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GENERAL INFORMATION

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OBSERVATIONS ON *ERGASILUS CHELANGULATUS* (COPEPODA: ERGASILIDAE) A GILL PARASITE OF *TILAPIA ZILLI* FROM MANZALLA LAKE

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Abstract

Ergasilus chelangulatus infested gill filaments of *Tilapia zilli* from Manzalla Lake. Only females were detected and the males are non parasitic. *E.chelangulatus* has a serrate seta on exopod one and a two-segmented first endopod. The body is elongate and protruded anteriorly. The antennae are elongate and have prominent sensilla on segments two and three. *E. chelangulatus* can be distinguished by the fourth antennal segment (claw) which is bent at nearly a right angle. Monthly study between June 2008 - May 2009, and a total of 463 fish were collected. The distribution of parasitic copepods in relation with the length, sex of fish was recorded. Mean intensity of 6.2/fish and a prevalence incidence of 34.2%. The prevalence and mean intensity were seasonal with higher levels in summer with 51.2% and 7 respectively. No significant differences were noticed in the infestation of male and female fish. A slight increase in the infestation prevalence was recorded as the size of fish increased. Gills showed severe pathological changes, where clubbing and fusing of the gill filaments were observed. Deep indentations were recorded in the tips of the damaged filaments which had broken off. Fish were infected at a similar rate, having 1 to 20 copepods attached to the end of the clubbed filaments or the proliferated epithelium . In histological sections the head part of the parasite was found in a deep cavity of the proliferated epithelium, piercing its antennae deep into the tissues. Only the end of the filaments showed changes. The proliferated epithelium was infiltrated by eosinophilic granular cells. In the central and basal parts of the hemibranchia the original structure of the filaments was preserved with intact secondary lamellae.

Key words: *Oreochromis zilli* parasite; copepod parasite; freshwater fish; pathological changes; Abbassa, Egypt.

INTRODUCTION

Ergasilidae von Nordmann, 1832 is one of the major families of Poecilostomatoida (Abdelhalim *et al*., 1993) and comprises 24 genera of parasitic copepods found in freshwater, brackish and coastal marine waters (El-Rashidi and Boxshall 2001). According to Amado *et al*., (1995) ergasilid adult females parasitize mainly teleosts with exception of species of *Teredophilus Rancurel*, 1954 that occur on annelids and bivalve molluscs. Ergasilus von Nordmann, 1832 includes species with high pathogenic potential some of them responsible for great mortality among cultured fishes in freshwater and brackish environments (Piasecki *et al*., 2004). *E. euripedesi* was redescribed and new combination *Gauchergasilus euripedesi* proposed (Montú and Boxshall 2002).

Species of *Ergasilus* are found world-wide and are considered to be one of the plagues of pisciculture. Only the females are found on fish hosts while the males are free-living in the zooplankton.

Most species are found on freshwater fishes but a few infect marine fishes of the littoral. Yamaguti (1963) reported 69 species of this genus world-wide. Hoffman (1998) listed 32 species for North America and Thatcher (2006) cited 15 species of from South America. While the pathological effect on the gills of the fish and the possible ways of control were investigated by Bauer *et al*., *(*1981). Damage caused by *Sinergasilus maior* is rather common in China (Nie and Yao, 2000; Wang *et al*., 2002). Also they, identified the parasite with the species *S. polycolpus.* Two of copopode, *E.sieboldi* and *L. cyprinacea*, proved to be pathogenic to fishes. *E. sieboldi* is a frequent parasite in natural waters, but the gills of pond-cultured fishes are only exceptionally infected by this parasite and there are no data on its occurrence on phytophagous fishes (Molnár and Székely, 2004). The prevalence of crustacean parasite infesting *Oreochromis niloticus* in Lower Egypt (KafreEl–Shiehk Governorate (1998-1999) fish farms were 30 % and the highest the prevalence was obtained during summer (56.6%.) and 44.8% in spring, while was 20.5 % in autumn and 0% in winter (Noor El Deen *et al*., 2010). The less polluted water can allow for or cause parasite proliferation,whilst higher level of contamination can have a negative effect on the survival of *Lamproglina clariae* (Avenant-Oldewage, (2003) and Eissa *et al.* 2011). There is more awareness of the importance of studying fish parasites as one of the major obstacles in fish production. About 80% of fish diseases are parasitic especially for warm water fish (Eissa *et al.*, 2000).

The present paper reports frequent and severe infections of branchial filaments of *Tilapia* ***zilli*** with *E.chelangulatus,* and gives a detailed histopathological description of the changes caused by these copepods. Also, described and identified *E.chelangulatus*. The prevalence and intensity of infection in relation to host age, length, sex, and habitat were studied. In addition, seasonal intensity of infection was monitored.

MATERIAL AND METHODS

Sample and localities

Fish were collected from some localities throughout a 20 km long; Fish hosts were captured with nets in the Manzalla Lake. Copepod parasites were removed from the gill filaments with dissecting needles and fixed in 70% alcohol. They were transported to a laboratory of Central Laboratory for Aquaculture Research, Abbassa, Egypt, where permanent slide preparations were made using the phenol-balsam method explained in Thatcher (2006). Digital photographs made through a light microscope were used to make the drawings. Measurements were made utilizing a measuring ocular and are expressed in micrometers.

Examination and analysis

Clinical Examination

Alive fish were clinically examined for general behaviors, changes in colour, respiratory manifestation, feeding and any clinical abnormalities of the gills according to the methods described by Noga (1996).

Parasitological examination

Crustaceans were refrigerated then fixed in 70% alcohol glycerin, passed through ascending grades of alcohol (70, 90,95% and absolute) then cleared in xylol, mounted in Canada balsam or byclearing in lacto phenol and mounted in glycerin-gelatin according to (Lucky 1977) and The gills were examined microscopically for copepod parasites as described in Cone and Marcogliese (1995).

Statistical analysis

The results of prevalance were statistically analyzed using analysis of variance procedure in SAS (Duncan 1955). It has been previously established that the abundance of both parasites correlated positively with fish length; only fish of the 15–22 cm length range were used in comparison among sample localities to prevent any statistical bias. The distribution of each parasite, Spearman's rank correlation coefficients (*r*) were calculated using the component population, means abundance, and prevalence of each parasite. Prevalence (number of hosts infected divided by the total number of hosts in a sample), and mean abundance (number of parasites divided by the total number of hosts in a sample) are the same as those described in *Bush et al*. (1997). Component population, prevalence, and mean abundance of each gill parasite were calculated (monthly and annually) for *Tilapia zilli* sampled from each locality. Mean values (component population, abundance, and prevalence) were compared using the non-parametric Kruskal–Wallis test while prevalence were compared using the *G*-test (Krebs, 1999).

RESULTS

Clinical picture

The first sign observed in *Tilapia zilli* infested with Ergasilus infestation was, swam rapidly in circles manner of the affected fish in which the fishes aggregate in groups around the water inlet. Most of these fishes showed dark discolourtion of the skin,emaciation, loss of appetite and eventually loss of escape reflex. The gills appeared pale in colour with numerous noduler like as white to yellowish colouration and appear as V or inverted V-shaped of the egg sacs on the attached gills in some examined fish during post mortem examination.

Description

Body elongate, tapered towards both extremities and projecting anteriorly; head incompletely fused to first thoracic segment; first and second thoracic segments completely fused (Fig. 1). Thorax of five free segments, including genital segment (Figs.1 and 2). Ventral pigment granules centrally located from eye to genital segment. Eye cobalt blue – color; body, indigo blue – color. Abdomen (Fig. 1-2) of three segments; segment two shortest of three. Uropod with two elongate caudal filaments(Fig. 2). Antennule of six articles, provided with simple setae. Antenna (Fig. 4) of four segments (including claw); prominent sensilla on segments two and three; claw bent to nearly a right angle. Mouthparts (Fig. 3). Mandible with posteriorly directed terminal bristles; palp denticulate posteriorly. Maxilla terminally bristled; maxillule not observed. Leg 1 (Fig.5) endopod two-segmented, exopod three segmented; first endopodal segment with a single pinnate medial seta; terminal endopodal segment with two stout spines and five pinnate medial setae; first exopodal segment with a single postero-lateral spine;

second exopodal segment with a single pinnate medial seta; terminal segment with four medial pinnate seta, one curved serate seta and two terminal spines. Leg 2 and leg 3 (Fig. 5) both rami three-segmented; first endopodal segment without spines and setae; second endopodal segment with two medial pinnate setae; terminal segment with four small pinnate setae and one small spine; first exopodal segment with one small lateral spine; second segment without spines and setae; terminal segment with six pinnate setae and one small spine. Leg 4 (Fig. 5) both rami two-segmented; first endopodal segment without spines and setae; terminal segment with five pinnate setae and one spine; first exopodal segment without spines and setae; terminal segment with five pinnate setae and one spine. Egg sac elongate with few rows of eggs.

Fig. (1). Female Ergasilus x100 .

Fig. (2). Uropod with two caudal filaments x100.

Fig. (3). Mouthparts x150 .

Fig. (4). Antenna of 4th segments (Claw); Prominent sensilla; x150.

Fig (5). Fifth pedigerous somite with leg 5, genital complex.

Fig **(6).** Egg sac elongate with few rows of eggs x150 .

Table (I). Measurements in micrometer (µm) of females *E. chelangulatus* collected from *Tilapia zilli*, in Manzalla Lake**.**

		Length	Width
Body (without caudal filaments)		530-630 (503)	145-200 (165)
Cephalothorax		222-302(300)	162-200 (160)
Free thoracic segments	**III**	56-87 (69)	115-138 (131)
	IV	48-62(54)	87-112(103)
	V	40-50(47)	58-768(66)
	VI	20-27 (24)	58-76 (64)
	VII genital	52-59 (53)	56-69 (64)
Abdomenal segments	**I**	14-22 (17)	45-55(50)
	II	6-11 (11)	40-50(45))
	III	13-20 (17)	38-50(42)
Uropod		24-30(25)	14-17 (15)
Caudal filament		150-250 (213)	
Egg sac		300-490(334)	50-70(59)
Egg diameter		35-38 (38)	

Table (2). Measurements in micrometer (µm) of the antennae of females of *E. chelangulatus* collected from *Tilapia zilli,* in Manzalla Lake.

		Length	Width
Antennules		50-11 0(101)	16-23 (20)
Antenna			
Segment	**1**	45-12(62)	44-55 (51)
	2	155-200 (176)	41-55(48)
	3	141-166 (155)	30-38(34)
	4	86-103 (78)	11-22(19)

Prevalence and Mean Intensity

Out of the 436 fish examined, 149 fish were infested with a prevalence 34.2% and mean intensity level of 6.2 *E.chelangulatus* per infested fish. Infestation prevalence levels in all the seasons stayed above 34% during the examination. In seasonally basis, the highest prevalence of *E. chelangulatus* was recorded with 51.2% in summer and the lowest prevalence with 17.5% in spring. The highest and lowest of mean intensity were recorded with 7 in summer, 5.2 in autumn, respectively (Table 3).

Table (3). Seasonal prevalence and Mean Intensity of *E. chelangulatus* In relation to the sex of *Tilapia zilli*.

Seasons		No of fish examined	No of fish infested	Prevalence (%)	Mean Intensity	Parasite/ infested fish
Summer		166	85	51.2	7	5-15
Autumn		93	33	35.5	5.2	1-6
Winter		74	13	17.6	6 .4	1-4
Spring		103	18	17.5	5.8	2-6
No.	**F.**	286	98	34.3	7	-
	M.	150	51	34	4.5	-
	Total	436	149	34.2	6.2	1- 20

A total of 286 female and 150 male fish were examined for the presence of *E. chelangulatus*. No significant differences were noticed in the infestation of male and female fishes. A slight decrease in the infestation prevalence was also recorded as the size of fish increased. Very close mean intensity values on each length class of fish were determined (Table 4).where the parasites invade more the smaller sized tilapia

Table (4). Infestation prevalence and Mean Intensity levels of *E. chelangulatus* in relation to fish lengths.

Length of fish (cm)	No of fish examined	No of fish infested	Prevalence (%)	Mean Intensity
8.5-10.0	146	65	44.5	6.8
15-20	98	39	39.8	6.7
20-25	56	21	37.5	6
25-30	136	34	25	5.7
Total	436	*149	-	-

Histopathological impact:

Among *Tilapia zilli*, *E.chelangulatus* infection was well observable by gross examination of the gills at the ponds. Most of the fish harvested showed macroscopic lesions on the gills. By opening the gill cover of the fish, gills showed a pale colour the tips of the filaments grew together into a whitish mass Fig. (7a), then deep indentations of the hemibranchia were seen.

Signs of a passed-off infection were encountered also in fish free from copepods. The number of copepods present on the gills of the 11 infected fish varied between 1 and 20 copepods. Infection was found both in the summer and the autumn months. Both the fingerlings and the small-sized fishes harbored parasitic copepods. All parasites were located at the edges of the gill filaments, some of them grabbing only a single filament (Fig. 7 b and c), while some others were attached to the proliferated epithelium of the fused gill filaments (Figs 7c). Parasites, both in solitary filaments and in the fused ones, formed a hole at their attachment site in the proliferated epithelium.

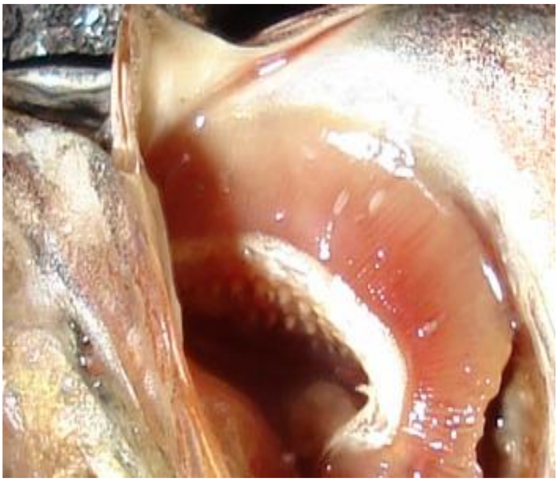

Fig. (7a): Attachment of adult female *E. chelangulatus* (arrowhead) to gills of *Tilapia zilli* in some parts of the pale or whitish hemibranchia.

Fig. (7 ,b and c): Parasite 8 to 27 copepods attached by clawed maxilliped deeply penetrating gill tissue, the head part(c): of the parasite was found in a deep cavity of the proliferated epithelium, piercing its antennae deep into the tissues.

All parasites found proved to be imago-stage females, some of them having egg-sacs at the tail. In some parts fusion of several filaments due to intensive epithelial proliferation was seen (Fig. 7 b and c) while other areas were characterized by clubbing of individual filaments. Both in fused areas and in separated filament regions attaching parasite could be observed fixed to the fish tissues by their antennae (Figs 8 and 9). The robust claws of the large antennae were deeply pierced into the proliferated epithelium of the filaments, reaching the connective tissue around the cartilage and the arteries running alongside it . Inside the affected area proliferation and degeneration of the connective tissue were also recorded (Fig. 8). Around the feeding organs of the copepods there was a deep depression in the proliferated epithelium. The superficial layer of the epithelium in these parts lost its original structure and epithelial cells became flattened like those of the skin, with a cuticle-like formation above the flattened cells (Figs 8 and 9), and cell debris was also seen around the damaged area. The proliferation, fusion and clubbing always affected only the distal part of the gill filaments but the central parts and portions close to the cartilaginous gill arch preserved their original structure (Fig. 10). Proliferation tissue was mostly composed of proliferating epithelial cell of the multilayered epithelium of the non-lamellar part of the filament, but it contained a large number of eosinophilic granular cells, which are normal elements of the gill tissue, but in this case their number was greatly increased. In some areas of the proliferated zone remnants of the secondary lamellae were also seen with damaged capillaries. Within the mass of epithelial cells and granular cells some free erythrocytes could also be recorded, but granulocytes or lymphocytes were not seen. In a similar way, there was a shortage in goblet cells which are normal elements of the healthy gill tissue (Fig. 10).

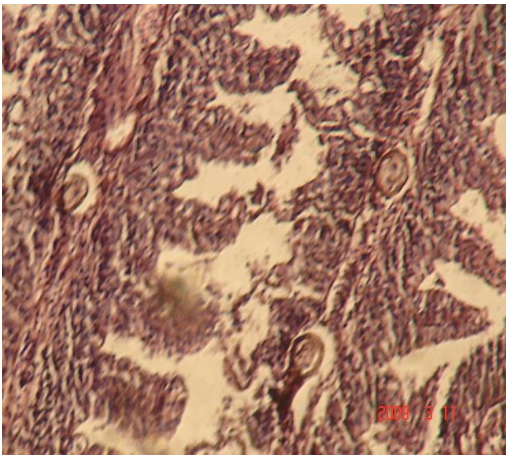

Fig. (8): The epithelium around the head shows a cuticle formation (arrowhead). Haematoxylin and eosin stained × 126.

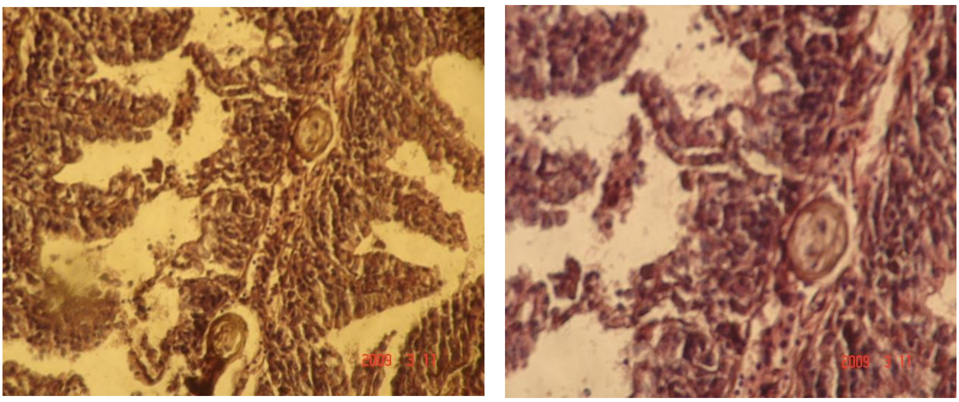

Fig. (9). *E. chelangulatus* female attaching to the clubbed end of a gill filament. The mouth part of the copepod (arrow) is located in a deep impression of the proliferated epithelium. × 250.

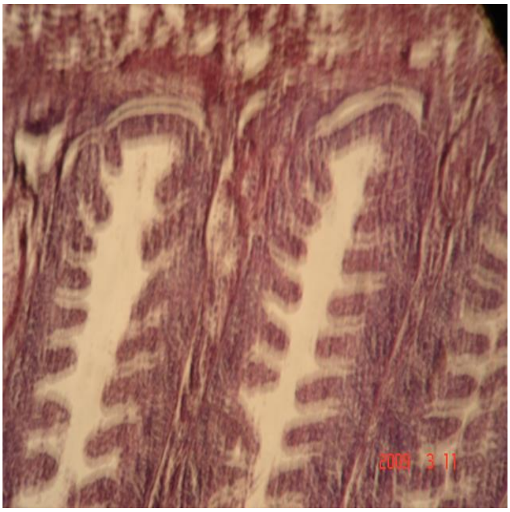

Fig. (10). Epithelial hyperplasia, which become extreme with extensive lamellar fusion, epithelial stratification and cellular necrosis. Elucidated by female copepods where clubbing and fusing of the gill filaments were observed × 250.

DISCUSSION

Ergasilus chelangulatus is not closely similar to any known species. It has a curved, serrate seta on the first exopod and a two-segmented first endopod as do most known species. The species bears a superficial resemblance to *E.cerastes* Roberts, 1969, from North American catfishes, and to *E.pitalicus* Thatcher (2006), from Colombian cichlids in that these have similar sensilla and two-segmented first endopods. Both of the latter have three-segmented fourth endopods, however, while our species has only two segments in that structure. The other two species also lack a curved, serrate seta on the first exopod. *E.chelangulatus* can be distinguished at a glance from all other known species in the genus by the form of the fourth antennal segment (claw) which is bent into a nearly right angle. Regarding to clinical signs of infested *Tilapia zilli* and exposed to natural cadmium showed that restlessness, respiratory distress and slimy pale skin. these results were in agreement with that recorded by (Allen, 1994). Concerning, the results of postmortem of examined *Tilapia zilli* infested with *Ergasilus* sp., it was found that there were increase of mucus producing cells in the gills and presence of white dots. These results agree with those recorded by Eissa (2004) who found that the postmortom emexamination of gills of tilapia sp revealed pale gill appearance with white dots. Dealing with parasitological examination of *Tilapia zilli* naturally infested with *Ergasilus* sp revealed that the *Ergasilus* appear as V or inverted V-shaped of the egg sacs on the attached gills. The results were coincided with those recorded by Eissa *et al.* (2010) and Noor El Deen *et al*. (2011) .

In the seasonally fluctuations, the highest prevalence of infestation of *Tilapia* ***zilli*** with *E.chelangulatus* was recorded with 51.2% in summer and the lowest prevalence with 17.5% in spring. The highest and lowest of mean intensity of E. *chelangulatus* intensity were recorded with 7 in summer, 5.2 in autumn respectively. This result coined with that of Noor El Deen *et al. (*2011) who reported that, seasonal prevalence of cultured investigated fish revealed that the highest percentage of infestation was in summer followed by spring and the lowest in autumn while no infestation in winter due to these crustacean parasites disappear at low temperature. Also, there was a relationship between cadmium residues in *Tilapia zilli* gills and its concentration in the water, the obtained results showed that the cadmium concentration in the gills were higher than that in the water.

From impact load of *E.chelangulatus* on *Tilapia* ***zilli***, the principle response and major drastic foot print is epithelial hyperplasia, which can become extreme with extensive lamellar fusion, epithelial stratification and cellular necrosis. Also many infections by crustaceans are innocuous or only cause localized feeding and attachment damage, Furthermore, these areas of damage can facilitate the entry and establishment of secondary bacterial, fungal and viral infections. Gill infections are typified by hyperplastic responses to the attachment sites and erosion by feeding parasites and by infiltration of inflammatory cells into the affected tissues (Smith *et al.*, 2007). While *Diplectanum aequans* destroys the secondary lamellae of European sea bass *Dicentrarchus labrax* (L.) gills, infections by *Neoheterobothrium hirame* on gills of Japanese flounder *Paralichthys olivaceus* only causes a pathological change when attached to the buccal wall leading to necrosis of the dermis and underlying tissue (Dezfuli *et al.*, 2007). Eosinophilic granular cells are reduced in number in *D. aequans* infections but elevated in *Ergasilus sieboldi* Nordmann infections on the gills of the common bream *Abramis brama* (L.) and infections by *Sinergasilus polycolpus* on gills of silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis* (Molnár and Székely, 2004; Williams, 2007).

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ملاحظات حول طفيل الارجاسيلس كيلانجيولاتس طفيل خيشومى الذى يصيب اسماك المياة العذبه البلطى الزيللى من بحيرة المنزله

رمضان أنور محمد رمضان

قسم أمراض الأسماك المعمل المركزى لبحوث الثروة السمكية– مركز البحوث الزراعية – وزارة الزراعة – مصر

الملخـص العربـى

تنتشر طفيل الارجاسيلس كيلانجيولاتس فى الخيوط الخيشومية فى أسماك المياه العذبة، البلطي زيلي من بحيرة المنزله وقد تم فى هذة الدراسه الكشف فقط انا للإناث فقط هى الطفيليه واما والذكور فغير معروف دورها الطفيلى. ويتميز جسم الطفيل بان له جسم طويل جزء امامىوهو الراس صدرى والجزء الخلفي وهو البطن والاجزاء التناسليه ويتميز الجزء الامامى بالاستطاله والجحوظ وبيه الإقدام الداخلية endopod والاقدام الخارجيه exopod . وهوائيات ولها الشعيرات الحسية بارز على القطعتين الثانية والثالثة .ولتصنيف هذا النوع فقد تم تمييزه بوجود قطعة antennal الرابعة (مخلب) الذي يعكف على ما يقرب من زاوية القائمة.

وقد تم جمع٤٦٣ سمكة بلطي زيللى ما بين يونيو ٢٠٠٨ حتى مايو ٢٠٠٩، وتمت دراسة شهرية. تمت دراسة معدل انتشار و توزيع تلك المجدافيات الطفيلية مع العلاقة مع طول و جنس السمكة.وقد وجد أن كثافة تلك الطفيليات بلغت ٦.٢ ومعدل انتشار ٣٤.٢٪. وتم تسجيل اعلي مستوى في فصل الصيف مع ٥١.٢٪ و ٧ على التوالي. وقد لوحظ عدم وجود فروق كبيرة في الإصابة من الأسماك الذكور والإناث. وسجلت زيادة طفيفة في معدل انتشار الإصابة مع زيادة حجم الاسماك وأظهرت الخياشيم تغيرات مرضية شديدة، حيث لوحظت التجمعات الخلويه ذات الألوان الشاحبة والتمزقات الخلوية و الشعيرات الخيشومية، وفي بعض أجزاء من شاحب أو بيضاء، ولوحظت الفجوات العميقة في الخيوط التالفة التي قطعت. أصيب الأسماك بمعدل مماثل، وبعد ١–٢٠ مجدافيات تعلق على نهاية الخيوط الخيشوميه. في المقاطع النسيجية تم العثور على جزء رئيسى من هذا الطفيلي في تجويف عميق فى الخياشيم.