j.Egypt.net.med.Assoc 79, no 4. 1007– 1027 (2019)

RELATIONSHIP BETWEEN ISOPODS PARASITE INFESTATION AND FISH QUALITY

By

Hanan, A.A.Elghayaty[1] and Samar W.A.Tadros[2]

(Agricultural research center)Animal health research institute, Port-Said lab department.

Food hygiene[1], parasitology[2]

ABSTRACT

Isopods are permanent ectoparasite of fishes that adversely affect the fish population and so on the economic gain, therefore the present study was conducted to evaluate the prevalence and identified these parasites in some important fish species of Manzala Lake at Port-Said Governorate (Egypt) and its effects on fish quality.

From February 2016 to January 2017 a total of 323 Fish sample was collected as 100 from Mugil species and 223 Tilapia species.The total prevalence was 36.22% with the highest prevalence at Spring by 43.67%; Summer 37.03% then Autumn 35.13% and the lowest incidence in Winter 28.39%In Tilapia the total prevalence 39.46 % highest season in Spring by 46. 03% then Summer 42. 85; Autumn 37. 77% and Winter 30.50% while in Mugil spp., the total prevalence 29% The highest prevalence was in Spring 37. 5% then Summer 24% and Autumn 31. 03 and Winter 22. 72%.

Two isopods spp., were isolated from Tilapia fish, *Nerocila Orbiganyi* and *Anilorca Physodes* with a total prevalence 70%and 30% respectively and one isopoda spp.,in Mugil fish *Livonica redmanii*. The fish quality assessment revealed non-significant changes observed in total volatile base nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid (TBA) and Histamine while there was a significant decrease in protein % in Tilapia fish with non-significant decrease in Mugil fish. The sensory quality and acceptability was very low for all panelists and author regard to appearance and the aesthetic presence of parasite itself on fishes.

Keywords:

Isopods, fish quality, protein, total volatile base nitrogen (TVBN), thiobarbituric acid (TBA), trimethyl amine (TMA), Histamine.

INTRODUCTION

Fish, is measured to be one of the essential element of the world economy.It is food of excellent nutritional value, providing high-quality protein rich in essential amino acids, minerals and a non-protein nitrogen fraction that plays a major role in fish quality **(Arino, *et al.* 2013).**

Ectoparasites not only damage the fish directly but also reduce the fish growth, and induce mortalities **(Piasecki *et al.*, 2004).**

Isopoda are the largest crustaceans ectoparasites of fish (20-50 mm long), they are preferred to inhibit the warmer marine, seas **(Raja *et al.*, 2014).** Cymothoids are parasites of fish, both as immature and adults, it is the largest family among isopods, includes at least 380 species **(Smit *et al.*, 2014),** its blood feeding, Settle in the buccal cavity of fish, the gill chamber or on the body surface and fins causing destruction of host tissue **(Lester and Hayward, 2006; Alas *et al*., 2008**; **Ravichandran *et al,* 2010 and Samn *et al.*, 2014)**, act as disease vectors **(Smit *et al.*, 2014)**. They feed on blood and macerated tissues **(Woo, 2006)**, causing anemia, reduction of fish quality, deaths in small fish and economic losses **(Bunkley-Williams *et al*., 2006 and Raja *et al.*, 2014).**

The term "quality" usually discusses as the aesthetic look and freshness degree of the fish also it involves safety aspects as being free from parasites, harmful bacteria,or chemicals **(Pearson, 1976, Gram and Dalgaard, 2002 and Venugopal, 2002**). Fish must be marketing free from parasites for human consumption **(EC, 2004).**

The quality of fish can be estimated by sensory, microbial or chemical methods and the human assessment considered the most, fastest and accurate technique **(Huss 1995, and Cesaretin *et al.*,2011).**Otherwise,itcan be used in collaboration with chemical assessment suchas measuringvolatilecompounds andhistamine**(Gulsun *et al.*, 2009).**

Volatile compounds are the distinctive particles responsible for the fishy odor and flavor present in fish several days after the catch, used as criteria for assessing the fish quality.

(Wu and Bechtel 2008; Amegovu *et al.*, 2012).

The amount of Trimethyl amine (TMA) and the Total Volatile Basic Nitrogen (TVB-N) are most commonly used for assessing the degree of quality in fish **(Pearson, 1976, Stamen1990, Castro *et al.*, 2006, Howgate, 2010 and Yusuf *et al.*, 2010).**The limit of acceptability, of (TVB-N) in fish muscle is usually ranged from 35-40 mg N /100 gm. increased than it

considered as spoiled, **(Lakshmanan, 2000).** Trimethyl amine (TMA) (produced by spoilage bacteria); a range between (10-15 mg/100 gm.) is the limit of acceptability, for, whole chilled fish as documented by **(Connell, 1995)**.

Thiobarbituric acid (TBA): It is determined as an index of oxidative rancidity in fish, **(Pearson *et al.*,1983;Regenesteinand Regenestein,1991)**.Biogenic amines such as histamine, are of importance due to the risk of food intoxication and also they serve as chemical indicators of fish quality **(Lehane and Olley, 2000, Kim *et al.*, 2009).** A hazardous level of histamine for human health has been suggested as 500 mg/kg although low levels as 50mg/kg (50 ppm) have been reported in histamine poisoning **(FDA, 2001, Huss *et al.*, 2003).**

The nonstop growing attentiveness by consumers and public food authorities as to the possible presence of parasites or parasite-related quality defects in fish highlights the demand of providing the fish processing industry with the latest information on the incidence, detection, and control about the most important parasite species of fish.So this study was carried out to identify isopods spp. that had affected some fishes of EL-Manzala Lake at Port-Said Governorate with concerning to its quality.

MATERIAL AND METHODS

A total of 323 fish including (223Tilapia species and 100 Mugil species) were captured from El-Manzala Lake at the periods from February 2016 to January 2017. Fish were transferred to the laboratory and observed for external parasites, lesions and quality.

Parasitological examination:

The isopods attached on the skin, fins or gills were collected, counted and preserved in 70% ethanol **(Bunkley - Williams *et al.*, 2006)** all collected fishes were clinically examined according the methods described by **Noga, (2010).** The morphological details of isopods spp. were examined using dissecting microscope. All measurements were done in millimetres and photographed using digital camera. The morpho-metric characters and classification of isopods spp., were done according to **Brusca, (1987) and Williams and Bunkely, (2003).**

Fish quality assessment:

A-Sensory analysis.

Sensory analysis was evaluated according to **(EOS: 3494/2005)** for chilled fresh fish using a panel of 10 panelists on each day of sampling. They were asked to evaluate the acceptability regarding to total appearance, odor, and color. Each evaluator was given scoring points from

0 to 5 Where 0 represented very poor quality 1, poor; 2 moderate; 3 good, 4 very good and any higher score indicated excellent quality.

B-chemical quality assessments:

1-Preparation of sample (EOS/ 2760-1:2006): About 250 grams of back muscles from the fish were collected, chopped and minced by a grinder, and kept freezing in a sealed container for chemical analysis.

2-TotalVolatileBaseNitrogen (TVN-B): according to **(EOS/ 2760- 1:2006).**

3- Trimethylamine (TMA): according to **(EOS/ 2760 - 1:2006).**

4- Thiobarbituric acid (TBA): according to **(EOS/ 2760-1:2006).**

5-Histamine: was analyzed using Ridascreen® Histamine (R- biopharm, Darmstadt Germany) kit. Preparation of fish samples and test procedure were carried out according to its instructions.

6-Protein % estimation: according to the **(AOAC, 2012).**

7- Statistical analysis: Data were presented as mean ± standard error and analysis of variance (one way ANOVA) the data was subjected to T test for significant differences ($p < 0.05$).

RESULTS

1-Clinical examination:

Fish affected with isopod (Tilapia and Mugil spp.,) suffer from severe haemorrhages, gill erosions, the isopods were severely attached leaving sever skin ulceration.

Unilaterally or bilaterally protrusions of gill cover, due to infestation by large isopod.

Two species of isopods were detected in Tilapia **(*Anilocra physodes* and *Neroclia orbignyi)*** and one species in Mugil **(*Livoneca redmanii*).**

Taxonomy of isopod species:

According to **Worms (2013),** all parasites belong to the family Cymothidae, superfamily, Cymothoodae, suborder, Cymothida; order Isopoda, superorder Peracarida, subclass Malacostraca and subphylum Crustacean of the phylum Arthropoda**.**

1- *Anilocra physodes*: (Linnaeus,1758) - Fig (3-A, and **4-E).**

Body: Is narrow and elongated with slight dorsal convexity measuring 13.2-16.4 mm long and 5-8mm width mostly pale creamy although some specimens was dark brown, with a **Cephalon:** narrow anteriorly and projected ventrally between the antennae of 9 segments and the antennules extends posterior to the eyes which are comparatively large and prominent. **Pereon:** consists of 7 segments in length and width progressively increase toward posterior.

The pereopod 7th is longer than the 6th and markedly produced.

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

2- *Nerocila orbignyi* (Guérin-Meneville, 1829-1832) Fig.4 (A, C, D).

It is (14.3-1 7.5 mm long and 5.5-9.5 mm width). Cephalon: Anterior margin has an indistinct medial point. Eyes are small. Articles I and II of antennule are partly fused; antenna consists of 11 articles.

Pereon: Pereonites I-IV posterolateral angles are not produced while pereonites V-VII posterolateral angles are produced and acute.

Pleon: Pleonite I is the longest. The ventrolateral margins of pleonites I and II are posteriorly directed and extend to pleonite V. Pereopods 6 and 7 are subequal in size, each with carina on mediolateral margin. Uropod exopod is curving medially and is about 1.5 times as long as endopod. Endopod is straight and its distal margin is obliquely truncate

***Livonica redmanii* (Leach, 1818): Fig: (2- D,Q) (3-B)** and **(4-B)** detected in Mugil spp., the body: is ovate and mostly twisted to one side, light brown with dark chromatophores measuring 18.3±2.30 mm in length and 7-12 mm in width.

Cephalon:

Is not projecting between the bases of the antennae. It`s Posterior margin appears trilobed. One pair of eyes is located laterally. 2 pairs of antennae appear.

Pereon: Composed of 7 segments, the last two appears narrower pereopods.

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.

Prevalence and seasonal dynamics. Table (1): 117 fish were infested from 323 examined with total incidence (36.22%). the highest season of infestation with isopoda was in spring (43.67%); summer (37.03%); autumn (35.13%) while the lowest was in winter (28.39%). Concerning with Tilapia spp., the highest season of infestation was in spring (46.03%); summer (42.85%); autumn (37.7%) while the lowest in winter (30.50%). While in Mugil spp., was in spring (37.5%); autumn (31.03%); summer (24%) and the low infestation was in winter (22.72%).

Quality assessment:

Sensory analysisy.

According to all panelists and authors the acceptability of the fish samples were much decreased related to appearance, lesions and founding of parasites itself (Table 3) and Fig.,1(A,B,C,D,E,F,G,H,I,J).

Chemical quality:

The results of Total volatile nitrogen (TVB-N), thiobarpituric acid (TBA) Trimethylamine (TMA), and the histamine level and protein % are seated in (Table 4).

Table (1): Seasonal and total prevalence of Isopods infestation in the examined fishes.

Season	Tilapia spp.,			Mugil			Total		
	No. examined	Infested		No. examined	Infested		No. examined	infested	
		No.	%		No.	%		No.	%
Winter	59	18	30.50	22	5	22.72	81	23	28.39
Spring	63	29	46.03	24	9	37.5	87	38	43.67
Summer	56	24	42.85	25	6	24	81	30	37.03
Autumn	45	17	37.77	29	9	31.03	74	26	35.13
Total	223	88	39.46	100	29	29	323	117	36.22

Table (2): Isopods spp., isolated from examined fish the site and % of attachment.

Isopods	Tilapia fish	Mugil	Attachment location	%
Nerocila orbigynii	+	−	**Gill, skin, fins**	**70%**
Anilorca physods	+	−	**skin, fins**	**30%**
Livonerca redmani	−	+	**Gills**	**100%**

Table (3): Sensory quality evaluations of isopods infested fish means± standard error.

parameter	Non-infested	infested
color	4.7±0.33	3.3±0.11
odor	4.9±0.25	3.9±0.33
appearance	4.5±0.6	1.9±0.63*
acceptability	4.9±0.011	1.5±0.6*

(*) of infested fish indicate significant difference at *P*< 0.05.

Table (4): Biochemical Assessment Result of Isopods infested fish means± standard error.

Fish sample / parameter		TVB.N	TMA	TBA	HIST.	P %
Tilapia	Non infested	25.2± 0.1	19.83± 2	3.9± 0.5	5.116± 0.3	24.2±1.2
	Infested	33.6±0.5	20.84±1	3.9±1.5	5.48± 0.2	17.4±2.1*
Mugil	Non infested	23.8±1.7	21.84±0.5	4.2±0.01	3.42± 0.08	21.8±6.3
	infested	28±1.3	25.4±0.1	3.1± 0.5	5.297± 0.6	19.95±4.2

TVB-N= total volatile base nitrogen.

TMA= trimethylamines.

TBA= thiobarpituricacid.

Hist. = histamine.

P % = protein %

*** Of infested fish indicate significant difference at *P*< 0.05.**

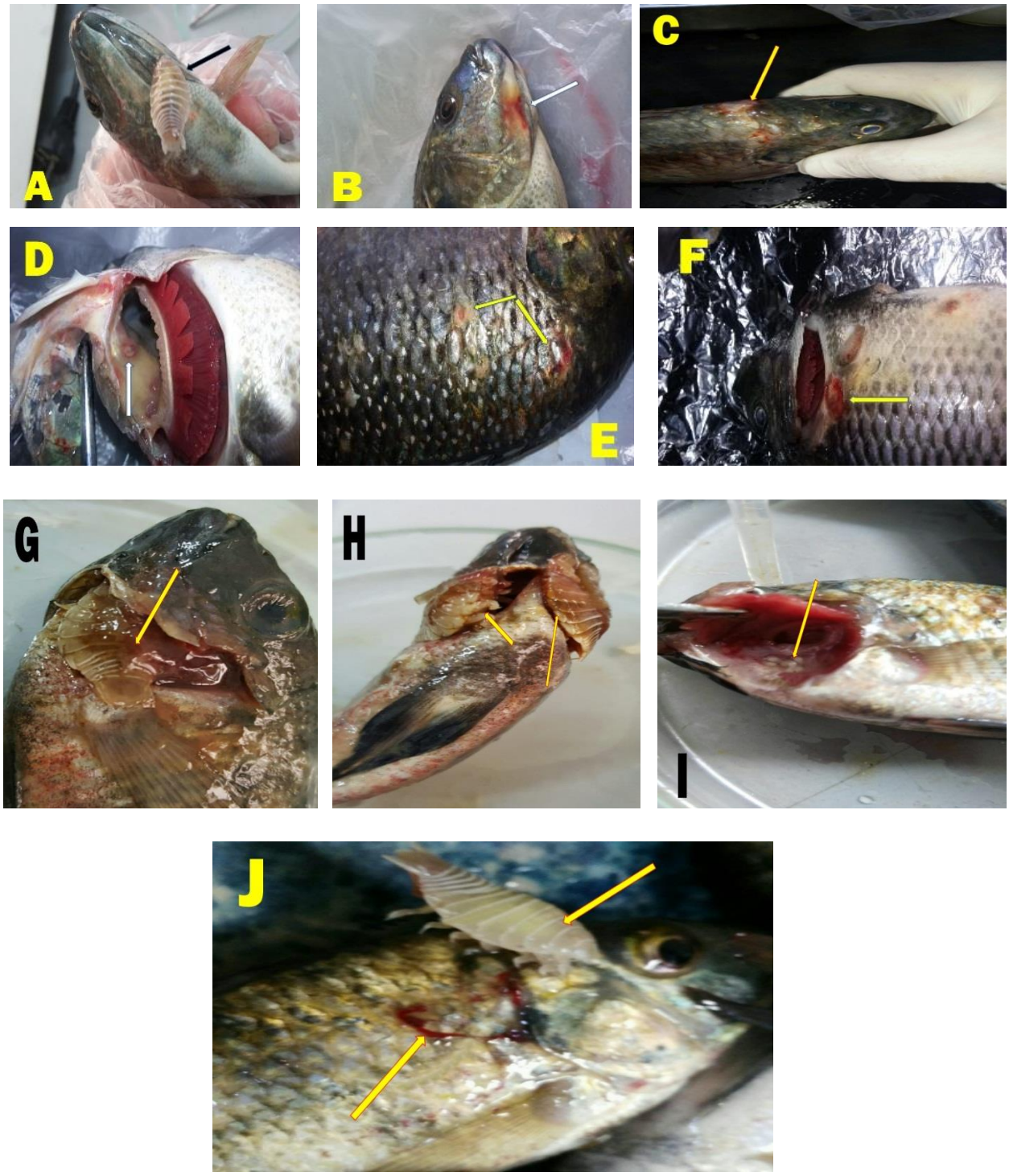

Fig. (1): Tilapia fish spp., infested with isopods **(A)** with inflammation and ulcer lesions **(B,C,D)** ulcers and wounds **(E,F)** excessive mucus and hemorrhage **(G,H)** the eggs of the isopods laying under operculum **(I)** and **(J)** Tilapia fish infested with *Nerocila orbignyi* causing sever hemorrhage.

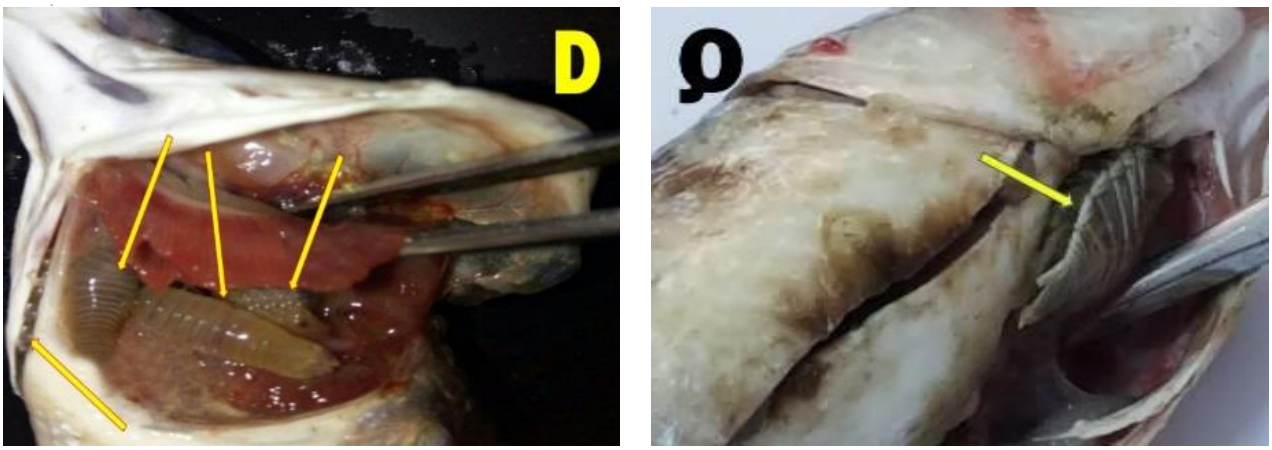

Fig. (2): Mugil spp., fish infested with isopods *Livonica redmanii*.

Fig. (3): A (Dorsal and ventral view of *Anilorca physodes*), **B** (*Livonica redmanii*).

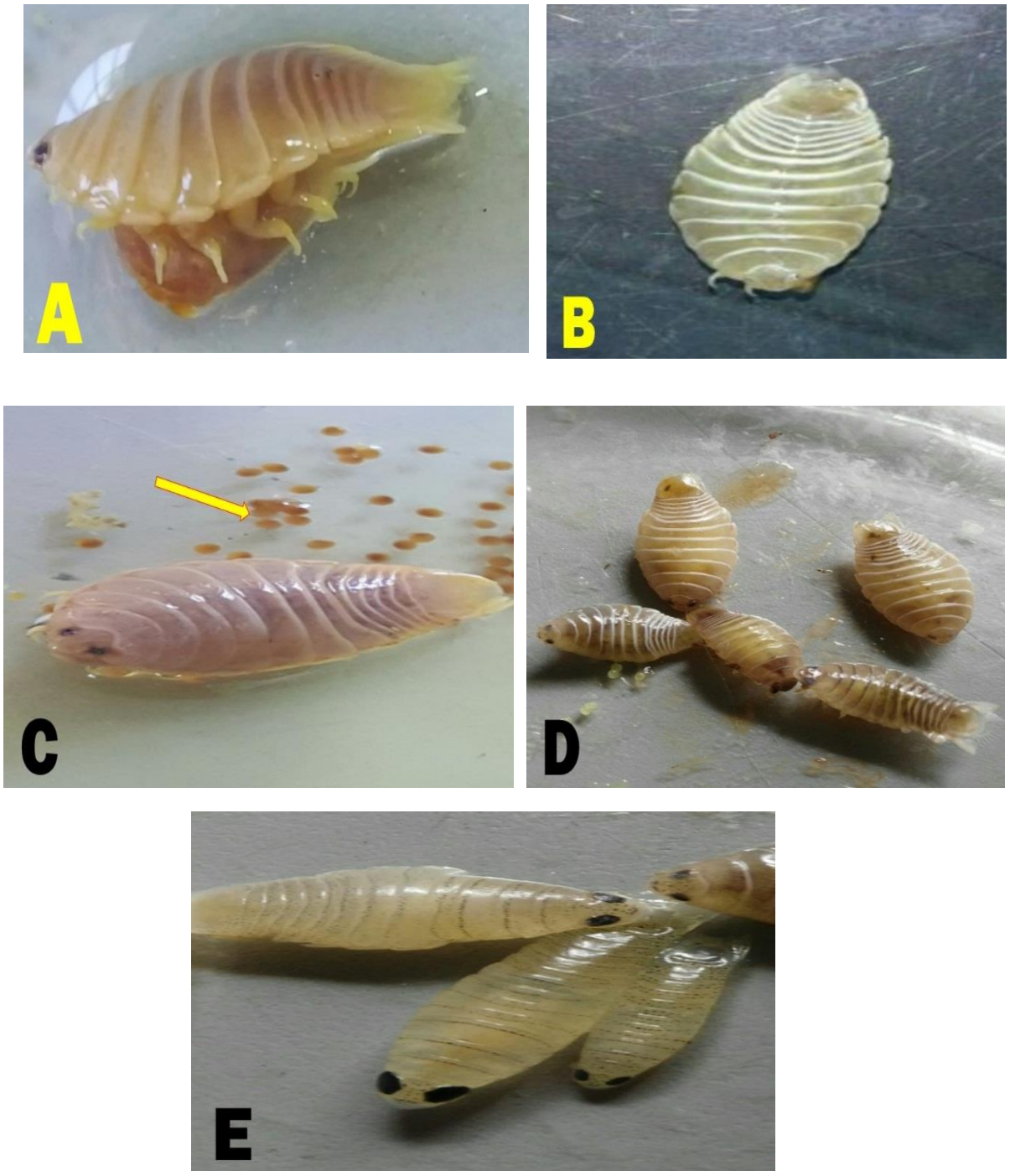

Fig. (4): Different isopods spp.isolated from Tilapia and Mugil fish:**(A)** ***Nerocila orbignyi*** female with miracipum**,(B)** ***Livonica redmanii*** male**,(C)** ***Nerocila orbignyi*** female with eggs**,(D)** ***Nerocila orbignyi*** male, female and juvenile**, (E)** ***Anilorca physodes***, male, female and juvenile **.**

DISCUSSION

Infested fish with isopoda was suffer from haemorrhage, ulcer and erosion of skin, gill filament inflamed and heavy mucus and the gill cover protruded due to embedded of large isopod parasite (unilateral or bilateral) this result agreed with **Dos Santos Costa and Chellapa (2010); Smit *et al.*, (2014); Eman Youssef *et al.*, (2014); El-Lamie and Abdel Mawla (2015); Mahmoud *et al.*, (2016).**

The total infestation with isopoda species was (36.22%) as 117 fishes were infested from 323 examined. fish collected from Lake El-Manzala ,Port-Said Province which nearly agreement with **Mahmoud *et al.*,(2016)** from Lake Qarun 32.66% and **Baradhirajan *et al.*,(2014)** in Parangipettai waters South east of India (34.19%). Higher than **Samn *et al.*, (2014)** from Abu Qir Bay, Alexandria; and **El-Lamie and Abdel Mawla (2015)** from Seuz Governorate as 10.26% and 22.6% respectively that is lower than **Shaheen *et al.*, (2017)** 50.7%; **Helal and Yousef (2018**) 46.7% and **Mahmoud *et al.*, (2017)**, 46.5% at Lake Qarun.

Regarding to seasonal dynamics, (Table 1) the highest prevalence was in spring (43.67%); summer (37.03%); autumn (35.13%) while the lower season was in winter (28.39%). This result disagree with **Mahmoud *et al.*, (2017)**; **Shaheen *et al.*,(2017)** in Lake Qarun and **El-Lamie and Abdel Mawla (2015)** from Seuz Governorate who detected higher season in Summer 73% ;82.5% and 30.2% respectively while agreed with them that, the lower season was in Winter as 28% ;20% and 11.6% respectively. The variation might be attributed to differences of examined fish species, geographical of fish hosts and parasites and periods of investigations.

Anilocra physodes morphologically agreement with **Ramdan *et al.*, (2007); Throrsen *et al.*, (2000); Mohmoud *et al.*, (2017) and Oktener *et al.*, (2018).**These detected as 30% in gill chamber and skin of Tilapia spp., which agreed with **Ramdan *et al.*, (2007)** from body of Spondyliosoma cantharus fish by prevalence 33.33% in Gulf of Jijel. **oktner *et al.*, (2018)** who examined *Anilocra physodes* according to family characteristics,28% belong to Sparidae, and %30 to Carangidae,Mugilidae,Centracanthidae, Sciaenidae,Mullidae, Scorpaenidae.

But disagreed with **Innal *et al.*,(2007**) who obtained that,the prevalence of ***Anilocra physodes*** was 4.76; 2.86 and 0.85% in Diplodus annularis; Sphyraena chrysotaenia and Liza aurata respectively.

Nerocila orbignyi detected by the prevalence of 70 % (Table 2) in skin, and gill of Tilapia spp., and morphologically agreement with **AL-Zubaidy and Mhaisen (2013),** who detected in Mugilid species (Moolgarda schili and Liza aurata) with infestation rate of 4.2% and 7.7% respectively in Yemen. Also it agrees with **Noor El-Deen *et al.*, (2013)** who isolated from D. Labrax with low infestation rate 6%. **Mahmoud *et al.*, (2016)** detected *Nerocila orbignyi* from skin of Solea vulgaris fish by prevalence of 18% at Lake Qarun. Also this parasite observed in gill chamber of T. zilli according to **Younes *et al.*, (2016) and Abdel-Latif (2016).**

Livonica redmanii is the only isopod detected in Mugil spp., by infestation rate of 100%. Identified according to **Brusca (1981) and Mark *et al.*,(1996).**The result agree with **Lima *et al.*, (2005)** when register a prevalence of 86% of *Livonica redmanii* in bronchial chamber of S. brasiliensis in the coastal waters of Rio Grande do Norte, Brazil, **Dos Santos Costa and Chellapa (2010),** in the costal water of Ponta Negera, Rio Grande Norte, Brazil but with low incidence 5.9% in the branchial chamber of C. Chrysurus.**Mahmoud *et al.*, (2017)** obtained it from branchial cavity of Mugil capito and D. labrax fish from Lake Quarn. Also **Helal and Yousef (2018)** who detected this isopod in gill (unilateral and bilateral) in Mugil cephalus fish by percentage 98% per hosted fish.

Sensory qualification (Table 3): the various sensory characteristics, as appearance, odor, or color are still very important points in fish quality assessment **(Alasalvar *et al.*, 2001).** The present study revealed that isopods can be detected by naked eyes attached on fish skin, fins, operculum, or impeded on gills filaments Fig. (1, 2). It is possessing adhesive organs and mouth parts adapted for piercing and sucking fish blood and macerated tissues causing inflammation, abrasions and wounds at the site of attachment on skin or Hemorrhage on gill and excessive mucus Fig.(1, 2) our finding agreed with many previous studying **(Kabata, 1985,Noga,2000.,Ostlund Nilson *et al.*, 2005;Grutter, *et al.*, 2008 El-Moghzy, 2008, Khalil *et al.*, 2014 Ravichandran and Rameshkumar, 2014 and EL-gendy, *et al.*, 2018)** the hook of pereopod penetrate into the skin to hold the parasite to the host body surface causing serious wounds, and the movement of isopods to feed cause inflammatory reaction and stimulate mucus production. According to sensory analysis results the presence of isopods parasites in fish severely reduced its quality due to its aesthetic appearance which agree with **(Levsen *et al.*, 2008; Zhu, *et al* 2011 and Dewi *et al.*, 2018).** Our finding disagree with

(Landau *et al.*, 1995; and Ostlund-Nilsson *et al.*, 2005) they reported no obvious harmful effects observed duo to isopods in fishes and it may be comes from different fishes or parasite species.

From the chemical quality aspect, (Table 4): The effect of isopods infestation on these parameter not obvious as the result indicating non-significant differences between infested and non-infested fish samples the result revealed that TVN.in isopods infested fish was 33.6 and 28 in Tilapia and Mugil fish spp., respectively and 25.2 and 23.8 in non-infested fish which considered within acceptable level established by the Egyptian Standard Organization which it is ranged as (25-35 mg TVB-N/100g fish muscle) **Reilly *et al*.,1985** stated that TVN are not reliable as indices of fish quality. During post harvesting (TMA-O) break down producing TMA which lead to significance loss of fish quality our study revealed TMA level 21.84 and 25.04 in infested fish and 19.83, 21.04 in non-infested fish respectively with non-significant differences. TMA always related to the pungent odor of fish and produced usually due to action of putrefaction bacteria and endogenous enzymes **(Connel, 1995; Yusuf *et al.*, 2010)**.Thiobarpituric acid (TBA) is considered as a helpful indicator for the degree of lipid oxidation and assessing fish freshness and usually expressed as mg malonaldehyd/kg fish muscle **(Tokur *et al.*, 2006)** it is increased significantly with storage time **(Talidauau *et al.*, 2003)**.The Egyptian Standard Organization has directed that,the permissible limit of histamine content in fresh fish shouldn't be increased than 10 mg/kg., and our results revealed histamine within permissible limits.

From the result seated in (Table 4) the protein % decreased significantly in Tilapia infested compared to parasites free fishes from 24.2±1.2 to17.4±2.1while in Mugil fish the infested fish protein level were reduced even non-significant, from 21.8±3.6 to 19.95±4.2 our finding is agree with **(Barber *et al.*, 2000; Barker *et al.*, 2005; Rameshkumar, and Ravichandran, 2013; and Rajaran *et al.*, 2018).** It may be due to that isopods are blood feeders which dependent on their hosts for nutrition, also it affects the food ingestion by the fish. In general parasites causes decrease of organic constituents such as protein **(Love, 1970)**

CONCLUSION

1- Isopods parasites infect Tilapia and Mugil species fishes at El-Manzala Lake and causing serious effects on fish.

2- Chemical quality of fishes not affected by Isopods and it may needs more investigation.

3- Isopods parasites affect sensory quality of fishes so unacceptable from consumers lead to decreasing marketability and so economic losses.

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العلاقة بين تواجد طفيل الأيزوبودا و جودة الأسماك

حنان عباس أحمد الغياتي [1],سمر وليم عبد الملك تادرس[2]

(مركز البحوث الزراعية) معهد بحوث الصحة الحيوانية-معمل بورسعيد[1]قسم صحة الأغذية, الطفيليات[2]

الملخص العربي

يعتبر طفيل الايزوبودا (متماثلات الارجل) من الطفيليات الخارجية للاسماك والتي لها تأثير عكسي علي نمو الاسماك وبالتالي مردود سئ علي الاقتصاد ولهذا اجريت هذه الدراسة لمعرفة مدي انتشار هذا الطفيل في بعض الانواع المهمة من اسماك بحيرة المنزلة في محافظة بورسعيد (مصر) ودراسة أثره علي جوده الاسماك المصابه ولهذا تم تجميع عدد 323 من العينات منهم 100 من اسماك البوري و223 من اسماك البلطي في الفترة من فبراير 2016 حتي يناير 2017 وأسفرت النتائج ما يلي كانت نسبه الاصابة الكلية 36,22 % بأعلي نسبه في فصل الربيع 43,67 % يليه الصيف 37,03 ثم الخريف 35,13% وأقل نسبه أصابه فصل الشتاء بمعدل 28,39 % وكان معدل الاصابه في اسماك البلطي 39,46% واعلي موسم لها فصل الربيع 46,53 % -يليه الصيف 42,85 –ثم الخريف 37,77 والشتاء 30,50 بينما مثلت نسبه الاصابة في اسماك البوري 29%وجاء الربيع اعلاها بنسبة 37,5 % يليله الصيف 34% ثم الخريف 31,53% ثم الشتاء 22,72% وقد تم العثور علي نوعين من الايزوبودا في اسماك البلطي هما نيروسيلا أو ربيجاني (من الخياشيم وعلي الجلد) ومثلت 70% من نسبه الاصابة الكلية وأنالوركا فيوسديس (علي الجلد والزعانف)بنسبه 30% اما في اسماك البوري فتم العثور علي نوع واحد فقط في الخياشيم بنسبه 100% هو ليفونيركا ريدماني .

أما من ناحيه تأثير الاصابه بطفيل الايزوبودا علي جوده الاسماك فان الاصابة لم تحدث تغيرات معنوية في مستوي كل من المركبات النيتروجينية الطياره الأمين ثلاثي المثيل , حمض الثيوباربتيوريك والهستامين ولكن أظهرت نسبه البروتين نقص معنوي في اسماك البلطي وكان النقص غير معنوي في اسماك البوري

وبالرغم من ذلك فإن الطفيل له تأثير سلبي علي جودة الاسماك الحسية من حيث الشكل والعلامات الظاهرة من جروح وانزفه والافرازات المخاطية المدممة علي الخياشيم ووجود الطفيل نفسه في الاسماك .