SYNERGISTIC EFFECT BETWEEN THE PARASITIC INFESTATION AND BIOACCUMULATION OF HEAVY METALS IN FRESHWATER FISH

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Abstract

In the present study, attempts were made to observe the synergistic effect between parasitic infestation and bioaccumulation of heavy metals in *O. niloticus*. Adult *Acanthocephalans* (*Acanthosentis tilapiae*) were isolated from the intestinal tract of *O. niloticus* (collected from EL- Manzala fish cultured ponds), with an incidence rate of 70% and intensity 15 worm / fish .The concentrations of heavy metals, namely, Lead and Cadmuim in both water samples collected from EL- Manzala fish cultured ponds and flesh samples of stressed *O. niloticus*, were higher than those of the permissible limits reported by WHO(1984). Moreover, there were higher levels of heavy metals in samples of fish infested with *Acanthocephalan*, while, non-infested samples contained the lowest level. The haematological picture of *O.niloticus* stressed by *Acanthocephala* and heavy metals pollution, revealed reduction in RBCs count and highly significant increase in WBCs count, resuling in raising of antibodies level. The total serum protein was significantly decreased in stressed *O. niloticus*. Electrophoretic patterns of fish blood serum protein, revealed polymorphism, which appeared between the serum protein fingerprint of stressed and non-stressed fish, which may be due to the synthesis of more gamma globulins in blood of stressed fish.

INTRODUCTION

Man-mad pollution and / or intensification of fish culture resulted in an increase of environmental changes which may be stressful to fish (Lio-Po and Lim,2002) .

The relationship of parasitism and pollution involves a double-edged phenomenon, in which parasitization may increase host susceptibility to toxic pollutants, or in which pollutants may result in an increase (or in some instances a decrease) in the prevalence of certain parasites (Sinderman, 1990) .

Intestinal helminthes as cestodes and *Acanthocephalans* of fish are of increasing interest as potential bioindicators of heavy metals contamination in aquatic habitats (Sures,2003). Moreover, the same author recorded that, metal concentrations may reach several thousand times higher in *Acanthocephalans* than in host tissues .The combined exposure of the European eel *(Anguilla anguilla)* to Cd and Pentachlorobiphenyl (PCB 126) ,resulted in a complete suppression of the antibody response, this may be the reason why hosts exposed to environmental pollution became often much more easily infected with parasites(Sures and

Knopf, 2004). Furthermore, parasitism and pollution affect the physiological homeostasis of aquatic hosts (Sures, 2006) .

Acanthocephalans or thorny–headed worms are represented usually as adults in the intestine of fishes or as larvae (Juvenile worms) in the viscera. The most obvious sign of infestation is protrusion of the adult worms from the rectum and production of fibrotic nodules in the intestinal wall with enlargement and inflammation of the intestine (Nickol, 1995). Abd EL-Aal (1996) isolated *Acanthosentis tilapiae* from intestine and stomach of *Oreochromis niloticus* with highest prevalence in summer (42.86%) .

The industrial and agricultural discharges are considered the primary sources of heavy metals pollution in fish . The heavy metals are recognized as toxic substances to the fish due to their presence in surface waters (as lead and cadmium) and to the low rate of elimination from the body (Lioyd, 1992). EL-Shebly (1998) recorded that all values of heavy metals (Zn, Pb, Cd) in *Oreochromis niloticus* tissues (in EL-Manzala fish- farm) and in the farm water were higher than those of the permissible limit of WHO (1984) .

Both *Acanthocephalans* and some heavy metals (Pb and Cd) affect the general health and growth of the fish, where they reduce in RBCs count and significantly increase the white blood cell count (Feldman *et al.*, 2000) .Cyprinid fish were found to produce precipitating antibody in response to natural and experimental infections of an *Acanthocephalan Pomphorhynchus laevis* (Harris,1972).Also, immunosuppression by certain pollutants has been demonstrated in fish (Sinderman, 1990) .

The aim of this study was planned to isolate and identify the intestinal *Acanthocephalans* from cultured *O.niloticus* and determine their effect on the blood picture and the immunity system. The second aim is to determine the heavy metals concentration (lead and cadmium) in the water of the aquaculture, and muscles of infected fish, where, the intestinal Acanthocephalans of fish are considered as potential bioindicators for heavy metal concentration in aquatic habitats .

MATERIALS AND METHODS

1-Fish

A total of 100 freshwater cultured *Oreochromis niloticus (O.niloticus)* of different sizes (16-25 cm) and weights (80-200 g) were collected alive from EL- Manzala fish farm. The fish were transported to the lab of Fish Diseases Department , Animal Health Research Institute, Dokki, Giza, in large plastic bags under good conditions .The fish were stocked in glass aquaria with the same water of the fish farm ponds and supplied with oxygen and feeding on a maintenance ration .Fish were examined clinically for any abnormal clinical signs accordining to Noga (1996) .

2-Parasitological examination

Parasitological examination was carried out to determine the incidence and intensity of *Acanthocephalans* infestation . The isolated worms from the intestinal tract were identified according to Soulsby (1968), then, washed with PBS, fixed in 10% formol–saline and stained in Borax carmine (Noga,1996).

3-Estimation of heavy metals (Lead and Cadmium) in the water and *O.niloticus* muscles

Ten water samples were collected from EL- Manzala fish cultured ponds , at various distances from the outlet .Water samples were preserved by the addition of 1 ml concentrated Nitric acid per liter (Noga, 1996) ,until the time of analysis by using the Atomic Absorption Spectrophotometer (A.A.S 3300) .Tewenty *O.niloticus* were obtained from the same fish farm. These fish were examined firstly for *Acanthocephalans* infection and were divided into infested and non-infested groups. A muscle sample from each fish (about 1 g) was taken .The samples were wrapped in aluminium foil and stored frozen until analysis was carried out .The muscle samples were digested by using wet digestion methods described by Chapman and Pratt (1979), then were analysed by using the Atomic Absorption Spectrophotometer (A.A.S 3300) . The samples were estimated at Nutrition Institute (Ministry of health ,Egypt) .

4-Haematological picture

A total of 16 fresh blood samples (with EDTA) were randomly collected from *O.niloticus* fish stressed by *Acanthocephala* and heavy metals pollution, and other blood samples from control non–stressed *O.niloticus* fish. Edetated blood was used for haemoglobin concentration (Hb), erythrocytic count (RBCs)and total leucocytic count (WBCs) according to Stoskopf (1993) .

5-Electrophoretic analysis

A total of 12 fresh blood samples were collected from stressed and control non–stressed *O.niloticus* fish, without EDTA , to collect serum . Serum total protein was estimated, according to Stoskopf (1993).

Electrophoresis of serum was performed as described by Laemmli (1970) . The gel was stained with Coomassie brilliant blue R-250 (Sigma), and destained with mixture of 45% methanol, 10% acetic acid and 45% distilled water .Polyacrylamide gels were subjected to densitometric analysis, and peaks integration were accomplished using video ultrascan densitometer.

RESULTS

1-Incidence and intensity of Acanthocephaliasis in *O.niloticus*

In the present study, the total incidence of *Acanthocephalans* in the intestine of investigated *O.niloticus* fish was 70%, with intensity of 13 adult worms /fish(Table 1).

Table 1 .The incidence and intensity of Acanthocephaliasis in *O. niloticus* fish .

Fish	No. of examined fish	No. of infested fish	Rate of infestation %	Intensity of infestation /fish	Species of *Acanthocephalans*
Oreochromis niloticus	130	91	70	15	*Acanthosentis tilapiae* (Adult worm)

2-Taxonomy and morphological criteria of recovered *Acanthocephalan*

Order : Neoechinorhnchidea , Southwell et macfie (1925)

Family : Quadrigyridae , Van Cleav (1920)

Subgenus : Acanthosentis , verma &Datta (1929)

Species : Acanthosentis tilapiae , Baylis (1947)

The body of both sexes is elongated, cylindrical, gradually enlarging from anterior end to its middle, then, narrowing until the posterior extremity . The forebody is provided anteriorly with a short, globular proboscis and a proboscis sac for its invagination. The proboscis measures 0.073-0.119 by 0.053-0.092 mm and provided with 3 rows of hooks .The first row is six in number,while, the hooks of the second and the third rows are much smaller .There is a pair of lemnisci projection . They are cylindrical, subequal and much longer than proboscis receptacle .

Male : Fig. 1(a & b)

The body measures 1.672-5.831 mm in length and 0.528-1.14 mm in a maximum width . The testes are ovoid in shape, tandem in position and situated just behind the middle of the body . Posterior to the testes,there is a single large cement gland containing up to 10 nuclei followed by cement reservoir which opens into the ejaculatory duct . The copulatory bursa is located at the posterior end of the body .

Female : Fig.2(a, b & c)

The body measures 3.190-8.561 mm in length and 0.672-2.110 mm in maximum width. The reproductive organs are represented by numerous ovarian follicles mixed with the eggs in the whole body parenchyma .The uterus is represented by a short uterine tube . The eggs are small in size, elliptical or fusiform and measuring 0.020-0.045 by 0.006-0.026 mm(Fig.3) .

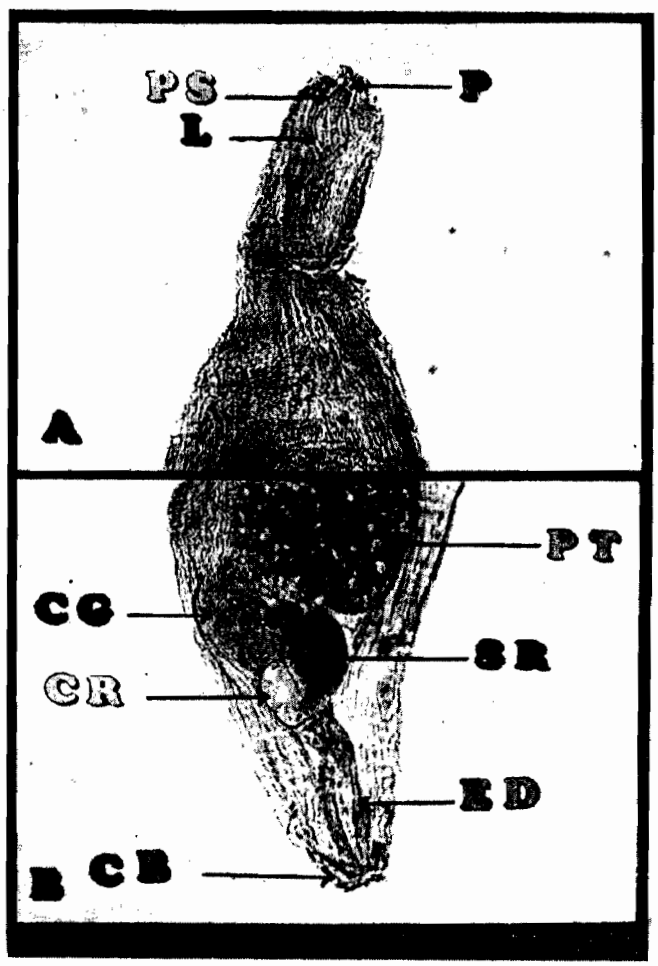

Fig. 1. Male of *Acanthosentins tilapiae*

A :Anterior end (X :100) .

P :Proboscis .

PS :Proboscis sac .

L :lemnisci

B : Posterior end (X :100) .

PT :Posterior testes .

CG :Cement gland .

SR :Seminal reservoir .

CR :Cement reservoir .

ED :Ejaculatory duct .

CB :Copulatory bursa .

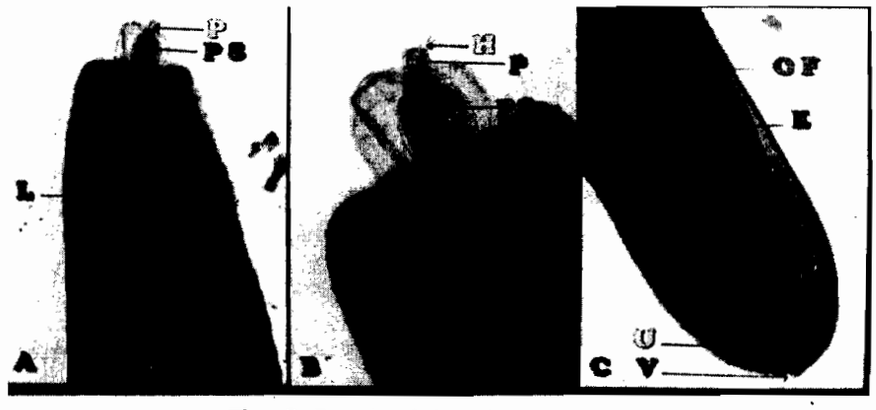

Fig . 2. Female of *Acanthosentis tilapiae*

A :Anterior end (X40) . B :Anterior end (X 100) .

P :Proboscis . H :Hooks .

PS :Proboscis sac . PS :Proboscis sac .

L :lemnisci .

C :Posterior end (X40) .

OF :Ovarian follicles . E :Eggs .

U :Uterus . V :Vagina .

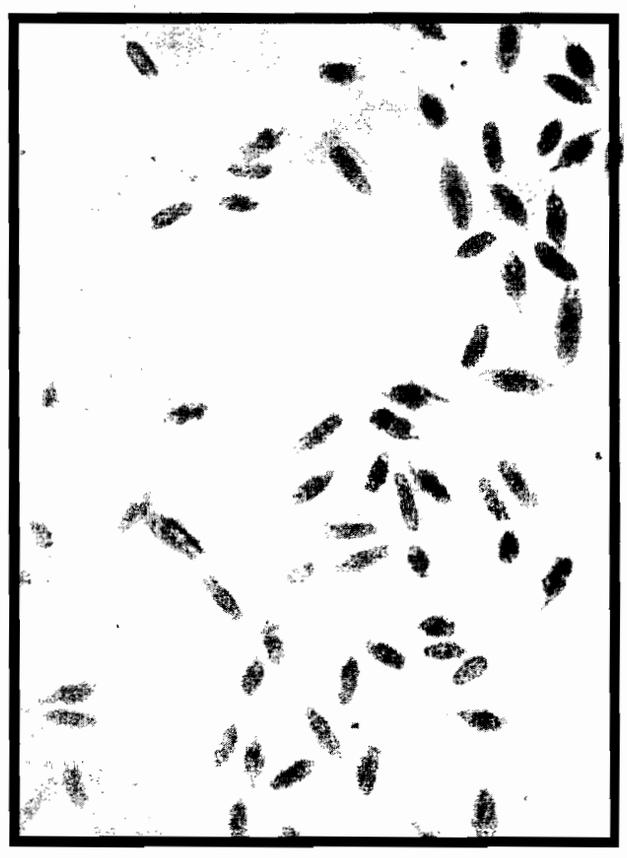

Fig . 3. Eggs of *Acanthosentis tilapiae* (X400)

3-Estimation of heavy metals (Lead and Cadmium)in the water and *O.niloticus* muscles

Table 2 denoted that the mean values of the heavy metals tested in water of EL-Manzala fish culture ponds and flesh of *O.niloticus* were higher than the permissible limit of WHO (1984) .Moreovere, there are higher levels of heavy metals recorded in samples of fish infested with *Acanthocephalan*, while non- infested samples contained the lowest levels.

Table 2. Estimation of heavy metals (Lead and Cadmium) in the water and flesh of *O.niloticus* from EL-Manzala fish cultured ponds .

Heavy metals	Permissible limit of WHO (1984)		Water of El-Manzala fish Cultured ponds	Flesh of *O. niloticus* infested with *Acanthocephala*	
	Water/ ppm	Fish flesh/ppm		Non- Infested	infested
Lead	0.050	0.6	0.42 ± 0.077	0.81 ± 0.11	0.92 ± 0.13
Cadmium	0.005	1.0	0.021 ± 0.016	2.82 ± 0.130	3.82 ± 0.230

4-Haematological picture

Table 3 showed the, reduction in RBCs count and the highly significant increase in WBCs count in blood samples stressed by *Acanthocephala* and heavy metals pollution. Also , the total serum protein was highly significantly decreased in stressed *O. niloticus* fish.

Table 3. Haematological picture of *O.niloticus* fish stressed by *Acanthosentis tilapiae* and heavy metals (Lead and Cadmium) pollution (X` ± S.E , n = 10) .

Haematological parameter / G Groups	RBCs (10)	Hb (g/100 ml)	PCV (%)	WBCs (10)	Differential lecuocytic count					Total protein (g/dl)
					Lymphocyte %	Monocytes %	Neutrophils %	Eosinophils%	Basophils%	
Control group	1.45 ± 0.12	6.9 ± 0.26	16.3 ± 0.42	6.2 ± 0.48	77.2 ± 2.6	3.6 ± 0.6	14.4 ± 0.8	4.1 ± 0.06	0.7 ± 0.02	4.49 ± 0.26
Acanthosentis tilapiae and heavy metals pollution group	1.12* ± 0.08	6.2 * ± o.12	13** ± 0.62	8.0 ± 0.76	76.4 ± 3.6	3.3 ± 0.8	14.6 ± 0.6	5.1*** ± 0.04	0.6 ± 0.03	2.65*** ± 0.20

* Significant at P 70.05 ** Significant at P 70.01 *** Significant at P70.00

5-Electrophoretic analysis

Polyacrylamide gel electrophoresis(Fig.4), showed the electrophoretic pattern of sera from non-stressed (lans-a,b and c) and stressed (lans-d, e and f) *O.niloticus* fish.The relative molecular weight value (Table 4, A) and the relative amount of serum protein (Table 4, B), in serum protein fractions, showed, polymorphism which appears between the serum protein fingerprint of stressed and non- stressed fish.

Fig. 4 . Electrophoretic pattern of sera from *O.niloticus* fish

non-stressed (lans-a,b and c) and stressed (lans-d, e,f)

Table 4 . The relative molecular weight value (Table 4, A) and the relative amount of serum protein (Table 4, B), in serum protein fractions of non- stressed and stressed fish.

A

Lanes:	Lane a	Lane b	Lane c	Lane d	Lane e	Lane f
Rows	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)
r1	0.061	0.055	0.065	0.056	0.067	0.065
r2	0.082	0.084	0.078	0.079		
r3				0.1	0.098	0.091
r4				0.15		
r5	0.16	0.16	0.016	0.16		
r6	0.19	0.19	0.019	0.18		0.18
r7	0.27	0.26	0.25	0.26	0.25	0.25
r8				0.28		
r9	0.36	0.36	0.35			
r10	0.45	0.45	0.44	0.45	0.46	0.46
r11	0.52	0.53	0.53			0.53
r12				0.55	0.54	
r13			0.59			
r14	0.61	0.64		0.62	0.62	0.62
r15	0.68		0.67	0.68	0.68	0.68
r16		0.71				
r17	0.73		0.74	0.73	0.73	0.73
r18	0.8	0.78		0.78	0.78	0.79
r19		0.83	0.81			
r20	0.87	0.89	0.87		0.89	0.89
r21	0.91			0.9		
r22		0.94	0.93			
r23				0.95	0.96	0.96

Table 4. (contd)

B

Lanes:	Lane a	Lane b	Lane c	Lane d	Lane e	Lane f
Rows	(amount)	(amount)	(amount)	(amount)	(amount)	(amount)
r1	2.22	2.28	1.99	1.47	2.51	2.61
r2	3.08	2.94	2.22	2.11		
r3				3.42	3.6	2.7
r4				1.78		
r5	2.56	3.02	0.49	2.45		
r6	3.13	3.29	2.04	3.36		2.74
r7	4.71	6.17	4.14	3.22	4.2	2.96
r8				3.64		
r9	4.88	4.3	4.77			
r10	2.86	4.01	3.99	5.53	3.8	2.46
r11	6.02	5.06	10			8.99
r12				10.1	7.76	
r13			10.6			
r14	7.82	6.13		8.29	8.44	8.69
r15	4.91		5.63	4.57	2.69	3.14
r16		4.31				
r17	3.22		4.56	4.94	2.35	4.2
r18	2.72	5.56		2.69	2.91	4.29
r19		2.11	2.2			
r20	3.76	4.36	4.59		2.54	3.21
r21	3.57			3.35		
r22		4.47	3.99			
r23				3.4	2.13	3.13
Sum	55.5	58	63.2	64.3	42.9	49.1
In lane	100	100	100	100	100	100

DISCUSSION

Acanthocephaliasis, a thorny -headed worm disease has been investigated as one of the important diseases in freshwater fish .*Acanthocephalans* embedded their spiny proboscis into the mucosal epithelium leading to chronic fibrinous inflammation.

In this work, the incidence and inensity of adult *Acanthosentis tilapiae* in the intestine of *O.niloticus* collected from EL-Manzala fish cultured ponds (Table 1) was70% and 13 worms /fish, respectively. These results coincided with Abd E- LAal (1996) who reported on the same parasite from the same host , but, in lower incidence . Such variation may be due to the difference in the water pollution from one locality to another and the environmental factors (as temperature) which affect abundance of *Acanthocephalans* intermediate hosts (microcrustaceans) .

Table 2 showed that the concentrations of heavy metals (Lead and Cadmium) in the water of EL-Manzala fish cultured ponds and the flesh of *O.niloticus* fish were higher than those of the permissible limit of WHO (1984) . These are due to presence of agriculture discharge (as Cadmium phosphate fertilizers), industrial discharge (from Superphosphate factories), and motor boats traffic, which are the main source of Lead pollution .These results are in agreement with those of Lioyd (1992) and El-Shebly (1998) . It is also evident from Table 2 that,higher levels of heavy metals were recorded in samples of fish infested with *Acanthocephalan*, while, non- infested samples contained the lowest level.This may indicate that there is a synergistic effect between parasitic infestation and bioaccumulation of heavy metals in fish,in which parasitism may increase host susceptibility to toxic pollutants, or in which pollutants may result in an increase in the prevalence of certain parasites . Moreovere, parasitism and pollution affect the physiological homeostasis of aquatic hosts . (Sinderman, 1990, Sures, 2003, Sures and Knopf 2004. Sures ,2006) .

The effects of *Acanthocephalans* infection and heavy metales pollution on the haematological pictures, in Table 3, showed the, significant decrease in the mean red cell counts, haemoglobin percent and the main haematocrit values in stressed *O.niloticus* fish in comparison to non stressed fish .The results may be attributed to *Acanthocephalans* embedding their spiny proboscis into mucosal epithelium and frequently between villi, leading to blood haemorrhage at the attachment site.Moreover, ther is highly significant increase in WBCs count of streesed fish, this could be referred to marked increase in the number of eosinophils and neutroplils . This result agrees with Feldman *et al.* (2000), who mentioned that total leukocytes increased in parasitic infection and some pollutant (as Cadmium).It is also is evident

from Table 3 that the total serum protein is highy significantly decreased in stressed *O. niloticus* fish, this result agrees with EL-Khatib and Elias (2003).

Polyacrylamide gel electrophoresis is the most effective method of investigation which provides an excellent opportunity to separate and calculate the ratio of protein fractions when influenced by any disease. The present study revealed polymorphism which appears between the serum protein fingerprint of stressed and non- stressed fish, which may be due to the synthesis of more antibodies (IgM) to *Acanthocephalans* antigens and pollutant. These antibodies have been circulating in the sera and are associated with gamma globulins in blood of stressed fish. (Harris, 1972, EL-Katib and Elias, 2003 and Sures, 2006).

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التأثير الإندماجى بين الإصابة الطفيلية و الأثر التراكمى للعناصر الثقيلة بأسماك المياه العذبة

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تناولت هذه الدراسة محاولات لمعرفة التأثير الأندماجى بين الاصابة الطفيلية و الأثر التراكمى للعناصر الثقيله بأسماك البلطى النيلى المستزرعه . وتم عزل ديدان بالغة من ديدان الأكانثوسيفالا (الديدان ذات الرأس الشوكية) من نوع الأكانثو سينتس من أمعاء أسماك البلطى النيلى والمجمعة من أحواض الإستزراع السمكى ببحيرة المنزلة و بلغت نسبة الإصابة 70 % وشدة الإصابة 15 دودة / سمكة .

وتم معايرة تركيزات المعادن الثقيلة (الرصاص و الكادميوم) بكل من عينات المياه وعضلات الأسماك (المصابة طفيليا ومعرضة للتلوث) المجمعة من أحواض الإستزراع السمكى ببحيرة المنزلة. أظهرت النتائج بأن نسب هذه العناصر فى كل من عينات المياه و عضلات الأسماك كانت تتعدى الحد المسموح به من قبل منظمة الصحة العالمية (1984). كما لوحظ أيضا أن هذه النسب كانت أعلى بعضلات الأسماك المصابة الأكانثو سينتس عن نظيرها الغير مصابة طفيليا.

تم تقييم تأثير الأصابة الطفيلية والتلوث على الأسماك من خلال صورة الدم و تشمل نسبة الهيموجلوبين وعدد كل من خلايا الدم الحمراء والبيضاء. وأظهرت النتائج إنخفاض نسبة الهيموجلوبين وزيادة عدد الخلايا البيضاء

وتم قياس نسب البروتين الكلى فى سيرم الأسماك حيث أثبتت التحاليل إنخفاضا ملحوظاً فى البروتين الكلى بالعينات المعرضة للإصابة الطفيلية والتلوث.

وأوضح التحليل الكهربى لسيرم الأسماك وجود تغيرات (بوليمورفيزم) ملحوظة والتى أستدل عليها من الإختلافات فى بصمة بروتينات السيرم بين الأسماك المعرضة للإصابة الطفيلية والتلوث و الأسماك الغير معرضة .ويرجع هذا البوليمورفيزم لتخليق أكثر للأجسام المناعية والتى تتحد مع الجاما جلوبيولين بسيرم الأسماك المصابة.