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An Investigation of Cymothoid Isopod Invasion in Lake Qarun Fishes with Preliminary Trial for Biological Control

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Abstract: Lake Qarun, Egypt was subjected to the problem of isopod infestation with the beginning of year 2015. The problem was gradually propagated reaching a catastrophic invasion causing fish mass loss, great marketing problem and decline of fish stock that consequently lead to economic losses in addition to the great impact on fishermen livelihood working in this area. Treatment and control of crustacean parasites especially isopods is a complicated process and the use of chemical therapeutics is illogical and difficult to be applied when the infestation being as epidemic in large water basin as in lake Qarun. The present work was to continue our previous investigation of the isopod invasion problem in lake Qarun, and also to estimate the possibility of using *portuniid* crab species as a biological agent to control cymothoid isopods through preliminary laboratory trial.
Key words: Lake Qarun, Fish, Cymothoid, Isopods, Biological Control.

Introduction

Deleterious tissue damage is considered the main indicator of parasitic infestation among fish population. Moreover, decreasing in growth and fecundity as well as mortality among both wild and cultured fishes are consequently cause great economic losses[1,2]. Family *Cymothoidae* includes numerous species of isopods that are always associated with many species of commercially important fishes in tropical and subtropical open water around the world. They are found in various parts of the fish body, such as buccal cavity, gills chamber and the body surface including the fins[3]. Cymothoid isopods are haematophagous [4] and also consume mucous, epithelium and subcutaneous tissues of their hosts[5,6].

Lake Qarun, is known as an old natural lake in Egypt, located in Fayoum depression about 90 km^2 southern Cairo. It is an inland closed basin of water with surface area of about 230 Km^2, characterized by high salinity especially with increasing evaporation during summer[7,8]. The lake was subjected to the problem of isopod infestation with the beginning of year 2015 [9] and the problem was gradually propagated reaching a catastrophic invasion causing fish mass loss, great marketing problem and gradual decline of fish stock that consequently lead to economic losses in addition to the great impact on fishermen livelihood working in this area. Treatment and control of crustacean parasites especially isopods is a complicated process and the use of chemical therapeutics is illogical and difficult to be applied when the infestation being as epidemic in large water basin as in lake Qarun. On the other side, although biological methods is not easy applied, but it is one of the promising ways to control such infestation. The object of the present work was to continue our previous study [9] for investigating the isopod invasion problem in lake Qarun, and also to estimate the possibility of using marine crab as a biological agent to control isopods through preliminary laboratory trial.

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Materials and methods:

Collection of fish samples

The present work based on 400 fish samples representing four fish species; *Dicentrarchus labrax, Mugil capito, Solea vulgaris. and Tilapia zilli* (100 samples from each species) which were captured seasonally with a help of local fishermen from various locations along lake Qarun, Fayoum province, Egypt during 2015 / 2016. Fish samples were macroscopically examined for isopod parasites mostly on spot to avoid the movement of the parasites from their original host. Fishes were transported alive in polyethylene bags and/or ice boxes with their natural water to the laboratory of parasitology department, Faculty of Veterinary Medicine, Cairo University where samples were measured to the nearest centimeters (cm) and subjected to examination.

Parasitological examination

Body surface, branchial chamber, gills and buccal cavity of each fish were examined. The detected isopods were collected from their host and preserved directly in 70% ethyl alcohol[10]. Isopods were measured to the nearest millimeters (mm) and photographed with a digital camera (Cannon), 12 mega pixels. The isolated parasites were identified according to the available literatures

Statistical analyses

Significant difference in mean length of infested and non infested fish were determined using Student's t-test [12]. The data on seasonal incidence of isopod infestation among fish species were analyzed statistically using chi square test using SPSS 20.0 statistics software for determined significant differences at $P<0.05$.

Evaluating the ability of *portuniid crab* to interact with parasitic cymothoid isopods

Experimental design:

Fish samples:

For each experiment, 15 of the naturally infested *Tilapia zilli* fish (body length10-15 cm) with *Nerocila orbegnyi* attached to their body surface (intensity one / fish) that were collected alive from lake Qarun; where 12 fishes were used in experiment and three were left as control positive.

Portuniid crab sample:

Fifteen *portuniid crab*s were collected alive by fishermen with their original water; where 5 of them were used in each experiment.

Fish and crab samples were transferred to the laboratory of Parasitology department, Faculty of Veterinary Medicine, Cairo University and kept under observation in separate aerated glass aquarium.

Procedure:

Twelve samples of the infested *T. zilli* with *N. orbegnyi* were transfered with their original water in a separate aerated glass aquarium and after 30 minutes 5 crab samples were introduced to the aquarium. Continuous observation for demonstrating the response and the ability of the crabs to interact with the isopods for the 1st two hours, hourly till 8 hours and after 12, 18 and 24 hours. The experiment was repeated for three times along the period of investigation. The conducted experiment was designed with the guide of [11] and modified to be suitable for the organisms used in the present trial.

Results:

Parasitological findings:

Macroscopically, unilateral and /or bilateral protrusion with marked elevation of the gill cover of the infested fish were noticed. No gross lesions were noticed in the skin of the infested fish except minor scratches at the sites of isopod attachment in some cases.

During the present study, four species belonged to four genera *(Anilocra, Livoneca, Nerocila ,and Renocila*) of cymothoid isopods (Crustacea: Isopoda; Cymothoidae) were isolated from the examined fish species sampled from Lake Qarun. All the detected isopods are females in addition to per-adult stages of *Livoneca redmani*. The isolated isopod species, their host and locality were shown in table 3.

Prevalence and seasonal variations

The current study revealed that, 186 out of 400 examined fish samples were found infested with isopods with a total prevalence of 46.5%.The highest rate of infestation with isopods was recorded during Summer (73%) while the lowest was during Winter (28%). There was a significant value between the rate of isopod infestation and seasons at $p<0.05$. Dealing with the isopod infestation rate among different fish species, *Tilapia zilli* showed the highest rate of infestation (51%) while *Mugil capito* recorded the lowest rate (42%) with a significance value between them (Table 1& Fig 1).

Concerning the seasonal dynamics of infestation among different examined fish species, *Tilapia zilli* recorded the highest rate during summer and winter (72%) while *Mugil capito* and *Dicentrarchus labrax* showed the highest rate during summer only (76% and 84% respectively) showing a strong relationship between the rate of infestation among these species and seasons (phi=0.0) noting that, this correlation is significant, with the same *p*-value that was given for the chi square test. On the other hand, *Solea vulgaris*, recorded its highest rate during summer and autumn (60%) with a weak positive relationship between the two variables (phi = 0.123) noting that, this correlation is flagged as non significant, with the same *p*-value that was given for the chi square test.

Statistically, there was a significant difference in mean length of infested (13.63±0.448) and non infested *Tilapia zilli* fish (18.67±0.458) condition; t (98)= -7.78, p=0.00.Also, There was a significant difference in mean length of infested (21.33±0.572) and non infested *Dicentrarchus labrax* fish .(26.27±0.6615) condition; t (98)= -5.777, p=0.00. The same result was recorded In *Solea vulgaris*, as there was a significant difference in mean length of infested (13.15±0.496) and non infested fish (18.36±0.218) condition; t (98)= -9.88, p=0.00. On the other hand, there was no significant difference in mean length of infested (15.97±0.445) and non infested *Mugil capito* fish species (17±0.326) condition; t (98)= -1.900, p=0.60.) (Table 2 & Fig 2).

Table (1): Prevalence and seasonal incidence of isopod infestation among examined fish species

Season **Type of fish**	**Autumn**	**Spring**	**Summer**	**Winter**	**Total% within species**
Tilapia zilli	44.0%	16.0%	72.0%	72.0%	51.0%
Mugil capito	20.0%	72.0%	76.0%	0.0%	42.0%
Dicentrarchus labrax	32.0%	60.0%	84.0%	4.0%	45.0%
Solea vulgaris	60.0%	36.0%	60.0%	36.0%	48.0%
Total % within season	39%	46%	73%	28%	46.5%

Total number examined from each fish species/ season =25

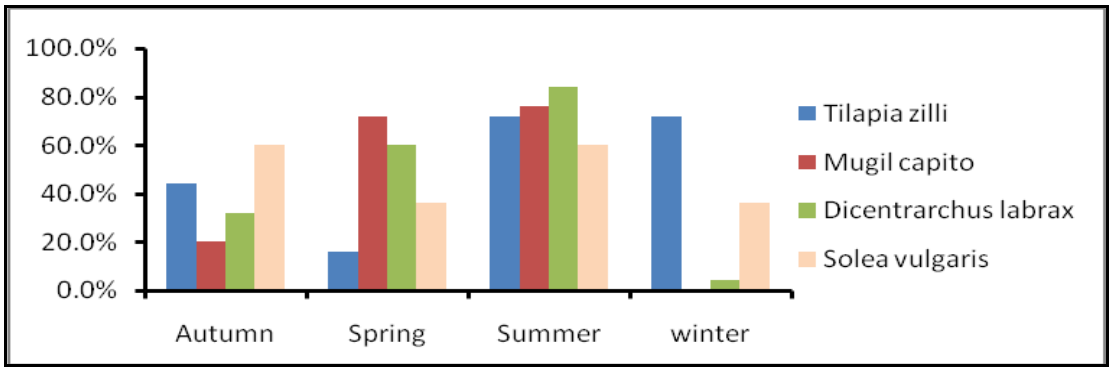


Fig (1): Seasonal incidence of isopod infestation among examined fish species.

Table (2): Mean length of infested and non infested fish of the examined species (Mean±SE).

Type of fish	Mean length of infested fish	Mean length of non infested fish
Tilapia zilli	13.63±0.448*	18.67±0.458**
Mugil capito	15.97±0.445	17±0.326
Dicentrarchus labrax	21.33±0.572*	26.27±0.6615**
Solea vulgaris.	13.15±0.496*	18.36±0.218**

SE= Standard error, *,** Different superscripts within the same raw between mean length of infested and non infested fish indicate significant difference at $P< 0.05$.

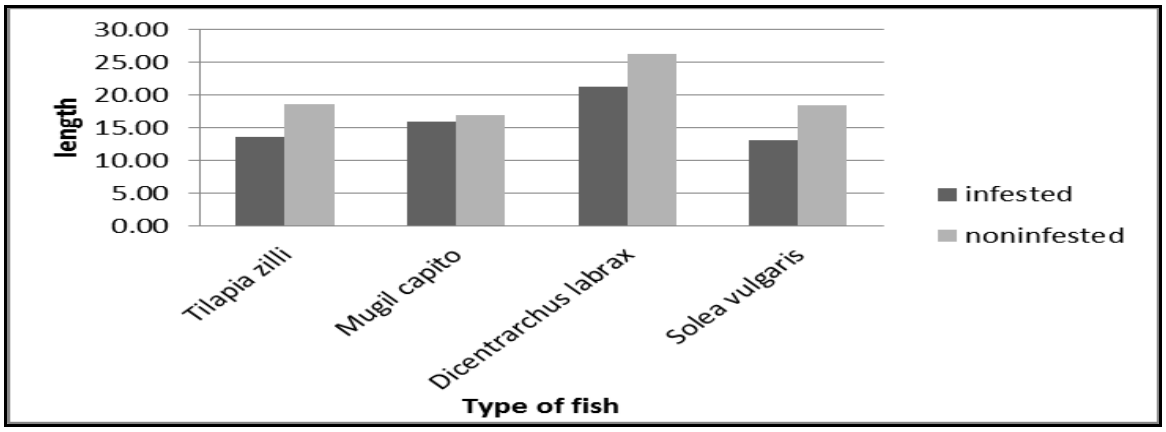


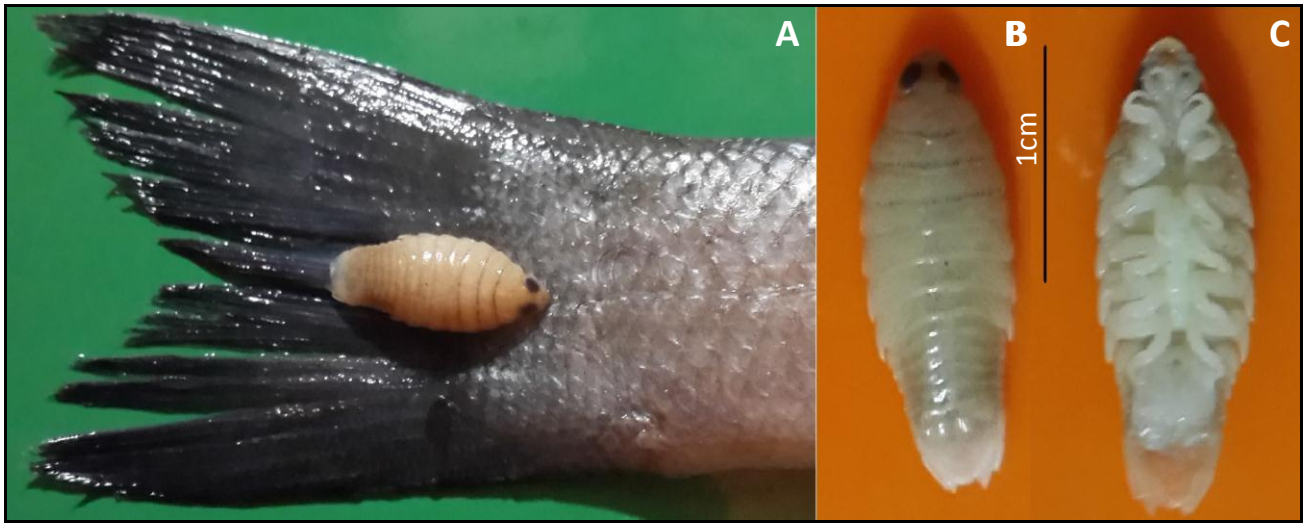
Fig (2): Mean length of infested and non infested fish of the examined species.

Table (3): The recorded isopod species and their location in the examined fish hosts.

Fish species Isopod species/location	*D. labrax*	*M. capito*	*Solea spp.*	*T. zilli*
Anilocra physodes	+ (Skin -The base of caudal fin)	-	-	-
Livoneca redmanni	+ **(**In the branchial cavity between gill and operculum)	+ **(**In the branchial cavity between gill and operculum)	-	-
Nerocila orbegnyi	+ (Skin- Base of fins)	+ (Skin -The base of caudal fin)	+ (Skin)	+ (Skin)
Renocila thresherorum	+ (Gill)	-	-	+ (Gill)

Morphological characters of the detected isopod species:

1-*Anilocra physodes* (Linnaeus., 1758) plate (1)



A*: A. physodes* attached to the base of caudal fin of *D. labrax*. B: *A. physodes* dorsal view. C*: A. physodes* ventral view.

Description: (based on 10 femal specimens).

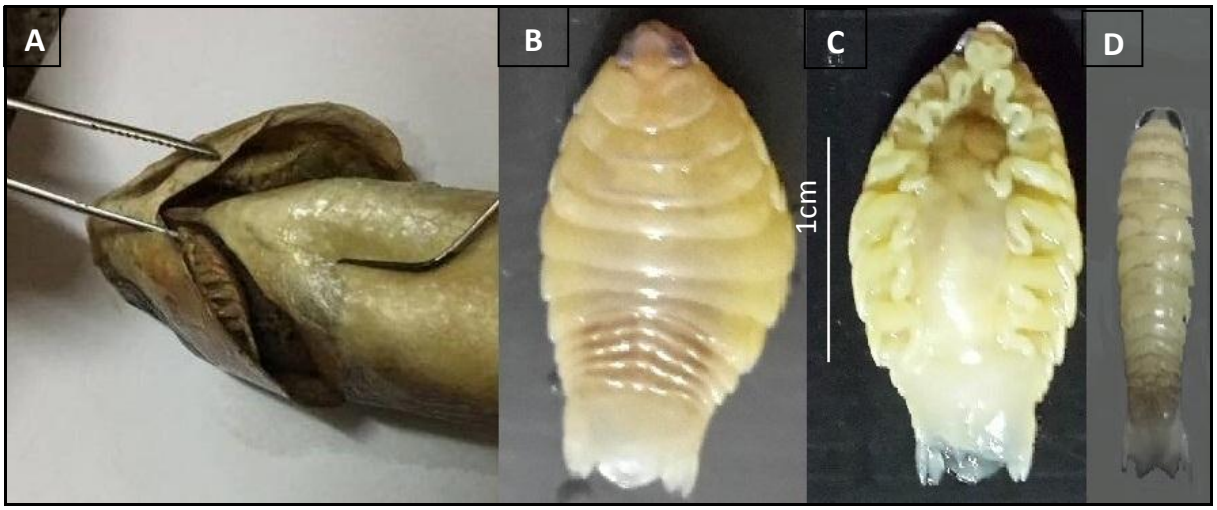
The body: Is narrow and elongated with slight dorsal convexity measuring 16.1± 1.59 mm long and mostly pale creamy although some specimens was dark brown.

Cephalon: It is narrow anteriorly and projected ventrally between the antennae with weak trilobed posterior margin. Eyes are comparatively large and prominent .antenna is of 9 segments and the antennules extends posterior to the eyes.

Pereon: It is of 7 segments of length and width progressively increase toward posterior. The pereopod 7^{th} is longer than the 6^{th} and markedly produced.

Pleon: Pleopod 3-5 are markedly narrower with highly folded medial lobe. The uropod is of rounded rami of subequal length and clearly extends posterior to the pleotelson.

2-*Levonica redmani* (Leach, 1818) plate (2)



A: *L. redmani* in branchial cavity of *M. capito* B: *L. redmani* female dorsal view C*: L. redmani* female ventral view D: *L. redmani* juvenile stage.

Description: (based on 10 femal specimens).

The body: Is ovate and mostly twisted to one side, light brown in color with dark chromatophores measuring 19.3±2.68 mm in length.

Cephalon: Is not projecting between the bases of the antennae .It`s Posterior margin appears trilobed .one pair of eyes are located laterally. 2 pairs of antennae appears, the 1st consisted of 6 segments and the 2nd of 8 segments.

Pereon: Composed of 7 segments, the last two appears narrower pereopods are robust and characterized by large dactyli**.**

Pleon: It is somewhat narrower than pereon and not immersed in it. It is of 6 segments that decrease gradually in width toward the posterior. The brood pouch extends over the pleopods. Uropoda are equal in length and extends beyond the pleotelson border**.**

Results of experimental trials:

The trial demonstrated that crab responded to the presence of the isopod attached to the body surface of *T .zilli* fish and detached the parasite from its fish host. Time of parasite detachment and their number in each trial was shown in table 4. It was noticed that all detached isopods (except 2 of them) showed noticeable damages in their legs and /or telson and did not reattached to the fish host till the end of experiment. No mortality occurs among control infested fish, Crabs or the exposed infected fish till the end of each experiment.

Table (4): The interaction between crabs and isopods infesting *T. zilli* after exposure*.

No. of fish in each experiment	Time of detachment	No. of detached isopodes	% of detachment
1st experiment	90 minutes	6	50
	190 minutes	3	25
	Till the end(24 hours)	0	0
2nd experiment	70 minutes	6	50
	Till the end(24 hours)	0	0
3rd experiment	65 minutes	6	50
	Till the end(24 hours)	0	0

NB: Crabs start to move toward infested fish after 40-50 minutes of exposure in all experiments
*** Number of infested fish used in each trials =12**

Discussion

The current investigation deal with the isopod invasion problem in lake Qarun. The isolated isopod species were identified according to[13,14] for *L. redmani*,[15,16,17] for *Anilocra physodes*. The detected *Renocila thresherorum* was found identical to that reported by [18,19] and to the specemins isolated by [9] from lake Qarun. The morphological characters of the detected *Nerocila orbegnyi* was also agreed with the description given by [20,21]. In the present work, only the isolated *A. physodes* and *L .redmani* were morphologically described as the other two isolated species were described in detail from the same locality in our previous investigation[9]. Results of the present study showed that the prevalence of isopod during year 2015/2016 reached 46.5%, the result which was much higher than that reported by[22] through his survey during year 2013 / 2014 , the data which indicated rapid progression of the isopod invasion so it is prudent to conclude that we face a serious disaster. Statistical analysis of the present result indicated a significant correlation between incidence of isopod infestation and fish host body length in most examined species, the result which came in agreement with [23] while disagree with that concluded by [24] who did not find correlation between the standard length of *P. nattereri* and isopod incidence.

As *Portuniid crabs* is considered to be an opportunistic predator and crustaceans are their principal food item [25], the demonstration of their ability in interacting with cymothoid isopods in the present work is a preliminary idea guided by that shrimps and other small aquatic predators are thought to remove and eat fish parasitic copepods and gnathiid isopods[11]. The laboratory trial was designed to establish and evaluate the

availability of using *Portuniid crab species* as a biological control agent against cymothoid isopods .Results showed that crabs can damage, detach and remove adult *Nerocila spp.* from their host body surface but the predation process was not accurately detected. The detached damaged isopods were slowly moved but couldn`t reattach to the body of fish again indicating that *Portuniid crabs* could play a role in controlling isopod infestation problem . Other studies and trials are currently conducted by our research team for evaluating the ability and importance of other cleaning organisms to be used for biological control of both adults and other developmental stages of isopods.

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