fish with Isopod	% of infestation
Winter	75	25	33.33
Spring	110	33	28.7
Summer	125	49	39.22
Autumn	60	15	25
Total	370	122	32.53

Table3. Isopod infestation rate among examined fish species.

Fish species	No. of infested fish	% of infested fish
<i>Mugil capito</i>	74	59.2
<i>Liza carinata</i>	38	30.4
<i>T.zilli</i>	10	8
Total	122	32.53

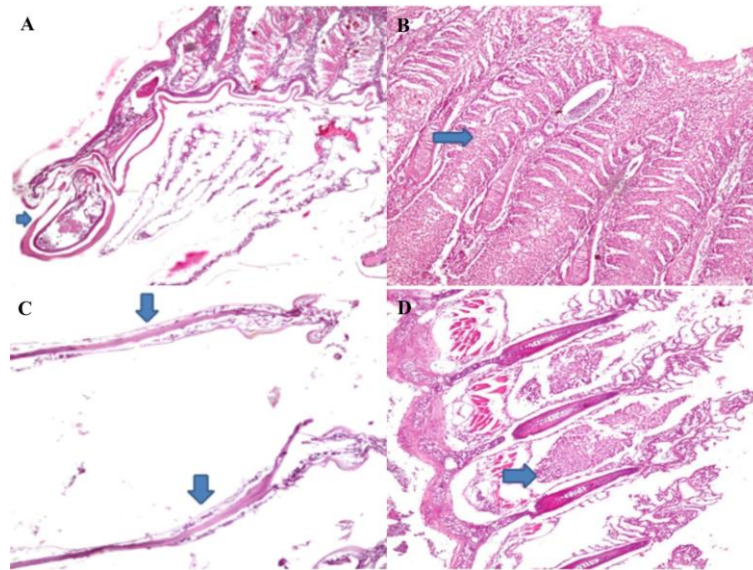
Total Number of examined fishes=375 (125 from each species),Total Number of infested fishes=122 fish, No. inf. = Number infested.,



4- Histopathological findings:

The infested gills of *Mugil capito* with *Levonica redmani* showed different parts of the parasites (Fig. A) and exhibited severe histopathological alterations. The gill lesions included severe hyperplasia of the epithelium of the secondary gill lamellae that lead to its fusion and even fusion of primary gill lamellae which in some parts was associated with leukocytic cells infiltration (Fig. B). Complete sloughing of

the secondary gill lamellae was also demonstrated (Fig. C). Sections of the parasite were seen between the primary gill lamellae compressing the adjacent secondary gill lamellae (Fig. D). There were also parts of gills that demonstrated congested blood vessels in the primary gill lamellae and eosinophilic granular cells infiltration of the secondary gill lamellae (Fig. D).



A: Mouth parts of the *Levonica redmani* (arrow). H & E stain X 100.

B: Gills of *Mugil capito* showing severe hyperplasia and fusion of secondary gill lamellae with eosinophilic granular cells infiltration (arrow). H & E stain X 100.

C: Gills of *Mugil capito* showing complete necrosis and sloughing of secondary gill lamellae (arrow) with few inflammatory cells infiltration in the primary gill lamellae. H & E stain X 200.

D: Gills of *Mugil capito* showing parts of the parasite (arrow) between the primary gill lamellae with necrosis and sloughing of secondary gill lamellae at the base of the gills. H & E stain X 10.

DISCUSSION

In the present study, two cymothoid isopods were detected; *Nerocila orbignyi* (identified according to **Al- Zubaidy and Mhaisen (2013)** who reported this species as the first record in Yemen from two Red Sea mugilid species) and *Levonica redmani* (identified according to **Mark et al. (1996)** and **Brusca (1981)**). In this work *Nerocila*

orbignyi was isolated from the skin of *Liza carinata*, *Mugil capito* and *Tilapia zilli*, this came in agreement with **Merella and Garippa (2001)**, **Marques et al. (2005)**, **Ramdan et al. (2007)**, **Kayis and Ceylan (2011)** and **Samm et al. (2014)** who isolated the same genus from caudal fin and body surface of another marine fish species including *Mugil cephalus*, *Crenilabrus*

pavo and *Solea solea* . This was also agreed with that reported by **Mahmoud et al. (2016)** who isolated the same species from the skin of *Solea vulgaris* of lake Qarun . On the other hand, *Nerocila orbignyi* was isolated from the gill chambers of *Tilapia zilli* collected from lake Qarun by **Younes et al. (2016)** and **Abdel-Latif (2016)** and **Shaheen et al. (2017)**. *Levonica redmani* isolated in the present work from gills and branchial cavity of *Liza carinata*, and *Mugil capito*, the same site of infestation tha reported by **Mahmoud et al. (2017)** and **Shaheen et al. (2017)**.

The recorded rate of isopod infestation in the present investigation was 32.53% which is being higher than that reported by **Youssef et al. (2014)(4%)** and **Abd El Aal and El Ashram(2011)(9%)**.on the other side, it was lower than that previously reported by **Mahmoud et al. (2016)** (32.66 %) , **Mahmoud et al. , (2017)** (46.5%) and **Shaheen et al. (2017)** (50.7%) through their investigation on lake Qarun fishes. These variations might be attributed to the difference of the examined fish species as well as to the continues changes of the environmental factors **Matthiessen et al. (1993)**.

Seasonally, the highest infestation rate with isopod in this investigation was noticed in summer (39.2%) while the lowest rate was recorded in autumn (25%). These results agreed with that obtained by **El-Lamie and Abdel-Mawla (2015)** **Shaheen et al. (2017)** and **Mahmoud et al. (2017)** .

Histopathological study was conducted for estimating the impacts of *L. redmani* infestation on the affected tissues of *M. capito* and revealed complete sloughing of the gill lamellae with eosinophilic granular

cells infiltration. There were also parts of gills that demonstrated congested blood vessels in the primary gill lamellae. This was inagreement with **Ramdane et al. (2007)** who found that cymothoids feed principally on host blood, but they may consume the mucus, epithelium and subcutaneous tissues of their hosts. Also, it agreed with **Lester and Hayward (2006)** and **Noor El-Deen et al. (2012)** who revealed that the infested gills showed congestion, oedema and necrosis. In addition **Eller (1975)**, **Romestand et al. (1977)** and **Ravichandran (2007)** recorded that isopod infestation caused erosion of gill lamellae, damage of gill rakers and pale gills. **Youssef et al. (2014)** said that infestation with Isopod parasites resulted in hyperplastic and hypertrophied reactions Lesions recorded in gills previously were congestion of lamellar vessels, adhesion of most gill filaments and lamellae, oedema and necrosis similar to the findings observed in the present study.

REFERENCES

- Abd El Aal, A. M. I. and El Ashram, A. M. M. (2011):** A morphological study (SEM) on a parasitic marine isopod, *Cymothoa spinipalpa* (Isopoda: Cymothoidae). Egyptian J. Aquac., 1 (1):17-26.
- Abdel-Latif, H.M. (2016):** Cymothoid parasite, *Nerocila orbigni* inflects great losses on *Tilapia zilli* in lake Qarun at Fayoum province. Int. J. of Innovative studies in Aquatic Biology and Fisheries., 2(3): 1-9.
- Alas, A., Öktener, A., Iscimen, A. and Trilles, J. P. (2008):** New host record, *Parablennius sanguinolentus* (Teleostei, Perciformes, Blenniidae) for *Nerocila*

bivittata (Crustacea, Isopoda, Cymothoidae). Parasitol. Res., 102, 645–646.

Al-Zubaidy, A.B. and Mhaisen, F.T. (2013): The first record of three cymothoid isopods from Red Sea fishes, Yemeni coastal waters. Inter. J. Mar. Sci., 3(21): 166-172.

Brusca, R. (1981): Amonograph on the Isopoda ; *Cymothoidae* (Crustacea) of the eastern pacific. Zoological Journal of Linnean Society, 73:117-199.

Bunkley-Williams, L., Williams, E. H. J. and Bashirullah, A. K. M. (2006): Isopods (Isopoda: Aegidae, Cymothoidae, Gnathiidae) associated with Venezuelan marine fishes (*Elasmobranchii, Actinopterygii*). Rev. Biol. Trop., 54: 175-18

Eissa, I. A. M., El-Lamie, M. M. and Zakai, M. (2012) Studies on crustacean diseases of Seabass, *Morone Labrax*, in Suez Canal, Ismailia governorate. Life Science J., 9 (3):512-518.

El-Lamie, M. M. and Abdel-Mawla, H. I. (2015): Isopods infestation in relation to vibriosis of some marine fishes. Egy. J. Aquac., 5 (2): 13-26.

Eller L. L. (1975): Gill lesions in fresh water teleosts. In: Ribelin WE, Migaki G. (eds) The pathology of fishes. The University of Wisconsin Press, Madison, 305–330.

Gupta, G and Abd El- Hamid,Z (2003): Water quality of lake Qarun, Egypt, Int .J. Environ.Stud.(60): 651-657.

Heckmann, R. (2003): Other ectoparasites infesting fish; copepods, *branchiurans*,

isopods, mites and bivalves. Aquaculture Magazine, 63:1-7.

Kayis S. and Ceylan Y. (2011): First Report of *Nerocila orbigny* (Crustacea, Isopoda, Cymothoidae) on *Solea solea* (Teleostei, Soleidae) from Turkish Sea. Turkish Journal of Fisheries and Aquatic Sciences, 11: 169-171.

Lester, R. J. G. and Hayward, C. J. (2006): Phylum Arthropoda, In: Woo, P.T.K. (Ed.), Fish Diseases and Disorders Volume 1: Protozoan and Metazoan Infections, 2nd ed. CAB international, London, pp. 466–565.

Mahmoud, N. E., Fahmy, M. M., Abuowarda, M. M. and Khattab, M. S. (2016): Parasitic cymothoid isopods and their impacts in commercially important fishes from lake Qarun, Egypt. Int. J. Chem. Tech. Research, 9 (12): 221-229.

Mahmoud, N. E., Fahmy, M. M. and Abuowarda, M. M. (2017): An investigation of Cymothoid isopod invasion in lake Qarun fishes with preliminary trial for biological control. Int. J. Chem. Tech. Research, 10 (2): 409-416.

Mark, R.; Juanes, F. and Hare, J. (1996): Occurrence and effect of the parasitic isopods *Leronica ovalis* ,on young of the year bluefish, *Pomatomus sasaltarix*. Canadian journal of Fisheries Aquatic Science.53:2052-2057.

Marques J. F., Santos M. J., Costa J. L., Costa M. J. and Cabral H. N. (2005): Metazoan Parasites as Biological Indicators of Population Structure of *Halobatrachus didactylus* on the Portuguese Coast. Journal of Applied Ichthyology, 21: 220-224.

- Matthiessen P., Thain J. E., Law R. J. and Fileman T. W. (1993):** Mar Pollut Bull; 26: 90-95.
- Merella P. and Garippa G. (2001):** Metazoan Parasites of Grey Mullet (Teleostea: *Mugilidae*) from the Mistras Lagoon (Sardinia, Western Mediterranean). *Scientia Marina*, 65(3): 201-206.
- Noga, E. J. (2010):** Fish disease Diagnosis and Treatment. Mosby-yearbook, Inc. Watsworth Publishing Co., USA. 2nd ed.
- Noor El-Deen, A.E., Abdel Hady, O. K., Shalaby, S. I. and Mona, S. Z. (2012):** Field studies on *Caligus* Disease among cultured *Mugil cephalus* in brackish water fish Life Sci. J., 9(3): 733-737.
- Osman, H. A. M., Hassan, M. A. and El-Refaey, A. M. E. (2014):** Studies on *Sarcotaces* Sp. (Copepoda, *Philichthyidae*) infestation (Black Bag Disease) among some marine fish species of Arabian Gulf, Saudi Arabia. *World Applied Sciences Journal*, 32 (9):1780-1788.
- Raja, K., Vijaya Kumar, V., Karthinkeyan, V., Saravanakumar, A., Sindhuja, K. and Gopalakrishnan, A. (2014):** Occurrence of isopode *Nerocila phaiopleura* infestation on white fin wolf herring (*Chirocentrus nudus*) from Southeast coast of India. *J. Parasitol. Dis.*, 38 (2): 205-207.
- Ramdan, Z., Bensouilah, M. A. and Trilles, J. (2007):** The *Cymothoidae* (Crustacea, Isopoda), parasites on marine fishes, from Algerian fauna. *Belg. J. Zool.*, 137 (1):67-74.
- Ravichandran S. (2007):** Infestation of isopod parasite *Lironeca puhi* in slender needle fish *Strongylura leiura*. *Res. J. Parasitol.*, 2: 87-93.
- Roberts, R. J. (2001):** Fish pathology. 3rd ed, W. B. Saunders, New York.
- Romestand B., Janicot M. and Trilles J. P. (1977):** Modifications tissulaires et reactions de defence chez quelques teleosteens parasites par les cymothoidae (crustaces, isopodes, hemalophages). *Annales de parasitologie Humaine et compare*, 52:171-180.
- Samn, A. A. M., Karima, M. M., Amr, F. Z. and Khalaf Allah, H. M. M. (2014):** First occurrence of *Nerocila bivittata*: parasitic isopods (skin shedders) on *Lithognathus mormyrus* (*Osteichthyes*, Sparidae) from Abu Qir Bay, Alexandria, Egypt. *J. of Amer. Sci.*, 10 (7):171-179.
- Shaheen, A. A., Abd El-Latif, A. M., El-Madawy, R. S. and Noor Eldeen, A. I. (2017):** Isopodiosis in Some fishes from Egyptian Qaroun lake: prevalence, identification, pathology and in vitro trials to get rid of it. *RJPBCS* 8 (1): 1971-1978.
- Timi, J. T. and Lanfranchi, A. L. (2006):** Size relationships between the parasitic copepod, *Lernanthropus cynoscicola*, and its fish host, *Cynoscion guatucupa*. *Parasitol.*, 132:207-213.
- Toksen E. (2007):** *Lernanthropus kroyeri* van Beneden, 1851 (Crustacea: Copepoda) infections of cultured Seabass (*Dicentrarchus labrax* L.). *Bulletin of the European Association of Fish Pathologists*, 27: 49553.
- Trilles, J. P. (1994):** Les Cymothoidae (Crustacea, Isopoda) du monde (Prodrome pour une faune). *Studia Marina*, 21/22 (1-2): 1-288.
- Woo, P. T. K. (2006):** Fish diseases and disorders. Volume 1, protozoan and

metazoan infections 2nd ed. CABI, U U.K.
22 pp.

Younes, A. M., Noor Eldin, A. I. and Abd Ellatif, M. A. (2016): A contribution of crustacean Isopoda, bacterial infections and physicochemical parameters in mass mortalities among fishes in lake Qarun. Research Journal of Pharmaceutical Biological and Chemical Science (RJPBCS) 7 (2): 1906- 1911.

Youssef, E. M., Salam, N. H., Eissa, I. A. M. and Zaki, M. S. (2014): Parasitological studies on the isopoda (*Cymothoidae*) parasites infesting some marine fishes at Suez Canal area at Ismailia Province, Egypt with a key to the *Cymothoid* genera. Life Science Journal, 11 (1):227-231.

الملخص العربي

مزيد من الدراسات حول متماثلات الأرجل من بعض أنواع الأسماك من بحيرة قارون، مصر

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تتطفل متماثلات الأرجل إجباريا على الأسماك البحرية والشروب والمياه العذبة. هي تسبب خسائر إقتصادية كبيرة لمصائد الأسماك، من خلال أحداث الوفيات أو عجز النمو أو تدمير أنسجة للأسماك. في السنوات الأربع الماضية عانت بحيرة قارون من الإصابة بمتماثلات الأرجل بين الأسماك مما تسبب في فقدان الأسماك وحدوث مشكلات تسويقيه. في هذه الدراسة تم تجميع و فحص ٣٧٥ عينة من الأسماك من بحيرة القارون بمحافظة الفيوم من أنواع الطوبار (١٢٥)، السهلية (١٢٥) والبطي الزيلي (١٢٥) للإصابة بمتماثلات الأرجل من بداية نوفمبر ٢٠١٥ حتى نهاية أكتوبر ٢٠١٦. قد كشفت الدراسة عن وجود نوعين من هذه الطفيليات هما نيروسيلأ أوربيغني وليفونيكا ريدماني وقد بلغ معدل الإصابة الكلي ٣٢.٥٣% (٢١.٠٧% و ١١.٤٧% ل نيروسيلأ أوربيغني و ليفونيكا ريدماني على التوالي) مع تسجيل أعلى معدل إصابة خلال فصل الصيف. كما أجريت دراسة نسيجية لتقدير آثار الإصابة بطفيل الليفونيكا ريدماني على أنسجة أسماك الطوبار المصاب وقد كشفت النتائج عن وجود تدمير كامل للصفائح الخيشومية مع وجود خلايا الإيزينوفيل الحبيبية. أوصت الدراسة بضرورة العمل على وضع استراتيجية علمية للسيطرة على الطفيل ومنع إنتشاره بالمسطحات المائية المصرية.