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Histopathological Studies on *Tilapia zillii* Fish in Relation to Parasitic Infestations at Lake Tamsah

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ABSTRACT

This study was carried out on 100 tilapias collected from Lake Tamsah, Egypt. All data collected seasonally. Body weight, total length and sex were examined for all fishes. Both parasitological and histopathological examinations of external and internal organs were carried out. The maximum growth in *T.zillii* was at summer season with body weight 112 g and length 18 cm, but the lowest growth was at spring season with body weight 20 g and length 9.5cm. The highly parasitic infestations appeared at winter season (40%), the parasites ranges between protozoa cysts which appeared clearly inside the intestine which was unidentified species and encysted metacercariae found in several organs such as gills, liver and spleen. Autumn season come next (36%) with infestation only by encysted metacercariae. The summer and spring seasons were equally in parasitic infestations (16%) and both seasons infested by encysted metacercariae in gills, liver, and spleen but at spring season the protozoa cysts appeared clearly inside the intestine. The parasitic infestations lead to severe histopathological alterations of infested organs ranged between low and high which were helped in disease diagnosis. These alterations included hyperplasia, degeneration and fusion of gill filaments. Vacuolar degeneration of hepatocytes, atrophy and necrosis of surrounding hepatocytes. Severe hyperplasia of melanomacrophage centers (MMC).

INTRODUCTION

Fish disease acts in cultured or wild fishes and diminishing the full fish production especially in tropic countries like Egypt. Parasitic diseases are considered an important problem than bacterial diseases in warm water fishes. Fish frequently serve as intermediate or transport host for larval stages of parasites in many animals, including humans.

Most of fish parasites are causing little or no harm to their hosts under the natural environmental conditions (Ali *et al.* 2015). In the same time, internal parasites are considered the first reason in the most fish diseases, causing low body gain, high mortality, immarketability and some of these diseases may have zoonotic importance. All degrees of harm may range in severity depending on the age and size of fish and parasite, as well as, parasites activity and the intensity of parasites (Eissa, 2002).

Many types of seafoods are a good source of vitamins B6 and B12 and also contain two other B vitamins known as niacin and riboflavin. Fish contain certain type of fatty acids called omega-3, which many scientists believe that it can reduce a person's risk for heart diseases and other illnesses. Minerals provided by various types of seafoods include magnesium, phosphorus, potassium, calcium, copper, iodine, selenium and zinc, so it is recommended that to eat seafoods which include the most important dietary supplements (WHO, 2003).

Fish suffer from diseases and parasites like humans and other animals. Fish defences against disease are specific and non-specific. Specific defences are specialized responses to specific pathogens known by the fish's body. Non-specific defences including skin, scales and the mucus layer which prevent microorganisms to sticks on the body and decreasing their growth. If pathogens penetrated these defences, fish can develop inflammatory responses that increase the flow of blood to infected areas and deliver white blood cells which destroy the pathogens. Lake Tamsah in Ismailia city, received a great amount of untreated domestic and industrial waste discharges and agricultural drainage return flows. This is because the lake is surrounded by industrial workshops for shipyards, residential areas and agricultural lands. Consequently, the lake and its beaches decrease the water quality in many locations. Moreover, substantial sediment loads entering the lake produce high accumulation substances rates and seriously obstruct lake transportation (Donia, 2011).

The histopathological studies are considered as a direct evidence referring to any adverse effect on fish. The most important fish organs affected by water pollution are gills, liver, and spleen (El-Sayed, 2015).

The present study aimed to investigate the most effective disease activity by making a general survey to parasites

affecting histopathology of green tilapia (*Tilapia zillii*) organs at Lake Tamsah.

MATERIALS AND METHODS

Collection of fish samples:

During the period extending from June 2014 to May 2015, a total number of (100) *Tilapia zillii* samples as (25/season) were collected from Lake Tamsah, The fish samples were kept at ice box with portable air pump with the same lake water to keep them approximately alive or almost in fresh state and transferred to the Fish Diseases lab in Faculty of "Veterinary Medicine, Suez Canal University, Ismailia, Egypt" to complete examinations.

Fish morphology:

Body weight, total length and sex of the collected fish samples were recorded.

Clinical and postmortem examinations:

Any clinical signs and postmortem lesions of the infested fishes were recorded to investigate any abnormalities using the methods described by Lucky (1977).

Parasitological examination:

External and internal parasitic examinations were carried out according to Bartholomew (2003).

Statistical analysis for parasitic prevalence seasonally:

Chi-square test was used for qualitative data and T-test for quantitative one to find statistical relations with parasitic infestations seasonally. All the analysis were performed using SPSS software.

Histopathological examination:

Gills, liver and spleen samples from infested fish were carefully removed and fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissue were embedded in paraffin wax and sectioned into five micrometers thickness, then stained with hematoxylin and eosin method according to Bancroft *et al.* (1996).

RESULTS

Fish morphology:

As shown in (Table 1) The maximum growth in *T. zillii* was in summer season with

body weight 112 g and length 18cm, while the lowest growth was in spring season with body weight 20 g and length 9.5cm.

Table 1: Fish body weight (g), total length (cm) and sex of tested *Tilapia zillii* at Lake Temsah.

Parameters		Summer	Autumn	Winter	Spring
Body Weight (g)		24-112	22-97	30-63	20-62
Total Length (cm)		10.5-18	10.5-16	10-14.5	9.5-15
Sex No.	M	14	15	10	15
	F	11	10	15	10
Total		25	25	25	25

(M) males, (F) females

Clinical picture of naturally infested fish:

The clinical symptoms ranged from dark coloration (Plate 1,a), erythema on the abdominal and gill parts (Plate 1,b) and enlargement in the internal organs *i* with dark intestinal coloration (Plate 1,c).

Parasitological Findings:

Protozoa:

The protozoa cysts are single celled with circle or oval shape, with medium wall thickness. The cysts could not be identified due to difficulties facing identification methods. It was found non motile free living inside the intestine, based on the morphological and parasitological investigations, protozoa considered as subkingdom of the kingdom Animalia. (Plate, 2 a).

Digenetic trematodes:

They are spherical or oval shape, double walled, outer thick and inner shade wall, separated from the metacercaria by a space containing fluid in which the metacercaria was moving. Pigmented granules were present in this space. The layers differed in thickness levels according to the place of metacercariae inside the host. As shown in gills the inner granules locks like H or Y-shaped (Plate, 2 b), but in liver

these granules was spread inside the inner wall, metacercariae usually found alone or collected at groups together (Plate,2 c). In spleen, metacercariae found large with inner granules collected in one side of the inner wall helped in movement (Plate, 2 d). Based on the morphological and parasitological investigations, trematodes identified as phylum Platyhelminthes, class Trematoda, subclass Digenea. All species of encysted metacercariae found as living motile organisms but of unknown species due to difficult methods of identifications.

Seasonal parasitic findings:

The highly parasitic infestations appeared in *T. zillii* fish was found at winter season at a rate of (40%) with protozoa cysts and encysted metacercariae in gills, liver and spleen, followed by autumn (36%) which infested by only encysted Metacercaria in gills, liver and spleen. The summer and spring seasons were equally in parasitic infestation percentage (16%), but in spring the protozoa cysts appeared inside the intestine. All parasitic species appeared during examination were unknown species due to complicated steps required for identification. (Tables 2 &3).

Table 2: Total and seasonal prevalence of parasitic infestations in *T. zillii*.

Season	No. of Examined fish	No. of Infested Fish	%	Parasites species
Summer	25	4	16	Encysted Metacercariae in liver and spleen
Autumn	25	9	36	Encysted Metacercaria in gills, liver and spleen
Winter	25	10	40	-Protozoa cysts -Encysted metacercariae in gills, liver and spleen
Spring	25	4	16	-Protozoa cysts Encysted metacercariae. in gills, liver and spleen
Total	100	27	27	

Table 3: Total and seasonal prevalence of parasitic infestations in males (M) and females (F) of *T. zillii*.

Season		No. of Examined fish	No. of Infested Fish	%	Parasite Species
Summer N=25	M	14	2	14.29	Encysted metacercariae in spleen
	F	11	2	18.18	Encysted metacercariae in spleen and liver
Autumn N=25	M	15	7	46.67	Encysted metacercariae in gills, spleen and liver
	F	10	2	20	Encysted metacercariae in gills, spleen and liver
Winter N=25	M	10	4	40	Encysted metacercariae in gills and spleen
	F	15	6	40	-Protozoa cysts -Encysted metacercariae in gills, spleen and liver
Spring N=25	M	15	2	13.33	-Protozoa cysts -Encysted metacercariae in gills
	F	10	2	20	-Protozoa cysts -Encysted metacercariae in gills, spleen and liver
Total		100	27	27	

N=Samples number

Seasonal single and mixed parasitic infestations in *T. zillii*

As shown in Table 4, the maximum single parasitic infestations were at autumn season (36%), followed by winter season (20%). The minimum single infestations recorded at spring (12%) and summer (16%) seasons. Mixed infestations recorded only in winter (20%) and spring (4%) seasons with protozoa cysts and encysted metacercariae in the same host.

Body weight and total length in relations with parasitic infestations seasonally in *T. zillii*.

Body weight:

As shown in Table 5, the maximum group examined was $\geq 30-50g$ (49fishes) with parasitic infestation 28.57% by Protozoa cysts inside the intestine and encysted metacercariae in gills, liver and spleen and the minimum group examined was $\geq 70-90g$ (5fishes) with no parasitic infestations.

Table 4: Single and mixed parasitic infestations in *T.zillii* seasonally.

Season	Single infestation	%	Parasitic species	Mixed infestation	%	Parasitic species
Summer N=25	4	16	-Encysted metacercariae In liver and spleen	0	0	-
Autumn N=25	9	36	-Encysted metacercariae in gills, liver and spleen)	0	0	-
Winter N=25	5	20	-Encysted metacercariae In gills, liver and spleen	5	20	Protozoal cysts inside the intestine+ Encysted metacercariae in gills, liver
Spring N=25	3	12	-Encysted metacercariae In gills, liver and spleen	1	4	Protozoal cysts inside the intestine +Encysted metacercariae in gills, liver and spleen
Total	21	21		6	6	-

N=Samples number

Table 5: Body weight range of *T.zillii* and relations between examined and infested fishes.

Weight Range (g)	Examined no.	Infested no.	%	Parasitic species
≥ 10-30	27	5	18.52	-Protozoa cysts inside the intestine -Encysted metacercariae in gills, liver and spleen
≥ 30-50	49	14	28.57	-Protozoa cysts inside the intestine -Encysted metacercariae in gills, liver and spleen
≥ 50-70	13	6	46.15	-Encysted metacercariae in gills and liver
≥ 70-90	5	0	0	
≥ 90-120	6	2	33.33	-Encysted metacercariae in spleen
Total	100	27	27	

Total length:

As shown in Table 6, the maximum group examined was ≥ 10-15cm (91fishes) with parasitic infestation 26.36% with Protozoal cysts inside the intestine and encysted metacercariae in gills, liver and spleen, while the minimum group examined was ≥ 15-20cm (4 fishes) with parasitic infestation 50% with only encysted metacercaria.

Statistical analysis for parasitic prevalence seasonally:

As shown in Table (7), *T. zillii* males recorded parasitic infestations 25% while

females was 28.3%, so that there were no statistically significant difference ($p=0.793$) between males and females parasitic infestations.

At the next Table 8, the mean values of body weights of *T. zillii* infested fishes were 46.73 ± 14.96 which indicated to no statistically significant difference ($p=0.114$) between the body weight and parasitic infestations, while total length means were 12.72 ± 1.27 which indicated that there were no statistically significant difference ($p=0.61$) between total length and the parasitic infestations.

Table 6: Total length range of *T.zillii* and relations between examined and infested fishes.

Total length range (cm)	Examined no.	Infested no.	%	Parasitic species
≥ 5-10	5	1	20	-Encysted metacercariae in liver
≥ 10-15	91	24	26.37	-Protozoal cysts inside the intestine -Encysted metacercariae in gills, liver and spleen
≥ 15-20	4	2	50	-Encysted metacercariae in liver and spleen
Total	100	27	27	

Table 7: Showing significant differences between males (M) and females (F) parasitic infestations

Fish Species	Infested No.	%	p value
<i>T.zillii</i> N=27	M	14	25.9
	F	13	28.3
			0.793

(M) Males, (F) Females, (N) Infested fish numbers, ($p<0.05$): means there were statistically significant difference between values.

Table 8: Showing relations between body weight and total length Mean±SD and p values in infested *T.zillii*.

Fish Species	Mean ± SD	p value
<i>T.zillii</i> N=27	Body Weight	46.73 ± 14.96 0.114
	Total Length	12.72 ± 1.27 0.61

(N) Infested fish numbers, (SD) Slanderred Deviation, ($p<0.05$): means there were statistically significant difference between values.

Histopathological Findings:

Micrographs of histological sections from *Tilapia zillii* fish revealed mild to severe changes in different infected organ. These changes included hyperplasia, degeneration and fusion of gill filaments (Plate 3 a). Concerning liver, vacuolar degeneration of hepatocytes and atrophy and necrosis of surrounding hepatocytes (Plate 3 b & c). However for spleen, severe hyperplasia of melanomacrophage centers (MMC) (Plate 3 d & e). for intestinal histopathological changes showing damage of the villi, inflammation and fibrosis associated with hyperplasia and metaplasia epithelial necrosis and dilation of blood vessels (Plate 3 f).

DISCUSSION

Fish in wild and aquaculture farms face a lot of enemies including rivals, predators and parasites, so this study introduced to suppose parasitological and histopathological changes in green tilapia (*Tilapia zillii*) which considered one of the most traditional species at Lake Tamsah in Ismailia city around whole of the year; from June 2014 to May 2015.

Clinical signs and postmortem examinations for the examined fishes showed little irritations with excessive mucus on gills and scales if low or moderate infestations, but the fish revealed severe infestations had hemorrhages on the gills and some body parts had dark coloration. These results were in agreement with what found by Elsheikha and Elshazly (2008); Abdallah *et al.*, (2009) and Taher (2009) about finding similar signs associated with presence of encysted metacercariae in *Oreochromis niloticus* and *Tilapia zillii* in Egypt.

Tilapia fishes are the second most popular cultured species in the world after the carps (FAO, 2009). The examined fishes listed their body weight and total length. The maximum body weight was (112g) and the maximum total length (18cm) which recorded during summer season. This was in agreement with Gophen (2016) who

mentioned that the spawning season for tilapia was summer season when the fishes recorded its maximum growth.

The maximum parasitic infestations noticed at winter season (40%) and the low infestations were at spring and summer (16%). This was in agreement with what found by Georges *et al.* (2016) who found that the presence of monogenea presented its maximal prevalence and mean intensity during the winter seasons which starts from November. Blahoua *et al.* (2009) also reported that *Scutogyrus minus* parasite appeared more widespread and more plentiful during the rainy season.

The highly parasitic infestations appeared in males at autumn season (46.67%) followed by males and females of winter season (40%). The lowest infestation appeared in males of spring season (13.33%), so that there was no significant correlation between the host sex and the infestation density seasonally. These were similar with those stated by Boungou *et al.* (2008) and Tombi *et al.* (2014) who observed no sex effect on the parasitism of the same host gill filaments of tilapia fishes.

The most common detected parasitic species were intestinal protozoa cysts, this was in agreement with what mentioned by Bichi and Ibrahim (2009) who detect the protozoa cysts inside the intestine, and digenetic trematodes as encysted metacercariae in different parts of the fish body, these findings were in agreement with what stated by Gamal (2009) who listed different species of encysted metacercariae in different body parts of the examined fishes in Assiut Governorate.

Histopathological sections could be used as identification tools for the studies deals with signs of disease Zakia *et al.* (2012). Fish diseases and histopathology, with a broad range of causes, are increasingly being used as indicators of environmental stress since they provide a definite biological end-point of historical exposure. Wild fish have greater parasite diversity but with lower population

abundance and the reverse is true for cultured fish. Consequently, the present study revealed that only undifferentiated nematode larvae and/or heterophyid metacercariae parasitized the gills, liver and spleen of a high percentage of examined cultured tilapia (El-Naggar *et al.*, 2009).

CONCLUSION

From the present study, it could be concluded that the highly parasitic distribution in *Tilapia zillii* fish was at winter season with clear histopathological alterations, and was shown in females higher than males.

It could be also concluded that, the areas of investigation at Lake Tamsah are affected by industrialization, agricultural and sewage effluents, which affect water quality, fauna, fish production and human health. It is recommended to treat different wastes before discharging to the lake.

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Plate 1: (a) Showing *T. zillii* fish with dark coloration.(b) Showing *T. zillii* fish erythema on the abdominal and gill parts. (c) Showing enlargement in the internal organs of *T. zillii* with dark intestinal coloration.

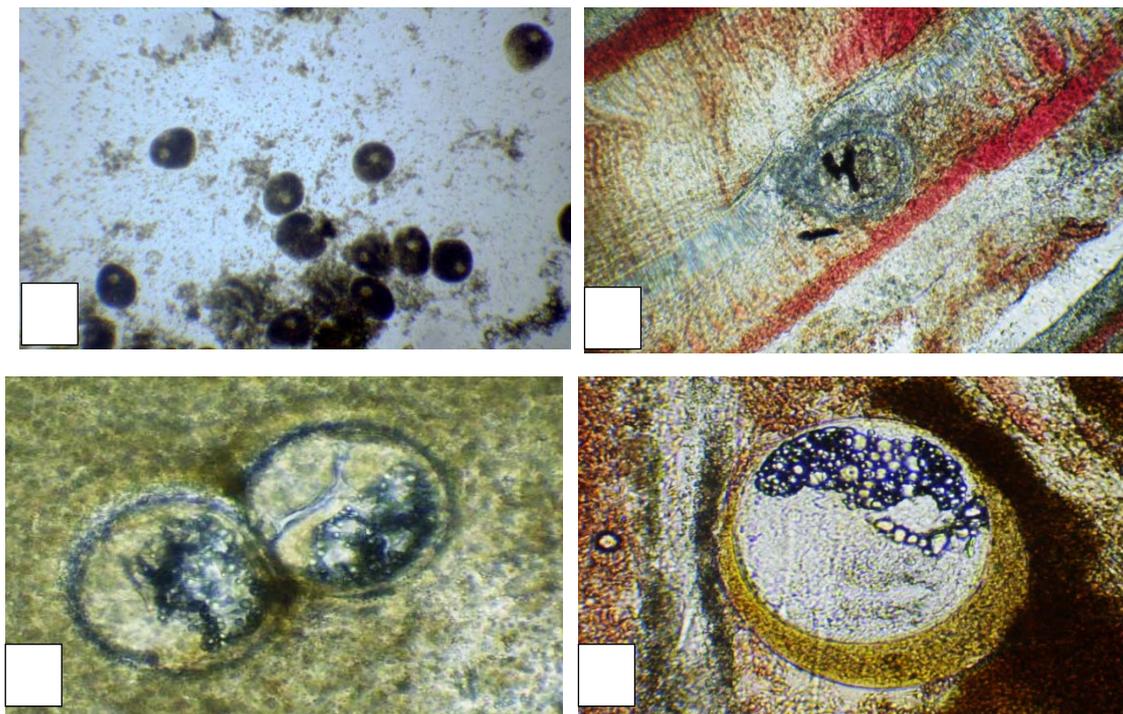


Plate 2: (a) Protozoa Cysts inside the intestine of *T. zillii* (Fresh specimen) 40X. (b) Encysd metacercaria embedded in gills of *T. zillii* (Fresh specimen) 40X. (c) Encysted metacercariae embedded in liver cells of *T. zillii*. (Fresh specimen) 40 X. (d) Encysted metacercaria embedded in spleen cells of *T. zillii*. (Stained with Acetic Acid Alum Carmine) 40X.

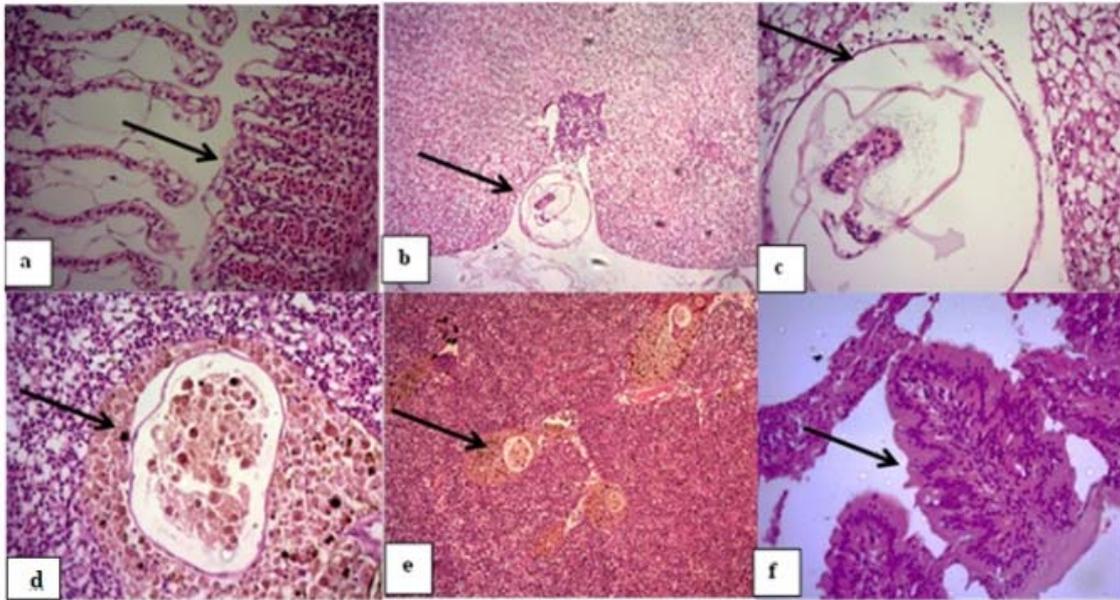


Plate 3: (a) Gills of *T. zillii* showing hyperplasia, degeneration and fusion of gill filaments. H&E. 400X. (b) Liver of *T. zillii* showing encysted metacercaria along with diffuse vacuolar degeneration of hepatocytes and atrophy and necrosis of surrounding hepatocytes. H&E 100X and (c) Liver of *T. zillii* showing encysted metacercaria with degeneration of hepatocytes and necrosis of surrounding hepatocytes. H & E. 400X (d) Spleen of *T. zillii* showing encysted metacercaria with severe hyperplasia of melanomacrophage centers (MMC). 400X (e) Spleen of *T. zillii* showing multiple encysted metacercariae along with severe hyperplasia of melanomacrophage centers (MMC). H&E. 100X. (f) Intestine of *T. zillii* showing damage of the villi, inflammation and fibrosis associated with hyperplasia and metaplasia epithelial necrosis and dilation of blood vessels. H&E. 100X.

ARABIC SUMMERY

دراسات هستوباثولوجيه على أسماك البلطى الأخضر (الزليلي) وعلاقتها بالاصابات الطفيليه فى بحيرة التمساح

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مى نشأت

أجريت هذه الدراسة على ١٠٠ من أسماك البلطى الأخضر (الزليلي) وقد جمعت هذه العينات عشوائيا بأوزان و أطوال مختلفه بواسطة الصيادين فى بحيرة التمساح بمحافظة الاسماعيلية وذلك على مدار الأربيع فصول من يونيو ٢٠١٤ حتى مايو ٢٠١٥.

وقد كانت النتائج المجمعمة كالأتى:

نتائج الفحص الطفيلي:

- بعد الفحص الأكلينيكي لأسماك الدراسة وجدت بعض الأعراض المرضيه الواضحه داخليا وخارجيا و كانت تتمثل فى بعض النزف والقروح.
- نتائج الفحص الطفيلي أوضحت وجود نوعين من الطفيليات وهى البروتوزوا وكانت حويصلات غير معرفه بداخل المعدة و الميتاسيركاريا المتحوصلة والتي ظهرت فى أكثر من عضومثل الخياشيم والكبد والطحال.
- سجلت أعلى نسبة إصابة طفيلية فى فصل الشتاء ٤٠% وكانت أكثرها فى الإناث ٢٤% أما الذكور فكانت ١٦%. أما أقل نسبة إصابة فقد سجلت فى فصلى الصيف والربيع ١٦% حيث تساوت الإصابة بين الذكور والإناث ٨%.
- تتنوع نسبة الإصابة بالأمراض الطفيليه حسب الأوزان و الأطوال، فقد سجلت مجموعة أوزان $50 \leq$ - ٧٠ جرام (٤٦.١٥%) و مجموعة أطوال $10-15$ سم (٢٦.٣٧%) أعلى نسبة إصابة وكانت معظمها بحويصلات البروتوزوا والميتاسيركاريا.
- أظهرت الدراسه عدم وجود فروق معنويه فى نسبة الإصابة الطفيليه بين الإناث والذكور فى أسماك البلطى الأخضر وأيضا سجلت عدم وجود فروق معنويه بين أطوال وأوزان الأسماك المصابة بالطفيليات على مدار مدة الدراسة

النتائج الفحص النسيجي

كل القطاعات النسيجه التي تمت للأعضاء المصابة بالطفيليات فى أسماك الدراسة أظهرت تأثير حاد فى الأنسجة كما أظهرت وجود بعض الطفيليات بداخل هذه الأنسجه والذى تسبب فى تدمير كامل لهذه الأنسجه ووظائفها.