Studies on Diseases caused by Isopods in relation to Bacterial Infection Among Some Wild Marine Fishes at Suez Canal Area

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Abstract:

A total number 200 different marine fish species represented as 100 Elsarah (*Nemipterus japonicus)* and 100 Mias (*Pomatomus saltatrix L.)* were collected seasonally from Suez and Ismailia provinces respectively . The infested marine fishes revealed no pathognomonic signs.They were investigated for detection of Isopoda parasites. It was revealed that 50% of the examined marine fishes was infested.The detected species were *Irona nanodies* from *Nemipterus japonicus* with a prevalence 60% , and *Lironeca ovalis* from *Pomatomus saltatrix L* with a prevalence 40%.The histopathological alterations were recorded and discussed.The secondary bacterial invasion was manifested by the isolation of *Pseudomonas aeruginosa* where it was experimentally infected into *Tilapia zillii* and reisolated again.

Key words: Isopoda , El-sarah , Mias , *Tilapia zillii , Pseudomonas aeruginosa*.

Introduction:

Parasites have been found to dominate food-web links ***(Lafferty et al, 2006 & 2008)*** and to be useful indicators of aquatic environmental conditions ***(MacKenzie, 1999; Hudson et al, 2006 and Sasal et al, 2007).*** They have also been shown to reflect the source population of fishes ***(MacKenzie , 2002)*** and are a major source of disease, either through the injuries they cause or through their activity as vectors ***(Panek, 2005).***

In recent years, crustacean parasitic diseases are becoming more frequent in aquaculture and considered the more parasitic problem on cultured marine fish ***(Tansel and Fatih , 2012) .***

Internal and external parasites may result in emaciated and physically weakened hosts that become susceptible to other causative agents of disease. Moreover , some of these parasites may have zoonotic importance ***(Eissa , 2002 and Woo, 2006).***

It was recorded that isopoda is an order of peracarid crustaceans including familiar animals such as woodlice and pill bugs. The name isopoda derives from the Greek roots, iso meaning same and podo meaning foot; they lack an obvious carapace, which is reduced to a cephalic shield covering only the head . Isopod crustaceans are part of the greatest fish ectoparasite group and are easy to identify due to their size and morphological aspects because they are easily found on the outer part of fish bodies ***(Thatcher, 2000 and Keable , 2002).*** Ecto or endoparasites of fish search mainly for both food and shelter . Adult isopods seem to have little direct effect on mortality and cymothoidae parasitic only as adults. Besides , for such isopoda , the fish is the total environment , so, they try to keep their victims that represented as shelter and source of feeding alive , with a little harm as possible as they can . In the same time , they can suck their food through pressure on gills. ***(Eissa, 2002; Rameshkumar and Ravichandran, 2013 and Bharadhirajan et al, 2014).***

The present study was aimed to investigate the role of isopoda in affecting some marine fishes in relation to bacterial infection.

Materials and Methods:

Fishes:

a- A total number of 200 different freshly caught wild marine fish species represented as 100 Elsarah (*Nemipterus japonicus*) ,each about 35±*4* g and 100 Mias (*Pomatomus saltatrix L.)* each about 100±6 g . They were collected seasonally from Suez and Ismailia provinces respectively .

b- A total number of 20 apparently healthy *Tilapia zillii* were collected alive , with an average body weight of 30±2 g .These fish were used for the experimental infection .

Aquaria:

Fish were kept in prepared full glass aquaria (100 x 50 x 50 cm) , these aquaria were used for holding the experimental fish throughout the period of the present study .They were supplied with chlorine free tap water according to ***Innes (1966),*** the continuous aeration was maintained using electric air pumping compressors (RINA, Italy). Water filtration was conducted through filters (Rena 225, Submersible water filters, France).Water temperature was controlled via thermostatic heaters (CMI , Germany).

Clinical picture :

The collected fishes were examined cinically according to the methods described by ***Noga (1996).*** The post mortem examination was carried out through the skin , inside of gills, branchial cavity as well as internal organs according to the methods adopted by ***Stoskopf (1988) .***

Parasitological examination:

The opercula were opened for removing the macroscopic parasite specimens from the infested fishes .The crustacean parasites were collected and washed several times in warm normal saline solution, transferred into test tubes, fixed in 3% formalin and preserved in 70% Ethyl alchol. There was no need for microscopical examination and staining, as these parasites were large enough to identify according to ***Lucky (1977)***

Bactrial examination:-

Isolation of bacterial species:

The isolation achieved as smears from the gills and the isopoda itself. Then , they were streaked on blood agar and macConkey agar for further identification . In addition , different selective media for accurate identification of isolates were used .

Identification of bacteria

The identification was carried using traditional methods (***MacFaddin, 2000).*** Also, using vitek2 system for accurate identification according to ***Pincus (2009).***

Experimental infection:-

The experiment was carried out using 20 apparently healthy alive *Tilapia zillii* with an average body weight 30±2 g . The fish were divided into two equal groups, each contained (10) fish .The first group was control and the second group was inoculated intraperitoneally. The used dose was 0.1ml of $2.4x10^8$ cfu\ml of 24 hours old broth culture of *Pseudomonas aeruginosa* **.** All experimentally infected fish were observed daily for any clinical signs and mortalities. Postmortem examination was done to record gross lesions. Bacterial reisolation of *P. aeruginosa* was attempted from such fish.

Histopathological examination

Spiecmens for histopathological techniques were freshly taken from affected gills of naturally infected fish.Specimens were trimmed and fixed in 10% phosphate buffered formalin. Then washing in running tap water for 24 hours, dehydrated in different concentration gradients of alcohol and cleared in xylol then embedded in paraffin wax and associated into thin sections 5 micron thickness . Sections were stained with H&E stain then examined microscopically ***(Roberts , 2001).***

Results:

Clinical picture of naturally infested fishes

The clinical examination of naturally infested fishes of different species including *(Nemipterus japonicus, and Pomatomus saltatrix L.)* that were collected from Suez Canal area, revealed no pathognomonic signs or lesions. Only, slight unilateral protrusion of the operculum (gill cover) revealing a small slit . After removal of the operculum, the gills were hyperemic with increase of mucus. Also , erosion of the gill filaments and adhesion of gill filaments at site of attachment. After examining the inside of operculum, there was only one isopoda lodging in the gill cavity in all infested fishes.

Parasitological examination:-

After examination of the gill cavity in *Nemipterus japonicus* ,it was found that the isopoda was singly and only unilateral in the branchial cavity of parasitized fish. Moreover, the ventral side of such isopod that contains the seven pairs of appendages was found facing the ventral side of the operculum. .Also, in *Pomatomus saltatrix L.* the isopoda was always found singly and was only unilateral in the branchial cavity of parasitized fish. However, the ventral side of such isopod that contains the seven pairs of appendages was found facing the dorsal side of the gill arches.

Description of the isopoda:-

Nemipterus japonicus

According to the parasitological examinations, these parasites are belonged to order Isopoda , suborder Flabellifera , family Cymothoidae , subfamily Anilocridae genus Irona , spp *Irona nanodies* . It was dorsoventerally flattened with symmetrical body. It was segmented , large (up to 2cm length and 1.5 g weight),creamy in color with small head as deeply embedded in the first segment of peraeon with two small black eyes . It consists of six segments.

Pomatomus saltatrix L.

According to the parasitological examinations, these parasites are belonged to order Isopoda , suborder Flabellifera , family Cymothoidae, genus Lironeca , spp *Lironeca ovalis* which was grey in color , segmented, large up to 2.5cm length and 2 g weight , head deeply embedded in first segment of peraeon with seven equal pair of legs and eyes well developed .

Prevalence rate :-

As in the table (1) the examination of different marine species revealed that 60 out of 100 were infested with (isopoda) in *Nemipterus japonicus* with its prevalence 60%, and 40 out of 100 were infested with (isopoda) in *Pomatomus saltatrix L.* with its prevalence 40% .The total prevalence rate was 50%.

Experimental infection:-

The intrapertoneally experimentally infected *Tilapia zillii* fish showed signs of septicemia as hyperemia , reddening of skin , hemorrhagic lesions , erosion of fins ,loss of scales , cloudness of the eyes and abdominal swelling .It was observed that there were no records of deaths in fish.The reisolation of bacteria was confirmed as *Pseudomonas aeruginosa*.

Histopathological changes:-

In Nemipterus japonicus Gill lamellae of the first and second gill arches were eroded .Secondary gill lamellae uneven clubbed and showed fusion. Some gills were stunted and atrophied. Degeneration and dwarfing of some lamellae along with leukocytic infiltration (plate 1).In *Pomatomus saltatrix*

*L.*The gills revealed congestion of lamellar vessels and adhesion of most gill filaments and lamellae. Destruction of some lamellae and lymphocytic infiltration was also observed(plate1).

Table 1: *Showing the prevalence of isopoda infection among the examined fishes.*

Fish	Number	No. of infest.fish	%of infest . fish
Nemipterus japonicus	100	60	60
Pomatomus saltatrix L.	100	40	40
total	200	100	50

Table 2: *Showing the seasonal prevalence of isopoda among the examined marine fishes.*

Season	**No of fish**	**No. of infested fish**	**%**
Summer	50	45	90
Autumn	50	15	30
Winter	50	10	20
Spring	50	30	60
Total	200	100	50

Table 3: *Showing the seasonal prevalence of isopoda among examined Nemipterus japonicus & Pomatomus saltatrix .*

Season	**No of fish**		**No. infested fish**		**%**	
	Nemipterus japonicus	Pomatomus saltatrix	Nemipterus japonicus	Pomatomus saltatrix	Nemipterus japonicus	Pomatomus saltatrix
Summer	25	25	23	22	92	88
Autumn	25	25	11	4	44	16
Winter	25	25	6	4	24	16
Spring	25	25	20	10	80	40
Total	100	100	60	40	60	40

Table 4: Showing morphological and culture characteristics of suspected *Pseudomonas aeruginosa* isolated from naturally infected marine fishes

Test	Reaction
Gram-reaction	negative
Shape	Short bacilli
Arrangement	Scattered
Motility	motile
Growth on pseudomonas agar	Mucoid white creamy colonies
Growth at 0°C 25°C 37°C 40°C	 - + + +
Catalase Oxidase Indole TSI Simmon citrate	+ve +ve -ve -ve +ve

Table 5: Showing the vitek 2 system results for identification of bacteria

Biochemical details	-	-	+	-	-	-	-	
	BGAL	O F F	BALaP	dSOR	5KG	PHOS	BGUR	
	9	1 5	22	32	39	45	57	
	-	+	-	-	+	-	+	+
	dCEL	G G T	BXYL	URE	MNT	AGAL	CMT	ILATa
	7	1 4	21	31	37	44	56	64
	-	+	(-)	+	+	-	-	-
	IARL	d G L U	dMNE	TYrA	C I T	NAGA	IHISA	ELLM
	5	1 3	20	29	36	43	53	62
	-	+	-	-	-	+	-	+
	PYra	A G L T P	dMAN	PLE	dTRE	SUCT	LDC	IMLTa
	4	1 2	19	27	35	42	48	61
	-	-	-	-	-	-	-	-
	ADO	B N A G	dMAL	LIP	dTAG	AGLU	ODC	GGAA
	3	1 1	18	26	34	41	47	59
	-	-	-	+	-	+	-	+
	APPA	H 2 S	BGLU	ProA	SAC	ILATK	GIYA	O129R
	2	1 0	17	23	33	40	46	58

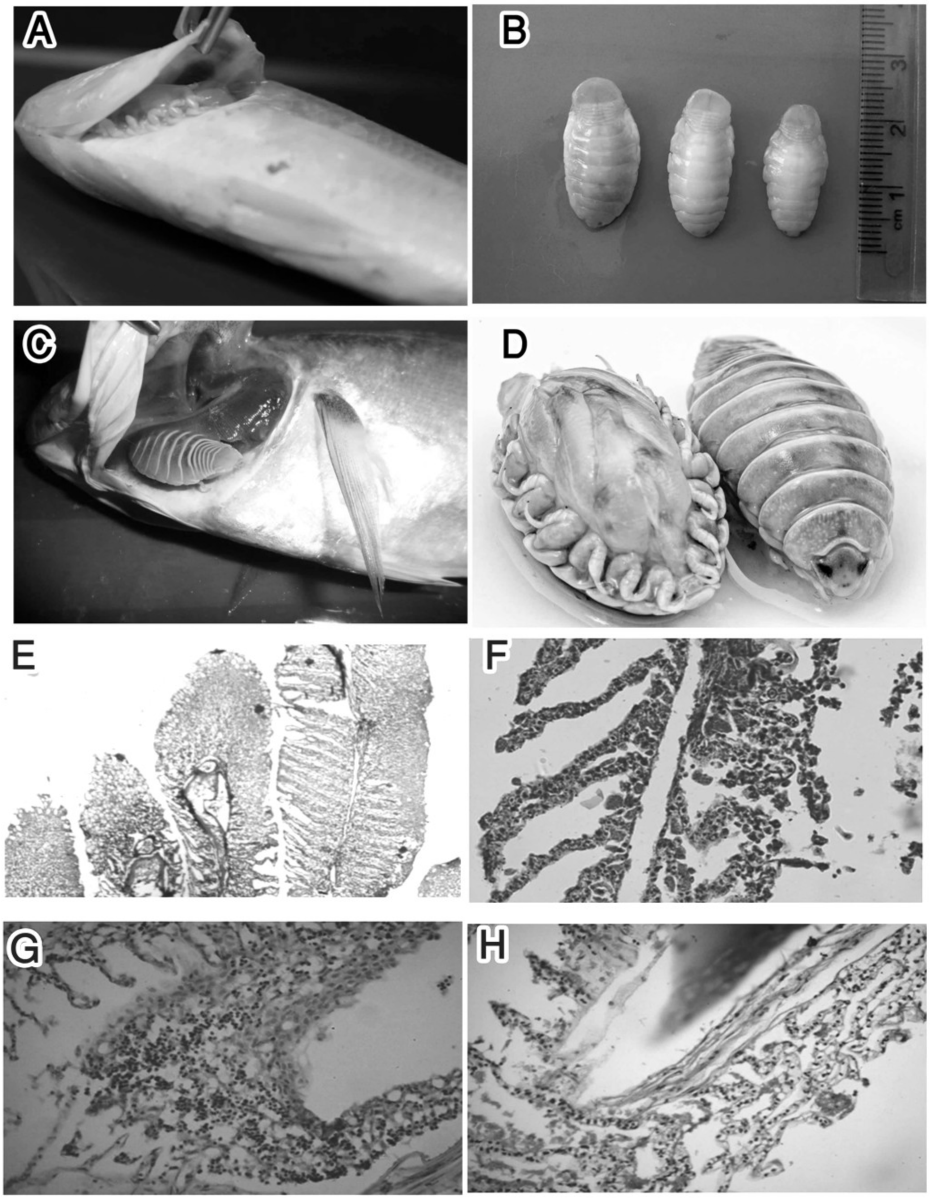

Plate 1 :

Figure (A): *Irona nanodies* inside the gill cavity of *Nemipterus japonicus.*

Figure (B):Showing *Irona nanodies* (dorsal view).

Figure (C):Showing *Lironeca ovalis* inside gill cavity of *Pomatomus saltatrix L* .

Figure (D): Dorsal and ventral view of *Lironeca ovalis* isolated from gill cavity of *P. saltatrix L*.

Figure (E): Gills of *N. japonicus* showing hyperplasia of primary and secondary lamellae, degeneration and dwarfing of some lamellae along with leukocytic infiltration. H&E. X 200.
Figure (F): Gills of *N. japonicus* showing leukocytic infiltration, with adhesion and erosion of some lamellae. H&E. X 400.
Figure (G): Gills of *P. saltatrix L* showing degeneration, atrophy and severe leukocytic infiltration. H&E. X 400.
Figure (H): Gills of *P.saltatrix L* showing hyperplasia of secondary lamellae along with adhesion and erosion of some lamellae. Atrophy of some lamellae. H&E. X 400.

Discussion

Isopod parasites have received considerable scientific attention because they cause serious damage to fishery resources ***(Ravichandran et al, 2009).*** Cymothoids (Crustacea, isopoda) are ectoparasites of marine and freshwater teleost fishes. The examination of *Nemipterus japonicus* and *Pomatomus saltatrix L* fishes resulted in the recovery of isopods belonged to Family Cymothoidae . Where, the infested fish harbor only one parasite in a unilateral gill chamber with slight protrusion of gill cover and retardation of growth in agreement with that described by ***(Eissa, 2002; Woo, 2006; Ravichandran et al., 2007; Ravichandran et al., 2009 and Bharadhidajan et al, 2014).***

In the present study, the clinical picture revealed slight inflammation in the gills with increased slime production and erosion with adhesion of the gill filaments. This result agrees with that described by ***Eissa (2002)*** and ***Eman, et al (2014)***. The *Irona nanodies* . which found inside gill cavity of *N. japonicus* caused slight protrusion of the operculum , infilammation of gills with increased slime production .This result agree with that described by ***Eissa (2002).*** While the *Lironeca ovalis* that found inside gill cavity of the *P.saltatrix L* caused protrusion of the gill cover ,erosion of gill filaments and adhesion of gill lamellae.This result agree with that described by ***Marks et al (1996) and Ali and Abo – Esa, (2007).***

In N. *japonicus* , the detected parasite was belonging to order Isopoda , suborder Flabellifera , family Cymothoidae , subfamily Anilocrinae , genus Irona , *Irona nanodies* . The parasite was segmented , creamy in color , and the small head was deeply embedded in the first segment of peraeon with small black eyes.This agree with the description given by ***Kabata (1985), Mahi Ghobashy (2000)*** *and* ***El-halafawy et al (2011).***While, isopoda found in

P.saltatrix L was detected to be belonging to order Isopoda , suborder Flabellifera , family Cymothoidae , genus Lironeca , *Lironeca ovalis* . Parasite was Large up to 2.5cm length , and 2 g weight. Head was well developed , with two black well developed eyes , grayish in color ,body is segmented, with seven equal pairs of legs. this description agrees with ***Kabata (1985), Sindermann (1990) , Marks et al (1996) and Eissa (2002).***

In the present study, only one parasitic isopoda was found lodging in the gill cavity and thus cause partial protrusion of the gill cover and this may impair the gill respiration of the fish. Also, this isopoda feed on the blood,mucus,epithelium and making the fish suffer. This agree with the results obtained by **Ravichandran *et al (2009)*.**

Regarding, the prevalence of *Irona nanodies* in *N. japonicus* was 60% and prevalence of Lironeca in *P. saltatrix L* was 40% , with atotal prevalence 50%, this nearly agreed with the result given by ***Eissa, (2002) and Mousa and Tantawy (2006)*** who recorded prevalence 47 and 40.8% in *Centropristis filamentosus* and *Seabastus marinus* fish respectively. Also , 40% for *Cymothoa indica* on the blue spot mullet *Moolgarda seheli* from the Yemeni coastal waters of the Red Sea ***(Al-Zubaidy and Mhaisen , 2014. Ali and Abo-Esa, 2007)*** recorded an isopoda, *Ovoinella obovata* in Red sea shrimp (*Penaeus semisulcatus*) but belonged to different family (Bopyridea) with incidence 32% . However,the infestation rate reached up to 100% in summer . This is may be attributed due to high water temperature and where such fish species exist naturally with abundance in markets.

In the present study , the prevalence of isopoda in *N. japonicus* was higher than in *P. saltatrix L* as 60 and 40 % respectively.was 40%. This may be due to the *N.japonicus* is a benthic fish , but the *P. saltatrix L* is a pelagic one, so the *N. japonicus* face the danger of isopoda more than *P. saltatrix L.* Moreover , the gill chamber of *N. japonicus* is more comfortable and wider than gill chamber of of *P. saltatrix L* so that facilitate the infestation of the *N. japonicus* with isopoda .Also, the tendency of *N. japonicus* to school thus increase the rate of infestation.

Ecto or endoparasites of fish search mainly for both food and shelter. Adult isopods seem to have little direct effect on mortality and cymothoidae parasitic only as adults ***(Segal, 1987; Adlard , 1990 and Sindermann , 1990).*** This fact coincides with our parasite, as it is an adult isopod .This explains why such isopod is always found singly and is only unilateral in the branchial cavity of the parasitized fish .This behavior may be taken to prevent their direct contact to the gills .Besides ,for such isopoda ,the fish is the total environment, so, they try to keep their victims that represented as shelter and source of feeding alive, with a little harm as possible as they can. In the same time, they can suck their food through pressure on gills ***Eissa (2002)***.

In relationships where survival of the host fish is important, parasitic species may consume tissues or fluids in a manner that does not cause serious harm to the host ***(Nikolsky, 1978).*** Several aspects of isopod life history support the concept that cymothoids may parasitize in a manner that does not constitute a serious threat to host well-being. In the case of Cymothoidae , swimming ability is vestigial in adults ***(Brusca 1978; Keusink, 1979; Sandifer and Kerby 1983).*** Thus, it may be critical for a young,mobile isopod to attach to a host and then mature as the host grows.This combination of life-history traits may result in cymothoids being adapted to semi-enclosed habitats where host density is high ***(Brusca, 1981and Rokicki, 1985)*** and where continued host survival is beneficial to the parasite.

However, parasitic isopoda may affect the growth and normal function of the fishes that suffer and lead to economic loss or the marketability may reduce then. ***(Bharadhirajan et al, 2014).***

The attachment of the parasitic isopod on marine fishes paved way for the entry of pathogenic microbes in to the attachment sites. But such behaviours also induce damage to skin and fins that is likely to increase the likelihood of secondary microbial parasite infections ***(Clayton et al, 1998).*** In the present study,it was found that *Pseudomonas aeruginosa* at the site of attachment at gill chamber . This agrees with the results obtained by ***Rameshkumar (2013)*** who identified isolates like flavobacterium, *Aeromonas hydrophila, Pseudomonas fluorescens, Pseudomonas putida,* Citrobacter, Photobacterium, Bacillus, *Mycobacterium marinum*, Flexibacter, *Aeromonas salmonicida* on the parasite attached region of host fishes.

In the present study , the pathogenicity test of *Tilapia zillii* infected with the *Ps auriginosa* revealed that it is pathogenic as it cause signs of septicemia as hyperemia , reddening of skin , hemorrhagic lesions and erosion of fins and loss of scales , cloudness of the eyes and abdominal swelling. These findings agree with the results given by ***Shayo et al (2013)*** . So , it was approved that the bacteria is pathogenic and cause high morbidity when it goes with or carried by the isopoda as secondary invader affecting the health of the fish and may finally cause death .

Regarding the histopathological findings , it was found that gill lamellae of the first and second gill arches were eroded .Secondary gill lamellae uneven clubbed and showed fusion. Some gills were stunted and atrophied in *N. japonicus*.Also , in *P. saltatrix L* the gills revealed congestion of lamellar vessels and adhesion of most gill filaments and lamellae. Destruction of some lamellae and lymphocytic infiltration was also observrd . These results nearly agree with results obtained by ***Rameshkumar (2013) and Eman et al (2014).***

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دراسات عن الامراض الناجمة عن الايزوبود وعلاقتها بالبكتريا فى بعض الأسماك البحرية بمنطقة قناة السويس

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تم تجميع ٢٠٠ سمكة بحرية متمثلة في ١٠٠ سمكة الصرع و١٠٠ مياس من محافظتي السويس والاسماعيلية .الاسماك المصابة كانت لا تعاني من اي اعراض مرضية وتم فحصها لوجود الايزوبودا وقد وجد ان ٥٠% من الاسماك البحرية المصابة بالايزوبودا وكانت انواعها كالتالي ايرونا نانوديز في سمكة الصرع وكانت نسبة الاصابة ٦٠% وليرونكا اوفاليس وكانت نسبة الاصابة ٤٠%.وتم تسجيل الاصابات النسجومرضية .وتبين من الفحوص البكتيرية ان وجود الايزوبودا قد سمح بدخول البكتريا الممرضة والتي تم عزلها والتعرف عليها السودومونس ايرجينوزا.وقد تم اجراء العدوي الصناعية علي اسماك البلطي الاخضر باستخدام السودومونس ايرجينوزا وقد سبب تسمم دموي للأسماك التي تم حقنها.