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Original Article

Some studies on plerocercoid of *Schistocephalus sp.* affecting Kawakawa (*Euthynnus affinis*) fish

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Abstract:

A total of 212 Kawakawa (*Euthynnus affinis*) fish is a descriptive name and it's called with "Balamyta" in the Egyptian markets. From which,108 fish (group a.) were frozen as 65 fishes under the weight (wt.) of 3kg/fish, 43 over such weight. The rest 104 fish (group b.) collected freshly dead as 62 fishes under the wt. of 3kg/fish, 42 fish over such weight . Fish were collected from Dakahlia and Damietta markets during the period from Nov. 2014 up to April 2015 and also, through the period from May 2015 up to October 2015 for the frozen and freshly dead fish respectively. The study elucidate the presence of an intermediate host (plerocercoid larvae) of a genus, Schistocephalus as a cestode (tape worm) within the family Diphyllobothridae affecting Kawakawa (Kaw.) fish. Plerocercoid larvae were isolated from the abdominal cavity of the affected fish. Case of the affected frozen fish under the wt. **3**kg/ fish revealed **3.07%** and over the wt. **3**kg/ fish showed **9.30%**. While under the wt. **3**kg/ fish was **4.83%** and over the wt. of **3**kg/ fish showed **11.90%** in the freshly dead fish. Musculature, internal organs and tissues in the abdominal cavity of the affected freshly dead fish were showed "fried" and dark brown colour. Histopathological findings revealed multiple deposits of haemosiderin as spots within the liver of the affected freshly dead fish and vacuolation of the muscle fibers. Besides,scanning electron Microscopy (SEM) exhibited the larval morphological outline which clarify the plerocercoid Schistocephalus sp. of the family Diphyllobothridae.

Key words: Kawakawa fish-Schistocephalus-Euthynnus affinis-Balamyta-SEM.

INTRODUCTION

One of the coastal species are the Kaw. fishes (*Euthynnus affinis*) which is identified by ***William and Earl, (1983)*** as a medium sized tuna and has had a blue back lines on the upper part of the body, it directed backwards and upwards. The belly is silvery white in colour. A few clear black spots are distributed on both sides of the body confined between the pectoral and pelvic fins.

Kaw. fish is living in temperatures ranging from 18-29^{o}C of waters. It is widely distributed in the tropical and subtropical waters of the indopacific region. So, money making occur during the fishing season through catching fishes ranging from 40-60cm in length. Mackerel tuna was catched, whenever

growed up to 100cm fork length (FL) and about 20kg in weight. Generally, it was catched around 60cm and 3kg .(***Collette and Nauen, 1983).***

The pathogenic effect of plerocercoids of ligula is associated with marked basophilic cells granulation in the middle glandular region of the pituitary gland ***(Arme,1975; Smyth and McManus, 1989).*** Accompanied with suppress in the growth of the gonads. This phenomenon has no clear mechanism confined.

Order Pseudophyllidae with a progenetic plerocercoids contain the general *Schistocephalus and Ligula*. These species have been received an special attention *in vitro* experimental work as it was used by the Danish worker Peter Abildgaard since 1970 which tried to conduct the first prove of a parasite life cycle, through feeding sticklebacks infected with plerocercoids to ducks. After several days autopsy revealed adult worms and also he wrote the transmission of the parasite from one host to another ***(Smyth, 1990 a, b).***

***Collette, (*2oo1)** was informed that the mackerel tune (*Euthynnus affinis*) have been related to the family Scombridae which includes 15 genera and about 50 species of epipelagic and a highly migratory species. The peak fishing seasons from April to November on west coast and from June to August on east coast areas . It contains species of a high economic values in which involved in food and marketed fresh, frozen and canned; also utilized dried, salted and smoked.

Pillai and Mallia, (2007) were reported that the environmental habit of Kawakawa populations of scanty knowledge, So, further investigations are needed to understand and evidence toward the fish behavior as one of the tuna fishes which had been collected in populations like aggregation. Althought, Fish Aggregation Devices (FADs) are structures identified that Kawakawa fishes moved at the surface or at midwater depths to get the advantage in picking of pelagic to fishes as a floating objects .

Roberts and Agius, (2003) were reported that any possible foreign material, microbial origin agent and metabolic waste products such as ceroid or haemosiderin selectively centres the circulating macrophages which considered as a metabolic dumps and then referred by melanomacrophages .These metabolic dumps represented as a collar of lymphocytes. The melanomacro-phages centres varied in degree according to the species .They were appeared as clusters of dark cells circulated the haemopoietic tissue. Also the amount of melanisation differ according to ages. The clusters were showed dark brown or black and have the same chemical and biochemical properties of melanin.

Many studies on the parasite schistocephalus sp. host range relationship were applied in which had been concluded that they more complex ***(Poulin and Morand, 2004; Miura et al.2006; Poulin and Keeney, 2008)*** .

Host specificity was the main obstacle faced who worked on the *Schistocephalus sp.* parasite due to the ecological and the physiological factors in which either the capability of the parasite overcome the host immune defense and infection occurs ***(Van Baarlen et al.,2007)*** or the parasite fail to develop and transmitted to another host ***(Combes,2001and Randhawa et al., 2007)***.

Helminth parasites like a schisto-cephalus solidus and schistocephalus pungitii were they characterized by a higher degree of host specificity and a complex life cycles ***(Nishmura et al., 2011)*** .Tape worm *Schistocephalus solidus* infections were inhibite the reproduction of the sticklebacks ***(Yitashao, 2012)***.

Individual plerocercoid may produce an extensive infection and a large size multiple infections leading to deprivation of host nutrients ***(Roberts, 2012)***. Moreover, in toxaemic conditions the melanomacro-phages centres of haemopoietic tissue were ruptured. Consequently, pigment granules sequestrated in blood .These pigment granules engulfed by macrophages formed dark granules inside their cytoplasm. While in haemolytic conditions breakdown of erythrocytes leads to deposition of haemosiderin in the melanomacro-phages and readily identified by Perl's Prussian blue staining technique.

Evidences of immune manipulation mediated with *Schistocephalus solidus* excretory and secretory products ***(Scharsack et al., 2013)***

The aim of this study was planned to investigate the prevalence of main parasites harbour the Kaw. fishes either frozen or freshly dead specimens with special respects to plerocercoid larvae in which the histopathological findings were done to study the effect of parasites on this fish . Sections for Scanning Electron Microscope (SEM) was carried out on plerocercoid.

MATERIAL AND METHODS

1- Specimens collection:

A total of 212 fish were collected , from which 108 (group a.) were frozen as 65 fish under the weight of 3kg./fish, 43 fish over the weight of 3kg./fish and 104 fish (group b.) were prepared freshly dead ,62 fishes under the weight of 3kg./fish, 42 fish over the weight of 4kg./fish. They were collected from the fish markets at Dakahlia and Damietta governorates during the periods from Nov. 2014 up to April 2015 for the frozen (group a.) and from the period from May 2015 up to October 2015 for the freshly dead (group b.) specimens respectively. The frozen fish exposed to thawing before examination. Both types (frozen and freshly dead) of fish specimens were weighted and the corresponding features were recorded to reach the diagnostic characters of the fish samples as divided into two weights under or over 3kg./fish.

2- Fish identification:

The collected fish samples during this study were identified according to ***William and Earl (1983)***.The fish (fig.1) were silvery white in colour dark blue back with oblique striped lines (Q) does not extend forward and many dark spots (S) distributed between the pelvic

and pectoral fins. The fork length (FL) of the examined specimens ranged from 20-25cm in length.

3- Specimens preparation:

The collected fish were necropsied through an incision with a scissor along the abdominal wall from the gills to the anal orifice. The abdominal cavity, internal organs, musculature were exhibited to parasitological and pathological examinations. The examination of plerocerocoid larvae were removed from the entire cavity and extracted from the adjacent organs and musculature.Each was transferred into petri dish containing normal saline for examination under a dissecting micro-scope.

a- Plecrocercoids procurement and preservation:

1-Scanning Electron Microscopy (SEM): According to ***Colwell and O'Connor (2000),*** visible sample of plerocercoid were fixed immediately overnight at 4^{o}c in 2.5% glutraldehyde solution in 0.1 phosphate buffer (PH 7.4) for 30 minutes. Samples were washed in the same phosphate buffer, then fixed in 1% osmium tetroxide (OSO4) for one hour and rewashed in phosphate buffer. Samples were dehydrated in ascending grades of ethanol (70 to 100 %), followed by a mixture of absolute ethanol and propylene oxide (1:1) and then in100% propylene oxide. Specimens were embedded in Epson resin and polymerized for 48 hours in 60^{o}c.The samples were examined with a high resolution JOEL (JSM-8510 Lv) at the Electron microscope unit, Faculty of Agriculture, Mansoura University.

2-Histopathological technique for examination of tissues:

2-1.Haematoxyline and Eosin staining technique: Histopathological sections obtained from the infected tissues(liver, musculature and abdominal cavity with larvae) were immediately removed out, fixed in 10% buffered formalin and then transferred into ascending grades of ethyl alcohol followed by clearing in methyl benzoate. Specimens were shortly washed in benzene, then embedded in paraffin wax (56^{o}c). Sections of 5-7 m were cut on rotatory microtome and stained with haematoxyline and Eosin and examined by light microscope according to ***Bancroft and Stevens, (1996).***

2-2.Perl's Prussian staining technique according to ***Denza and Barbara, (1973).***

Staining method for showing non –haemoglobin iron particulate in erythrocytes, macrophages, normo-blasts, and other cells. The result of staining appears bright blue wherever iron particle (ferric form) present, and the other tissue cells emerge red nuclei and pink cytoplasm.

b-Plerocercoid description:

The collected plerocercoid larvae were measured for the mean of lengths, widths, classified and identified according to ***Theodore et al.(2008).***

4- Statistical analysis:

All statistical analysis Student-test after ***Steel and Torrie (1980)*** to determine the significance of differences between groups.

RESULTS

Kawakawa history array:

A discussion with a seller and fishery were supplied with a considerable amount of data to understand the fish population seasons (whenever, is it freshly or frozen fish) behaviors and to study the prevalence. Fishermen described the method of catching the kawakawa and some other species of scombroidae fish with a "shinshela types".

Fig.(1):Kawakawa (*Euthynnus affinis)* fish.

A prevalence of the parasitic plerocercoid during this study was identified as a plerocercoid of the tape worm *Schistocephalus* which belonging to the family Diphyllobothridae were observed and classified according to ***Theodore et al. (2008)*** which were eventually parasitizing the Kaw. fish. They were found occurs in the abdominal cavities, musculature and adjacent to the internal organs (plate I: photo a and b).

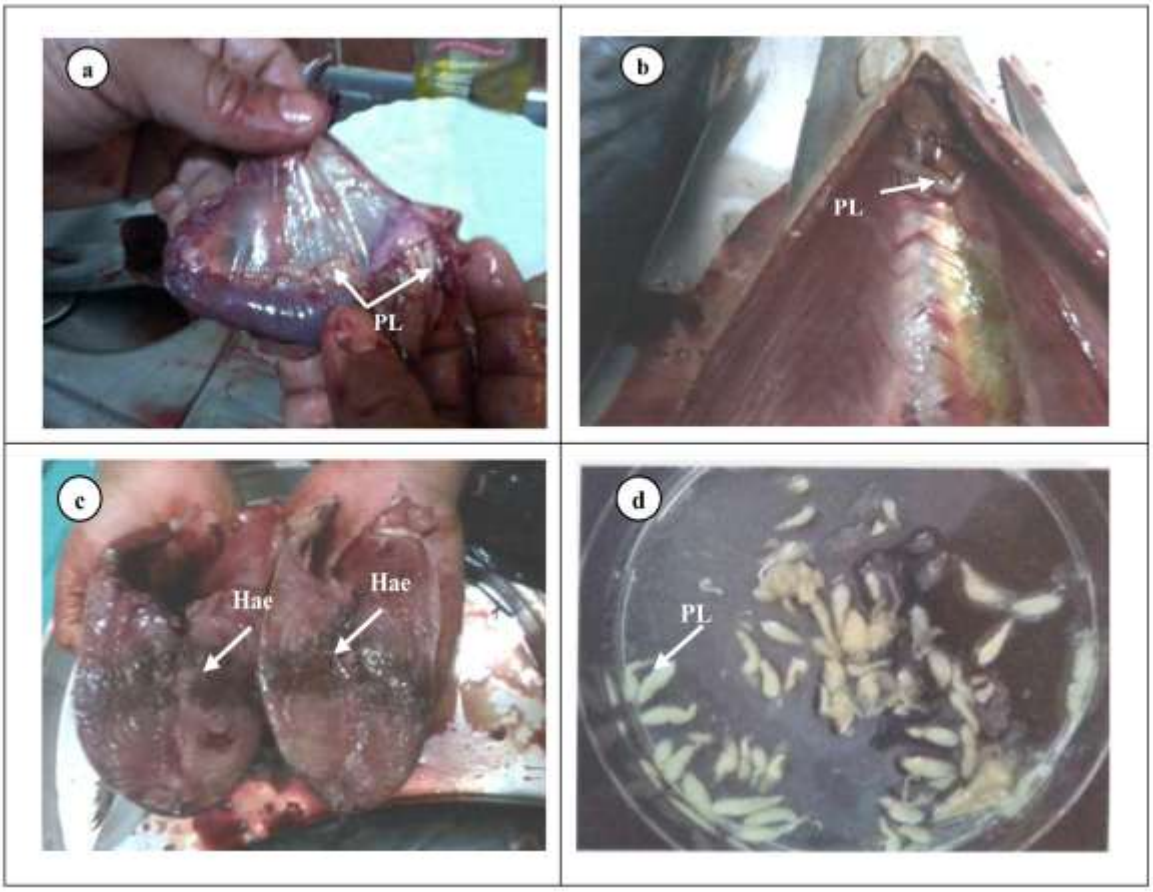


Plate I: Showing the macroscopic correlation between the affected freshly dead Kaw. fish and the parasite ***Schistocephalus sp.***plerocercoids:

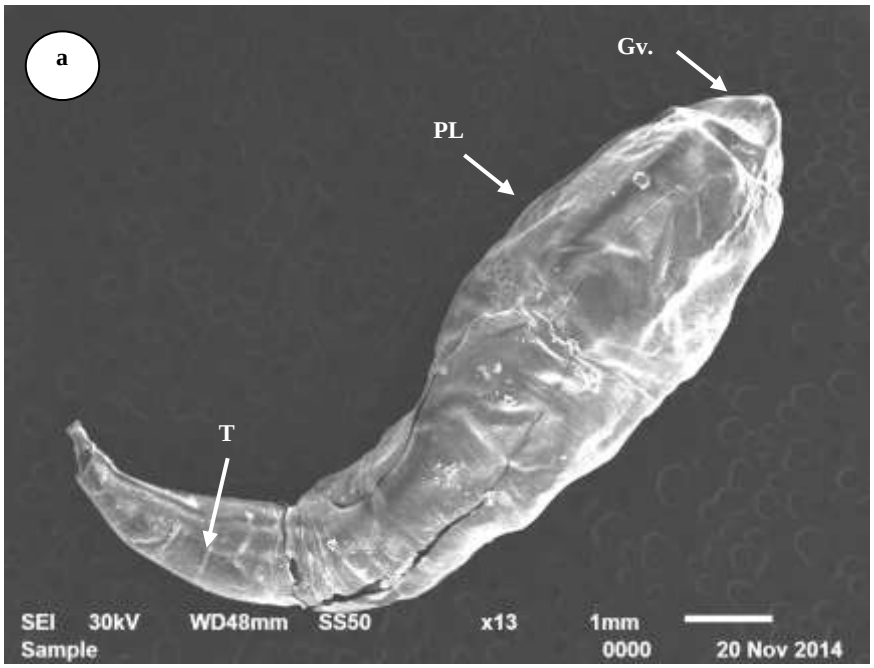
Photo. (a)The plerocercoid (PL) adjacent to the internal organ and musculature after were extracted from the abdominal cavity.

Photo. (b)The abdominal cavity wall containing the plerocercoid(PL).

Photo. (c) Haemorrhagic (Hae) line of dark brown coloured musculature.

Photo. (d) Collected plerocercoid (PL); whole larvae after tissue dissection in petri dishes.

Extracted *Schistocephalus sp.* Plerocercoid in petri dish examined with dissecting and ordinary microscope (plate1: photo d) were whitish body in colour, fusiform broader at anterior and was striated with shallow grooves on the scolex and pointed at the posterior end. They were ranged from 2-3mm up to 1.3-1.4cm in length; 1-3mm in width.



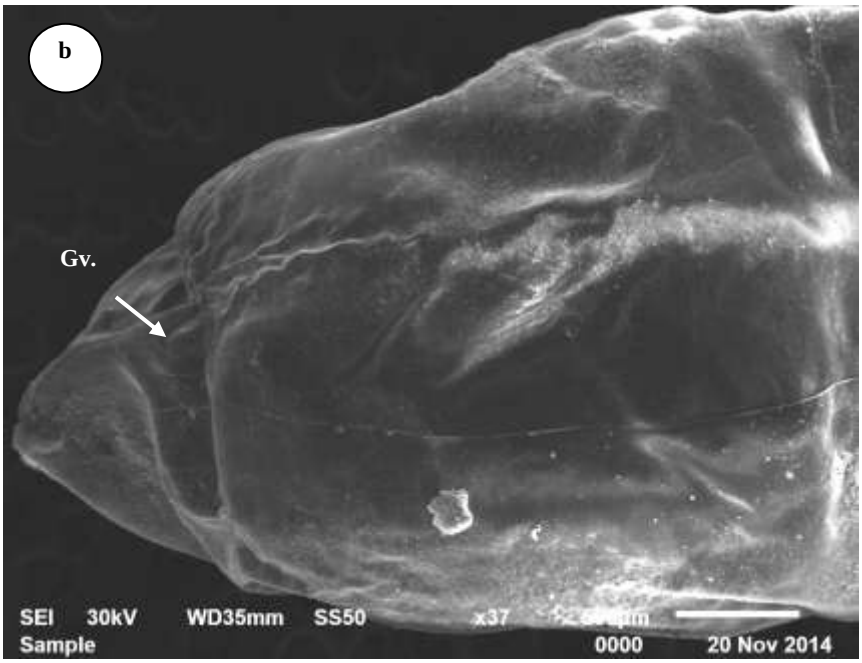


Plate II: Scanning electron microscope(SEM).

photo a) Clarifying the whole size of the *schistosephalus sp.* plerocercoid larvae (PL) and was showed a convex shape anterior and a concave posterior ornamented with tubercles(T).

photo b) Plerocercoid showed broad anterior end containing a groove(Gv.) on the head with a convex dorsal aspect .

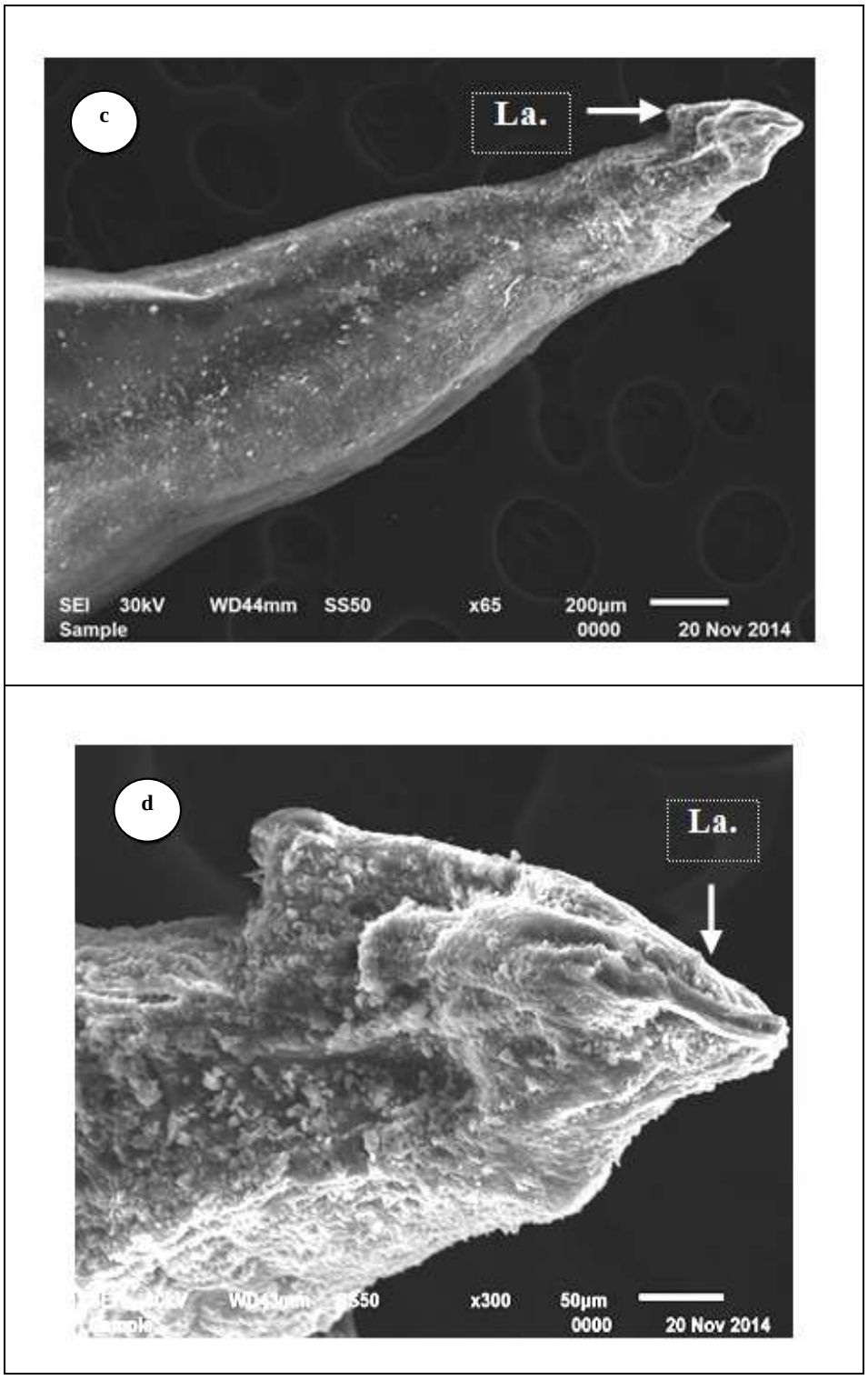


Plate III: Scanning electron microscope was showed the posterior end of the ***Schistocephalus sp.*** Having a laciform (La) shape tail (photo c) and a magnified lancet shape (photo d).

Table (1): Monthly prevalence of *Schistocephalus sp.* plerocercoid from frozen (Balamyta) Kawakawa fish (Group a).

Date of collection	Frozen kawakawa fish (group a.)							
	Weight under 3 kg/fish				Weight over 3kg/fish			
	No. of samples	-ve plercocercoid	+ve plercocercoid	% of +ve	No. of samples	-ve plercocercoid	+ve plercocercoid	% of +ve
Nov. 2014	10	10	-	-	6	5	1	16.66
Dec. 2014	13	12	1	7.69	8	8	-	-
Jan. 2015	9	9	-	-	6	5	1	16.66
Feb. 2015	13	13	-	-	7	6	1	14.28
March. 2015	10	9	1	10.00	9	8	1	11.11
April 2015	10	10	-	-	7	7	-	-
Total	65	63	2	3.07	43	39	4	9.30

Table (2): Monthly prevalence of *Schistocephalus sp.* plerocercoid from freshly dead (Balamyta) Kawakawa fish(Group b).

Date of collection	Freshly dead kawakawa fish (group b.)							
	Weight under 3 kg/fish				Weight over 3kg/fish			
	No. of samples	-ve plercocercoid	+ve plercocercoid	% of +ve	No. of samples	-ve plercocercoid	+ve plercocercoid	% of +ve
May 2015	10	10	-	-	9	8	1	11.11
Jun. 2015	8	7	1	12.5	7	6	1	14.28
July 2015	13	12	1	7.69	7	5	2	28.57
August 2015	9	8	1	11.11	8	7	1	12.50
Sept. 2015	12	12	-	-	6	6	0	-
Oct. 2015	10	10	-	-	5	5	-	-
Total	62	59	3	4.83	42	37	5	11.90

Results revealed the increased prevalence of infestation of Kawakawa fish with a *Schistocephalus sp.* plerocercoids larvae in freshly dead to a frozen fish with 11.90 and 9.30% in over 3kg./fish than the freshly dead to a frozen fish with 4.83 and 3.07% of under 3kg./fish respectively. Also, the freshly dead kawakawa started with May up to October rather than the frozen fish which have been arise from Nov. up to April Table (1), Table (2).

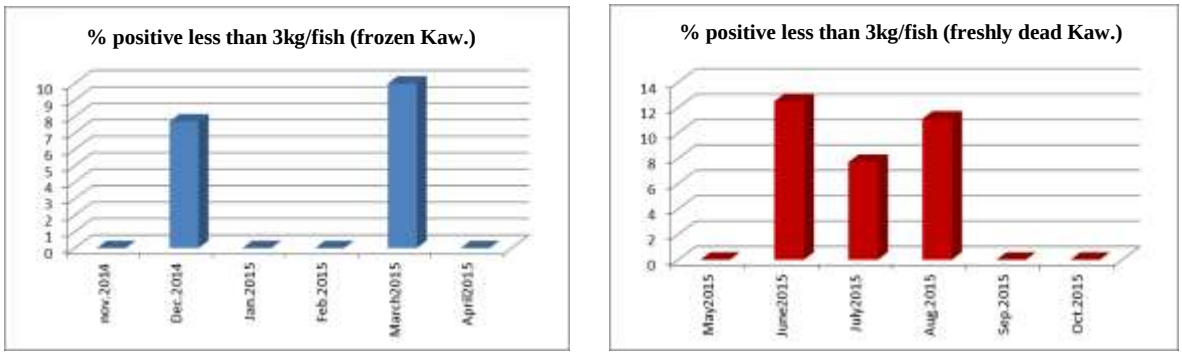


Fig. (2): Comparison between positive percentages of *Schistocephalus sp.* plerocercoid in frozen and freshly dead fish of less than 3kg/fish body weight.

Table (3): Monthly correlation of *Schistocephalus sp.* Plerocercoid infested Kawakawa fish in both frozen and freshly dead fish under the wt. of 3kg./fish during the period of Nov. 2014 up to April 2015 and May 2015 up to Oct. 2015 at Dakahlia and Damietta Governorates.

Date of collection		Mean ± SE
Frozen	November 2014	0.00 ± 0.00^{c}
	December 2014	7.64 ± 0.06^{b}
	January 2015	0.00 ± 0.00^{c}
	February 2015	0.00 ± 0.00^{c}
	March 2015	10.00 ± 0.00^{a}
	April 2015	0.00 ± 0.00^{c}
freshly dead	May 2015	0.00 ± 0.00^{c}
	June 2015	12.70 ± 0.09^{a}
	July 2015	7.70 ± 0.06^{b}
	August 2015	11.36 ± 0.11^{a}
	September 2015	0.00 ± 0.00^{c}
	October 2015	0.00 ± 0.00^{c}

The different letters in the same column means the mean difference is significant at the > 0.05 level.

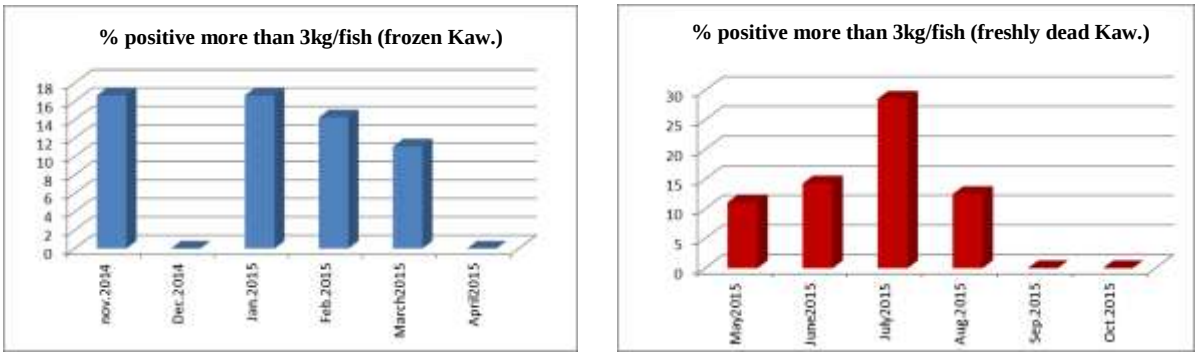


Fig. (3): Comparison between positive percentages of *Schistocephalus sp.* in frozen and freshly dead fish of more than 3 kg body weight.

Table (4):Monthly correlation of *Schistocephalus sp.* plerocercoid infested Kawakawa fish in both frozen and freshly dead fish over the wt. of 3kg/fish during the period of Nov. 2014 up to April 2015 and May 2015 up to Oct. 2015 at Dakahlia and Damietta Governorates.

Date of collection		Mean ± Std. Error
Frozen	November 2014	16.54±0.05[a]
	December 2014	0.00±0.00[d]
	January 2015	16.54±0.05[a]
	February 2015	14.52±0.08[b]
	March 2015	11.26±0.06[c]
	April 2015	0.00±0.00[d]
freshly dead	May 2015	11.26±0.06[d]
	June 2015	14.52±0.08[b]
	July 2015	28.30±0.07[a]
	August 2015	12.38±0.07[c]
	September 2015	00.00±0.00[e]
	October 2015	0.00±0.00[e]

The different letters in the same column means the mean difference is significant at the > 0.05Level.

In the present study, *Schistocephalus sp.* were a cestode tape worm harbour the abdominal cavity and may produce a pressure on the musculature and internal organs leads to swelling the abdomen and consequently atrophy of this organs (plate 1: photo a and b) . This observation was agreed with that which explained ***(Arme, 1975; Smyth and MacManus, 1989)*** who have been studied the pathogenic effect of plerocercoid on fish. As, the parasites themselves may produce enzymes that damage host cells and inhibit the physiological process of fish .These metabolic products which called with metabolic dumps ***(Roberts and Agius 2003)*** can be toxic to the host causing fish deterioration.

Similar resultive side-effect for infestation with a ***Schistocephalus sp.*** in this study were revealed that the infested fish showed dark brown line of haemorrhagic musculature (plate1: photo c), in which were seemed disintegrated rapidly (fried). Another finding was reported by ***Robert, (2012)*** wherever, in toxemic conditions, the melanomacrophage centers of the haemopoietic tissue were usually burst-up and their pigment granules disseminated, consequently, may be readily recognized in any tissue section.

Histopathological examinations of liver tissue of infested freshly dead fish with ***Schistocephalus sp.*** revealed an extensive basophili cells aggregation With sequestrated melanin granules disseminated in the melano-macrophage centers (photo H).To investigate and expose the haemorrhagic elements, differential staining of Perl's Prussian blue was carried out which display a large amounts of haemosiderin dispersed and circulate in liver tissue which appear as a bright blue dotings (photo S). Further histopathological myopathy was showed in muscle with follicular degeneration and vaculation of muscle fiberes (photo N).

Longtudinal(Lon.) and transverse (Tr.) sections were clearly showed (photo M) the *Schistocephalus sp.* plerocercoid as a concerned parasite of Diphyllbothriidae drawing this study.

To the best of our knowledge this is the first record showing the infestation in Kawakawa fish (mackrel tuna) with a monthly prevalence rate n Dakahlia and Damietta governorates. We are concluded that the heavy parasitological infestations of fishes may lead to deterioration of their meat in addition to other organisms.

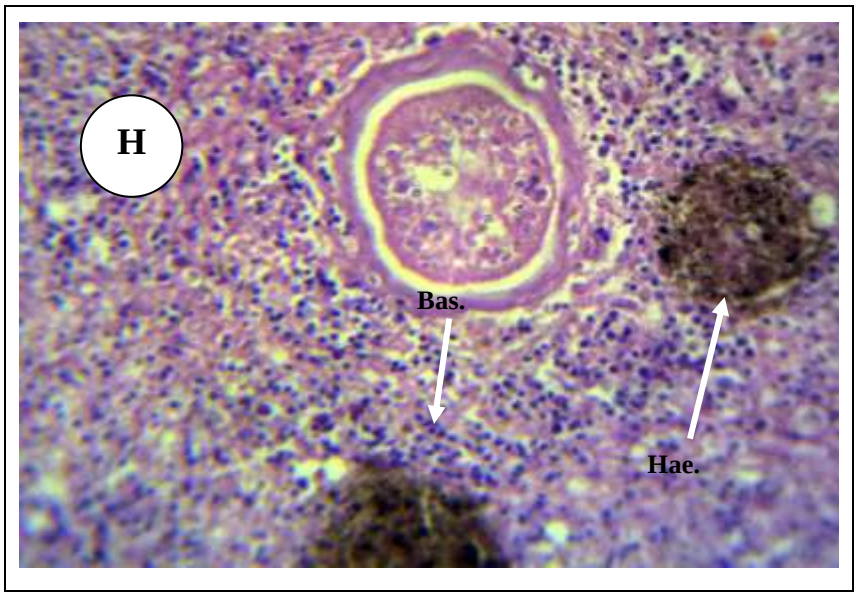


Photo H: Liver showing sequestered melanin granules (Hae.) disseminated in the melanomacrophages centres of the liver tissue with basophilic cell (Bas.) aggregation. H. & E ×400.

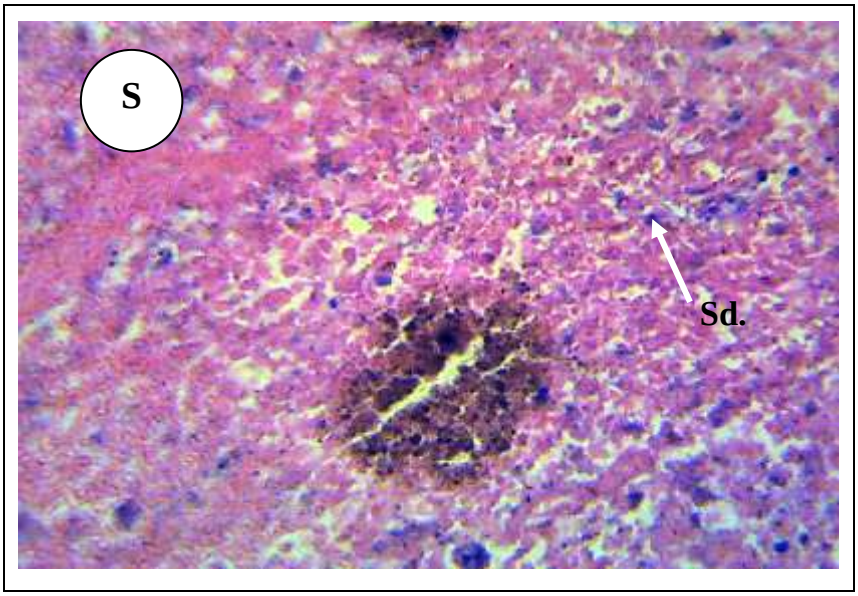


Photo S: Liver tissue with an extensive focal deposition of haemosiderin (Sd.) (macrophage laden haemosiderin) stained bright blue and dark brown melanomacrophages. Stained by Perl's Prussian blue, x= 400.

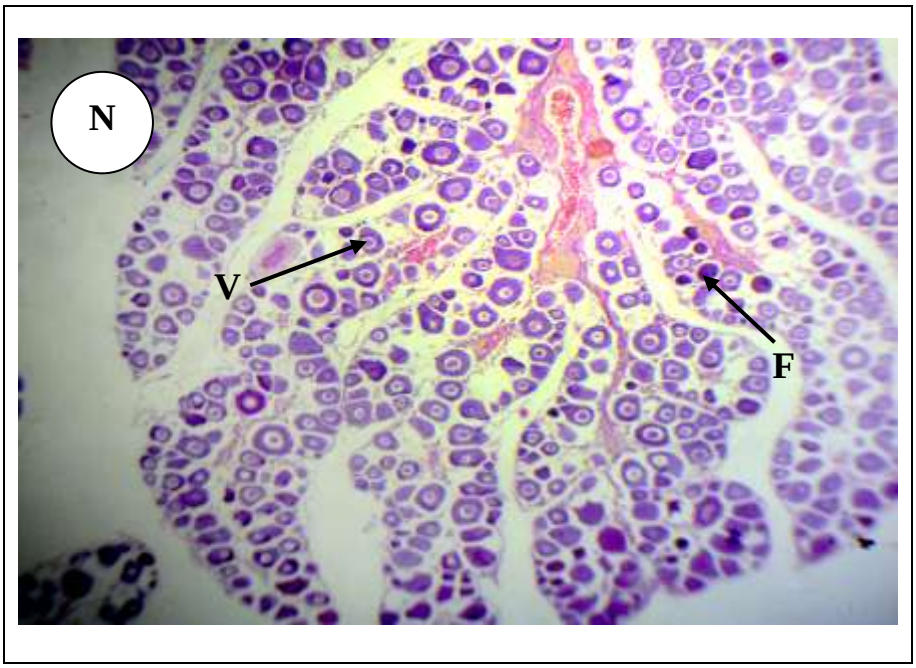


Photo N: section of musculature of the affected freshly dead Kaw. Fish infested with a plerocercoid of ***Schistocephalus sp.*** undergo multiple follicular (F) degeneration and vacuolation (V) of muscle fibers. H. & E ×400

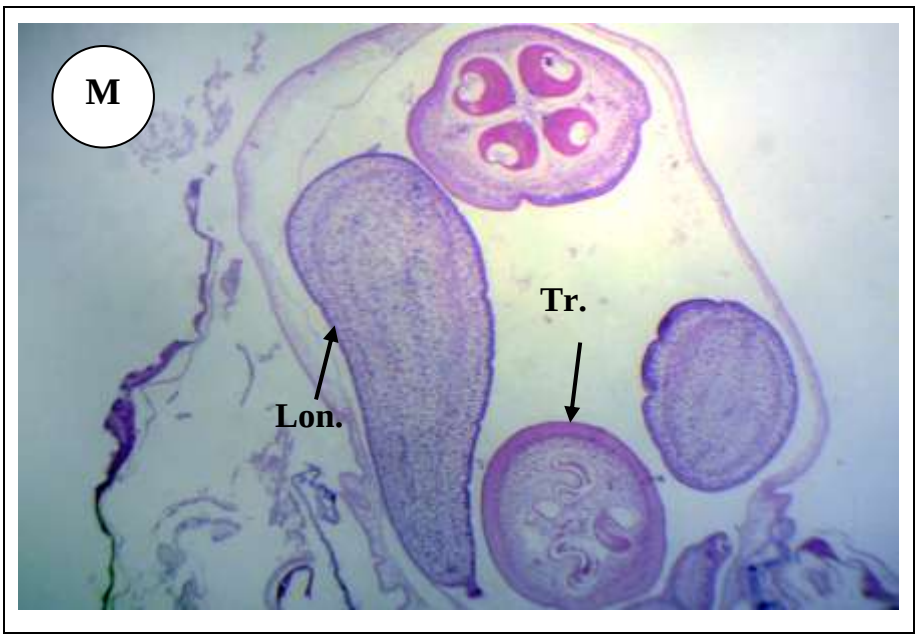


Photo M: sections of plerocercoid ***Schistocephalus sp*** were showing a longitudinal (Lon.) and transevese (Tr.)***.*** was taken from the abdominal cavity. Stained by H. & E ×100

REFERENCES

Arme, C. (1975): Tapeworm – interactions. *Symposia of the society for experimental biology* 29:505-32.

Bancroft, J.D. and Stevens, A.(1999): Theory and practice of histological techniques 4th.ed.Chukhull Livingston, New York.

Collette, B.B (2001): Scombridae. Tunas also, (albacore, bonitos, mackerels, seerfishes, and wahoo). In: K.E. Carpenter, V. Niem, (Eds.), FAO species identification guide for fishery purposes. The living marine resources of the western central pacific. Bony fishes (Labridae to Latimeriidae), estuarine crocodiles. FAO, Rom: 3721-3756.

Collette, B.B. and Nauen, C.E. (1983): FAO species catalogue, scombrides of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos, and related species known to date. FAO fish synop., 125:1-137.

Colwell, D. and O'connor, M. (2000): Scanning Electron Microscopy of sarcophagid (Dipetra) larvae recovered from a case of human cutaneous myiasis. J .Med. Entomol., 37(6):854-9.

Combes, C. (2001): Parasitism: The ecology and evolution of intimate interactions. University of Chicago press.

Dezna C. Sheehan and Barbara B. Hrapchak (1973): Theory and practice of histotechnology.

Miura, O.; Torchin, M.E; Kuris, A.M., Hechinger, R.F., and Chiba, S. (2006): Introduced cryptic species of parasites exhibit different invasion pathways. Proceedings of the National Academy of Sciences, 103 (52), 19818-19823.

Nishimura, N.; Heins, D.C.; Andersen, R.O.; Barber, I., and Cresko, W.A. (2011): Distinct lineages of *Schistocephalus* parasites in the three spine and nine spine stickleback hosts revealed by DNA sequence analysis. PLOS ONE,6(7),1-10.

Pillai, N.G. and Mallia, J.V. (2007): Fish identification of tunas. Bibliography on tunas. No.92.

Poulin, R. and Morand, S. (2004): Parasite biodiversity, smithsonian Books.

Poulin, R., and Keeney, D.B., (2008): Host specificity under molecular and experimental scrutiny. Trends in parasitology, 24 (1),24-28.

Randhawa, H.S., Saunders, G.W, and Burt, M.D.B. (2007): Establishment of the onset of host specificity in four phyllobothriid tapeworm species (cestoda: Tetraphyllidea) using a molecular approach. Parasitology, 134 (pt 9), 1291-1300.

Roberts, R.J. and Agius, C. (2003): Melano-macrophage centres and their role in fish pathology, J. *Fish Dis.* 26, 499-509.

Ronald J. Robert, (2012): The patho-physiology and systematic pathology of Teleosts. Fish pathology 92-137.

Scharsack,J.P.,Gossens ,A., Franke, F. and Kurtz, J.(2013):Excretory products of the cestode, *Schistocephalus solidus*,*modulate* in vitro responses of leukocytes from its specific host, the three-spined sticklebacks (*Gasterosteus aculeatus)*. Fish and shellfish immunology,35(6),1779-1787.

Smyth, J. D. (1990b): Peter Abildgaard-forgotten pioneer of parasitology, parasitology tody,6:337-339.

Smyth, J.D and McManus, D.P. (1989): The *physiology* and *biochemistry* of *cestodes*. Cambridge university press. ISBN 0521355575.

Smyth, J.D. (1990a): Parasitological serendipity: From ***Schistocephalus*** to ***Echinococaus.*** International Journal for parasitology, 20:411-423.

Steel, R.G. and Torrie, J.H. (1980): Principle and procedures of statistics. McGraw-Hill publishers New York, 2nd edition.

Theodore M., Tamara B., Collette B. and Norman, S. (2008): Common diseases of wild and cultured fishes in Alaska p. 72-73.

Van Baarlen, P.; Van Belkum, A.; Summerbell, R.C.; Crous, P.W., and Thomma, B.P.H.J. (2007): Molecular mechanisms of pathogenicity: How do pathogenic microorganisms develop cross-kingdom host jumps. FEMS Microbiology Reviews,31(3),239-277.

William N. Eschmeyer and Earl S. Herald (1983): A field guide to pacific coast fishes. The peterson field guide series. Illustrations by Howard Hammann and Katherine P. Smith. Page 70-71.

Yitashao, (2012): The brain-pituitary-gonadal axis of the three-spined stickleback, Gasterosteus aculeatus, Doctral dissertation-Dept. of zoology. Stockholm University –Sweden.

الملخص العربي

بعض الدراسات على يرقات الشيستوسفالس الشريطية التي تصيب أسماك الكاواكاوا

حامد ربيع حامد*، سعد نخلة أبوالسعود*، نسمة رشد مختار**

*****ماري بشرى بانوب**

***قسم الطفيليات – ****قسم الباثولوجي – معمل المنصورة – معهد بحوث الصحة الحيوانية**

***** قسم الطفيليات – معهد بحوث الصحة الحيوانية بالدقي**

سمكة الكاواكاوا هو الاسم العلمى وأيضا تسمى "البلاميطة" تجاريا في الأسواق المصرية. أجريت الدراسة على عدد 212 سمكة كان منها عدد 108 سمكة مجمدة (مجموعة أ) (عدد 65 سمكة تحت وزن 3كجم/ سمكة، عدد 43 سمكة فوق 3كجم/ سمكة) والباقي (مجموعة ب) (عدد 104 سمكة مصادة حديثا، عدد 62 سمكة تحت وزن 3كجم/ سمكة ، عدد 42 سمكة فوق وزن 3كجم/ سمكة). جمعت الأسماك من أسواق محافظتي الدقهلية ودمياط في الفترة من نوفمبر 2014 وحتى أبريل 2015 وخلال الفترة من مايو 2015 وحتى أكتوبر 2015 للأسماك المجمدة والمصادة حديثا على التوالى. أسفرت الدراسة عن اكتشاف يرقات عائل وسيط "بليروسركويد" لنوع يسمى "شيستوسيفلس" ينتمي لعائلة "دايفلوبوثريدي". تم الكشف عن اليرقات في التجويف البطني للأسماك المصابة. سجلت النتائج في الأسماك المجمدة تحت وزن 3كجم/ سمكة عدد 2 من إجمالي عدد 65 سمكة بنسبة إصابة قدرها 3,07% والأسماك فوق وزن 3كجم/سمكة عدد 4 سمكة من إجمالي عدد 43 سمكة بنسبة إصابة قدرها 9,30%. بينما كانت الأسماك المصادة حديثا تحت وزن 3كجم/ سمكة عدد 3 سمكة من إجمالي عدد 62 سمكة بنسبة إصابة قدرها 4,83% والأسماك فوق وزن 3كجم/ سمكة عدد 5 سمكة من إجمالي عدد 42 سمكة بنسبة إصابة قدرها 11,90%. أظهرت الدراسة أن العضلات والكبد من الأعضاء الداخلية وأنسجة من التجويف البطنى للأسماك المصابة والمصادة حديثا كانت "متهتكة" وبنية قاتمة اللون. أسفر الفحص الهستوباثولوجي عن ظهور بقع ومساحات نزفية بصورة كثيفة داخل كبد السمكة المصابة. أما الدراسة بالميكروسكوب الالكتروني أوضحت مقاطع طولية وعرضية ليرقات الطفيل التابع لعائلة "داي فيلوبوثريدي".