Endoparasitic Diseases in Relation to Heavy Metal Pollution among Some Marine Fishes in Port-Said Governorate

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Abstract

This study was carried out to detect the endoparasites affecting *Pomatomus saltatrix, Caranx rhonchus,Platycephalus indicus* and *Epinephelus aeneus* collected from Port Said Province at different seasons. The highest infestation rate was in *P. saltatrix*(89%) followed by *C. rhonchus* (75%), *P. indicus*(68%) and *E. aeneus* (57%). The isolated parasites were represented as nematodes (*Anisakis simplex*, *Hysterothylacium aduncum* and *Philometra saltatrix*), cestodes (Plerocercoides of *Scolex pleuronectis)* and acanthocephalan parasites (*Andracantha tandemtesticulata* and *Hesterosentismongcia*). Seasonal prevalence, detection of iron, lead and copper in water and histopathological picture were also recorded and discussed.

Introduction

Parasites play a principale role in the ecology of aquatic ecosystems as well as in the mariculture industries ***(Ekanem et al.2010).*** Parasites of fish are a concern since they often produce a weakening of the fish`s immune system and that lead to increasing their susceptibility to secondary infections, resulting in the nutritive devaluation of fish and economic losses ***(Onyedineke et al.2010).***Heavy metals are defined as naturally occurring metallic elements that have a relatively high density compared to water ***(Tchounwou et al. 2013).***The relationship of parasitism and water pollution is complicated and in essence involves a double-edged phenomenon, in which parasitism may increases host susceptibility to toxic pollutants or pollutants may result in an increase or decrease in the prevalence of certain parasites. Heavey metals may effect on intermediate hosts in parasite life cycle or free living life cycle stage of parasite ***(Sindermann, 1990 and Eissa et al, 2012 and 2013).***Therefore, the present study was directed towards further understanding of the effect of dissolved heavy metals on prevalence of endoparasitic infestation in some Mediterranean Sea fishes at (Port Said province).

Materials and methods

Fishes:

A total of 400 alive marine fishes were represented as100 of each *Pomatomus saltatrix,Caranx rhonchus, Platycephalus indicus* and *Epinephelus aeneus* of different body weights were collected from Suez Canal area (Port-Said Province) and Mediterranean coast of Egypt in different seasons between December 2014 and the end of November 2015. They were obtained by the aid of fishermen and fishing gears and then transportedalive in thick polyethylene bags containing 1/3 of its volume water where the remaining volume was filled with airto laboratory of Fish Diseases and Management Dept, Faculty of Veterinary Medicine,Suez Canal Univ.

Clinical picture:

Clinical and postmortem examinations were done on the examined fishes according to ***Amlacker (1970).***

Parasitological examination:

The collected fishes were macroscopically and microscopically examined for presence of parasites according to ***Conoroy and Hierman(1981).***The collected parasites were washed several times in a petridishes containg normal saline to get rid of debris and to be relaxed.

The collected cestodes:were fixed in 5% formalin, stained in Semichon'sacetocarmin thendehydrated in ascending grades of ethyl alcohol (30, 50, 70, 80. 90 and 100%), cleared with clove oil, then xylene and mounted in Canada balsam then left to dry in horizontal position in hot air ovenaccordinig to ***Lucky (1977).***

The collected acanthocephala: were compresed in between 2 slides then fixed, stained and mounted as cestodes.

The collected nematodes (adult and larvae) : wererelaxed in saline inside refrigerator and fixed in hot alcohol 70%.Serial passage was done in glycerin alcohol ascending grades 30, 50, 70, 80, 90 and 100% according to ***Meyer and Olsen (1992),*** then cleared and mounted.

Analysis of water samples for heavy metals:

Five water samples (2 L) were collected seasonally at the time of fish collection into pre-cleaned plastic containers using a vertical water sampler.For detection of Pb, Cu and Fe water samples were fixed with 2% nitric acid then analyzed for presence of heavy metals according to ***APHA (1992).***The samples were measured in Toxicology and Micro-analytical Research Unit at Faculty of Science, Suez Canal Univ.

Histopathological examination:

Small tissue specimens about 0.5 cm in thickness were collected from ovaries and intestines affected with different parasites were rapidly fixed in 10% neutral buffered formalin and histopathologically examined according ***toTakashima and Hibiy (1995*)**.

Results

Clinical picture:

The clinical signs in the four naturally infested species revealed abdominal distension with slight emaciation,stuntinggrowth, reduced appetite, anal prolapse. *Platycephalus indicus* showed no external abnormalities except protrusion of nematodes from anus in few cases. Also, *P. indicus,* and *E. aeneus* showed no characteristic lesion.Internal examination of spleen and kidneys showed no abnormality in all examined fishes. On the other hand, liver was haemorrhagic in some examined *P.saltatrix* and*C. rhonchus* while it was pale in some infested *P. indicus*. Stomach and intestines showed congestion, enlargement, thickening and inflammation of their walls in some examined *P.saltatrix* and *C.rhonchus*. In case of *P.saltatrix* females, there were - in all examined cases- philomitried infestation in ovaries or abdominal cavity as shown in **photo (1**). Infested *P. indicus* showed free living nematodes in abdominal cavity, stomach or intestines.

Results of parasitological examination:

Based on the morphological and parasitological investigations, the isolated parasites, were identified as larval nematodes: *(Anisakis simplex* Rudolphi 1809 and *Hysterothylacium aduncum* Rudolphi 1802) from body cavity and internal organs of *Platycephalus indicus, Caranx rhonchu, Pomatomus saltatrix* and *Epinephelus aeneus*. Adult nematodes: *Philometra saltatrix.* Costa 1845 which was only isolated from *P.saltatrix* ovaries. Regards Cestode larvae: Plerocercoides of *Scolex pleuronectis* Müller 1788 from body cavity and internal organs of all examined fishes.Intestinal Acanthocephalans were represented as:*Andracantha tandemtesticulata* Yamaguti 1939.and *Heterosentis mongcai* Van cleave 1931 from intestines of *Platycephalus indicus* as shown in **plates(1 to6).**

Seasonal prevalence of endoparasitic infestation among different examined fishes:

Table (1) and **fig (1)** revealed that the total prevalence of endoparasitic infestation was 72.25%.The highest prevalence was in spring (80%) and the lowest was in summer (59%). The prevalences, in *Pomatomus saltatrix*,*Caranx rhonchus*,*Platycephalus indicus* and *Epinephelus aeneu*s were 89, 75, 68 and 57% respectively.

Result of heavy metal analysis of water samples:

Table (2)showed that during winter, iron was 0.78 ,copper was 0.02 and lead was 1.06ppm.During spring it was 1.91, 0.07 and 1.01 ppm for iron, copper andlead respectively. During summer, lead was 0.043, iron and copper was zero. During autumn it was 0.088ppm for iron but copper and lead were zero.

Results of histopathological examination of the infested fishes:

Plate (7) showed the histopathological alterations in different affected organs. In nematode infestation, intestines showed destruction, necrosis and atrophy of intestinal mucosa along with necrosis and degeneration of the submucosa. Lamina propria and submucosa were edematous and infiltrated with mononuclear cells.In ovarian infestation with *Philometra saltatrix,*ovarian tissue showed degenerated atretic follicles and fibrosis. In acanthocephalan infestation intestine revealedepithelial lining desquamation, destruction of intestinal villi and mucosal lining of intestine along with damage to the mucosal wall. The intestinal lumen showed cross section in addition to degeneration, desquamation, hyperplasia and necrosis of epithelial cell lining the intestinal villi.

Table(1)*Seasonal prevalence of internal parasitic infestation among examined fishes:*

Season	Winter (n = 25)		Spring (n = 25)		Summer (n = 25)		Autumn (n = 25)		Total	
Pomatomussaltatrix	No	%	No	%	No	%	No	%	No	%
	20	80	23	92	23	92	23	92	89	89
Caranx rhonchus	25	100	20	80	9	36	21	84	75	75
Platycephalusindicus	18	72	22	88	14	56	14	56	68	68
Epinephelusaeneus	13	52	15	60	13	52	16	64	57	57
Total(n = 100)	76	76	80	80	59	59	74	74	289	72.25

Table (2): *showing heavy metal analysis of water samples*

Season	Fe (ppm)	Cu (ppm)	Pb (ppm)
Winter	0.78	0.02	1.06
Spring	1.91	0.07	1.01
Summer	zero	zero	0.043
Autumn	0.088	zero	zero
Mean	0.69	0.02	0.53

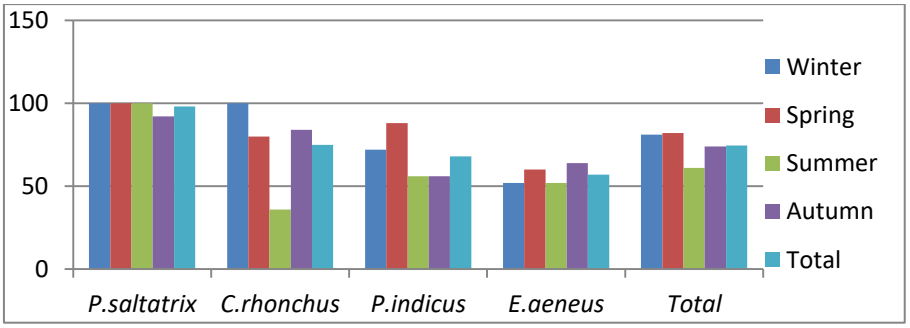

Fig (1): *Seasonal prevalence of endoparasitic infestation among examined fishes:*

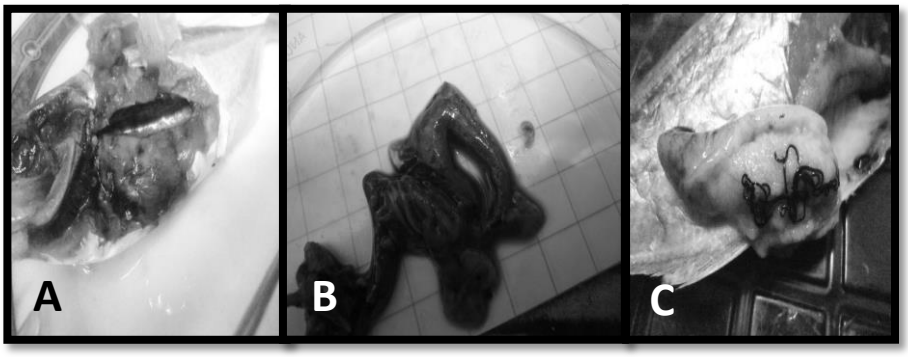

Photo (1) *a.Caranx rhonchus* showing haemorrhagic liver and intestines due to *Scolex pleuronectis* larva infestation.
b.*Caranx rhonchus* stomach and intestine showing congestion, enlargement, thickening and inflammation of their walls due to acanthocephalan infestaion.
c.Pomatomus saltatrix ovaries showing live *Philometra* spp.

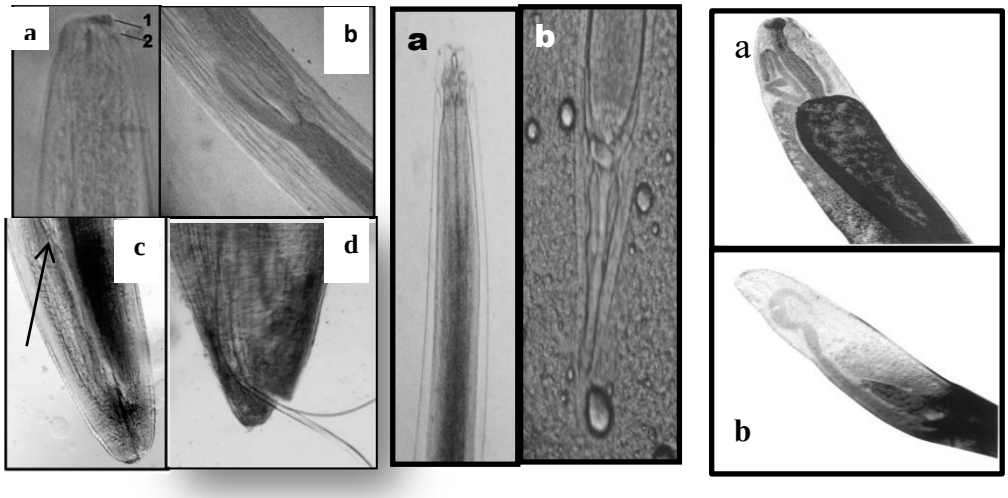

Plate (1) showing *Anisakis simplex* (L3): a.The anterior end 1.Boring tooth, 2.Excretory pore. b.Oesophagus with anterior muscular part and a glandular ventriculus .Long intestinal caeca with clear demarcation. c. Excretory duct. d. Male posterior end with a short mucron
Plate(2): Showing*Hysterothylacium aduncum: a.*Cephalic end b.Posterior end.
Plate(3): Showing*Philometra saltatrix:*, a.Gravid female cephalic end b. gravid female posterior end.

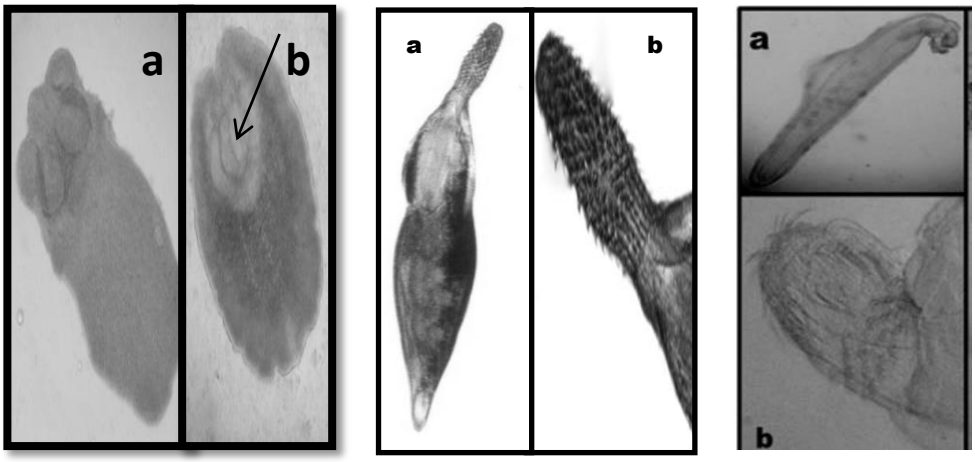

Plate (4): Showing *Scolex pleuronectis*larva with a.clear four rounded and cup-shaped sucker s. b.well differentiated bothridia (arrow).
Plate (5): *Andracantha tandemtesticulata:* a. Male, b.Prersoma (proboscis+neck)
Plate (6): *Heterosentis mongcaia*. a.Holotype female, b. Proboscis

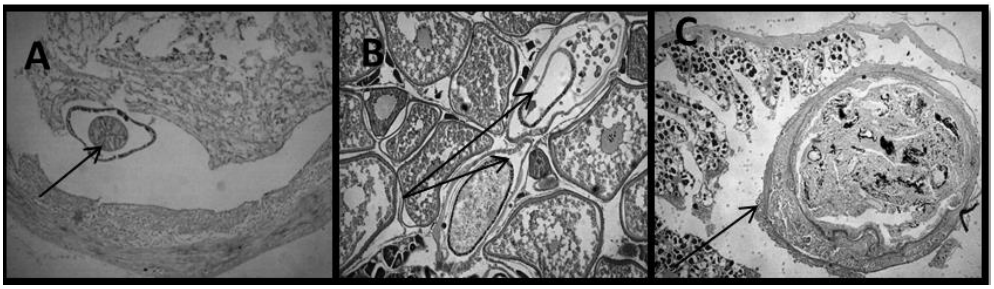

Plate (7) A. Intestine of *P. indicus* infested with the nematode showing cross section of nematode along with degeneration and atrophy of mucosa and submucosa. **B**.Ovarian tissue from *P. saltatrix* infested with *Philometra saltatrix* showing cross section of the worm along with follicular degeneration. **C.**Intestine of *C. rhonchus* infested with acanthocephala showing cross section of the parasite and ulceration with sloughed parts of mucosa.

Discussion

Marine fishesare valuable and favorable fishes all over the world butunfortunatelysuffered significant population declines.

Regarding the total Prevalence ofendoparasitic infestation, it was 72.25% which is nearly similar of ***Luque and Alves (2001)*** 76.4% and near to that obtained by ***Bayoumy et al. (2012)*** 61.25% but it was higher

than that obtained by ***Roumbedakis et al. (2012)*** which was less than 10%.

In this study, seasonal prevalence was the highest in spring (80%) this was disagreed with ***Maather El-lamie (2007)*** who recordedthe highest prevelance in winter83.1% and the lowest in summer (59%),this in agreement with ***Maather El-lamie (2007)*** who recorded 53%. But these results disagree with ***Leonardos and Sinis (2003).***The highest prevalence in *Pomatomus saltatrix* as (89%) this was in agreement ***Lora Clarke et al. (2006).*** *Caranx rhonchus* showed the highest prevelance in winter (100%) and it was the lowest in summer (36%). *Platycephalus indicus* showed the highest prevalence in spring (88%) and the lowest in summer and autumn (56%). Concerning to *Epinephelus aeneu*s the highest prevalence was in spring (60%) and the lowest in winter and summer (52%).These variations may be attributed to species difference, water temperature, site and number of fish.

The concentrations of dissolved heavy metals in water samples were 0.69 for iron, 0.02 for copper and 0.53 ppm for lead.These results exceed the ***EPA (2002)*** who recoded that iron and lead limit must be less than 0.3 and 0.05 ppm respectively while it was within the normal level for copper 0.05 ppm. These parameters were much more than that recorded by ***Mai Abd-El Azeem (2012)*** and this may be due to presence of industrial area in Port Said***, (Zahran et al. 2015).***

Concerning with iron, it was lower than those recorded by ***Saeed and Shaker (2008)*** in Mediterranean coastal area which as 1.42 ppm but it was near to that recorded by ***Goher et al. (2014)*** that was 0.57 from Ismailia Canal water.

Heavy metal concentration tended to vary significantly from season to season; this was agreed with ***Sahajrao and Rajkumar (2015).*** During winter iron was 0.78 , Cupper was 0.02 and lead was 1.06ppm.During spring it was 1.91 for iron ,copper was 0.07 and lead was 1.01ppm.During summer iron and copper were zero and lead was 0.043ppm. During autumn it was 0.088ppm for iron, zero for copper and lead.

For iron, the highest level was in spring and the lowest was in summer that disagree with ***Sahajrao and Rajkumar (2015)*** as the highest was in summer.Also, ***Shembekar (2015)*** indicated that the highest was in winter.

Heavy metals were observed in the following order Fe>pb>Cu this result is in agreement with ***Sahajrao and Rajkumar (2015), Shembekar (2015)*** and ***Zahran et al. (2015),***but disagree with ***Juan et al. (2009)*** who found that Cu >Fe, ***Zahang et al. (2010)*** who found that Pb> Fe>Cu and ***Mai Abd-El Azeem (2012)*** who found that Cu>Pb.

Presence of high level of lead and iron was one of the important factors responsible for decrease in fish immunity that was clear in spring season as iron recorded 1.91 and lead 1.01ppm. The internal parasitic infestation recorded the highest prevalence 82%.Inspite of presence of suitable temperature during autumn but internal infestation recorded lower levels which was 74% this may be as a result of very low levels of iron 0.088 ppm and complete absence of lead in water. On the other hand, during winter lead recorded 1.06ppm and iron 0.78 ppm and in spite of coldness of water the internal infestation recorded the second highest parasitic infestation among the year which was 80%.

Concerning the histopathological changes in ovarian tissue infested with *Philometra*, it was shown atretic follicles, fibrosis with degenerated follicles.These results were in agreement with ***Burak (2007)*** and much less series than those described by ***Ercument et al. (2005)*** and ***Lora Clark et al. (2006)*** who recorded hemorrhage, inflammation, edema, prenecrotic and necrotic changes, and follicular atresia.

Intestines which were infested by nematode showed destruction, necrosis and atrophy of mucosa with necrosis, degeneration of the submucosa, edematous lamina propria and submucosa with infiltrated with mononuclear cells,these results were in agreement with ***Heba Abdel-Moula (2005) and Akhtar (2008).***

In case of acanthocephalan infestation, intestines revealed desquamation of the epithelial lining, destruction of intestinalvilli and mucosal lining of intestines along with damage to the mucosal wall. The intestines showeddegeneration, desquamation, hyperplasia and necrosis of epithelial cell lining. These results were in agreement with ***Martins et al. (2001) and Snail et al. (2011).***

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الملخص العربي

أجريت هذه الدراسة للتعرف علي الطفيليات الداخليه التي تصيب أسماك المياس ،الباغا, الرقاد و الوقار المجمعه من محافظة بورسعيد في المواسم المختلفة. أعلي نسبة اصابة كانت في أسماك المياس 89% تليها أسماك الباغا 75%،الرقاد68% وأخيرا الوقار57%.الطفيليات المعزوله كانت ممثلة كلأتي ديدان اسطوانية (اناساكس سيمبلكسوهيستيروثيالوسيكم ادنيكم) من أسماك المياس والباغا والرقاد والوقار و(فلوميترا سلت تريكس) من أسماك المياس ، ديدان رأس شوكية(اندراكنثاتندمتيستكيلاتاو هتيروسينتس منجكيا) من أسماك الرقاد و يرقات ديدان شريطيه (سكولكس بلورينكس) من أسماك المياس ،الباغا ،الرقاد والوقار.نسبة الاصابة الموسمية، الصورة الهيستوباثولوجية ونسبة الحديد، النحاس والرصاص في الماء تمرصدها أيضا.