

## Impact of Parasitic Infection and Water Quality on the Bagrid Fish, *Bagrus bajad*, Inhabiting Ismailia Canal Waters, Egypt

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### ABSTRACT

A histopathological study was organized addressing the impact of endo-parasitic infection and water quality on 296 *Bagrus bajad* collected from Ismailia Canal waters from March 2021 to September 2021. Endoparasites were shown to infect 68.92% of fish. Based on the type of parasite, the highest parasitic infection was associated with trematodes, followed by nematodes and cestodes. Some physico-chemical parameters such as water temperature, pH, ammonia, nitrite, nitrate, total alkalinity, dissolved oxygen, hardness and total dissolved solids have been simultaneously investigated. The prevalence of parasites recorded an increase during summer. Histopathological changes in the infected fish's intestine, liver and muscles were also recorded. Pathological alterations in the intestine of the infected fishes showed necrotic enteritis, manifested by desquamated and necrotic villus epithelium and inflammatory cell infiltration within lamina propria and submucosa. Whereas, the liver of the infected fishes showed devastating focal areas of congested necrosis, represented by losses of cellular details. Moreover, the muscles of the infected fishes showed chronic inflammatory reactions around the remnant of parasites and degeneration of myofibers with focal areas. In conclusion, the parasitic infection might have negative impacts on fish wealth and human health.

### INTRODUCTION

Aquaculture has become a major source of low-cost protein in many countries. Fish production has doubled in the last 10 years, especially in Egypt, where aquaculture accounts for more than 80% of the total fish production (Gafred, 2019; David *et al.*, 2021). Moreover, aquaculture is considered one of the fastest-growing food industry sectors, and it plays an essential role in mitigating the increasing demand for animal protein (Shah & Mraz, 2020; Mansour *et al.*, 2021). Parasitism is still considered an important factor for causing morbidity and mortality worldwide, resulting in heavy economic loss in fishing crops (Khanum *et al.*, 2015). The endoparasites of fish are highly prevalent in different waterbodies. Therefore, there should be a nationwide strategy for preventing and controlling endoparasites of the fish, and further study should

be conducted on the economic and public health impacted by these parasites (Gebremedhn & Tsegay, 2017). Parasites constitute a major limiting factor to fish growth (Bichi & Yelwa, 2010). Helminth infections in fish have a great impact on its production in relation to fish health, such as declining the host's immune system due to the pathogenic effects of secondary infections, depriving fish of essential nutrients, causing morbidity, mortality, and subsequently economic loss (El-Seify *et al.*, 2015; Nguyen *et al.*, 2021). Water spreads most of the pathogens easily. Therefore, it is necessary to have an understanding of water quality in order to successfully diagnose and correct aquarium diseases. Stress has been linked as the primary factor to fish disease and mortality in aquaculture (Petri *et al.*, 2006). Fish diseases and histopathology, with a broad range of causes, can be used as indicators of environmental stress since they provide a definite biological endpoint of histological exposure; it is a mechanism that can indicate fish health by determining early injury to cells and can therefore be considered an important tool to determine the effect of parasites on fish tissues (Fartade & Fartade, 2016). Moreover, histopathological biomarkers can be sensitive indicators of subcellular stress in organisms exposed to a range of pollutants over short and long periods (Adams *et al.*, 2000).

The present work aimed to study the impact of some environmental factors such as water temperature, pH, ammonia, nitrite, nitrate, total alkalinity, dissolved oxygen, total hardness and total dissolved solids; seasonal variations of parasitic infection and prevalence of endoparasites that infect the freshwater fishes, *Bagrus bajad*, inhabiting Ismailia Canal waters, as well as investigating the histopathological alteration of different target organs of the infected fishes.

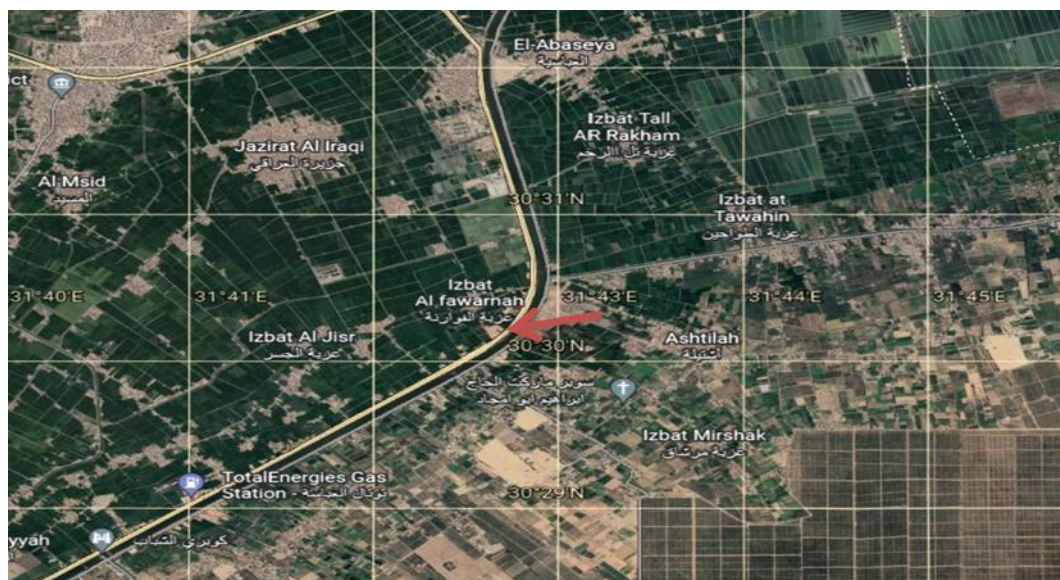
## MATERIALS AND METHODS

### Water quality parameters

Water temperature, pH and total dissolved solids were determined on spot during sampling using a portable pH meter with a thermometer (Adwa 11,31). A dissolved oxygen meter measured the dissolved oxygen concentration in water (Hanna model HI 9146). Total alkalinity, total hardness, ammonia, nitrate and nitrite concentrations were evaluated according to methods described by APHA (2000).

### Collection of fish samples

A total number of 296 freshwater bajaded fish, *Bagrus bajad*, were collected from Ismailia Canal waters during the period from March 2021 to September 2021, forming the material for the present study (Fig. 1). After collection, fish were transferred to the Marine Biology Department, Faculty of Science, Al-Azhar University, Egypt in ice box after packing in plastic bags. Male and female fish were individually examined and recorded.



**Fig. 1.** Image of google earth map showing the location of the study area

### Parasitic infection

After collection, fish were immediately dissected. A cut was made on the ventral side from the anal opening to the lower jaw. Then, two more cuts were made on the lateral side to expose the body cavity with the alimentary canal and other internal organs. The surface of the visceral organs, body cavities and serous membranes were examined for parasitic infection by using a hand lens. The alimentary tract was removed, cut into parts (stomach and intestine) and placed in 0.09% physiological saline for parasite recovery under a dissecting microscope. Each part was further carefully slit open to aid the emergence of parasites. Gastrointestinal parasites were further recognized by their wriggling movements on emergence in normal saline under the microscope (Marcogliese, 2011). Moreover, fish specimens were macroscopically examined, both externally and internally, for the presence of macroscopic encysted metacercariae with the aid of a magnifying hand lens to detect any changes in the visceral organs and muscles and microscopically by using direct compression between two glass slides (Mwita & Nkwengulila, 2008). Metacercariae identification was based on those dissected directly from the tissues. The general feature was separately collected and tentatively identified to species level based on the morphological details; their dimensions were measured, and their occurrence in single or in groups (EL-Shahawy *et al.*, 2017) was determined, and they were directly photographed without staining.

The collected trematodes and cestodes were fixed in a 5% formalin solution, stained with acetic acid alum carmine, cleared in xylol, mounted in Canada balsam and photographed. Nematodes were kept in glycerin alcohol, washed in 70% ethyl alcohol, cleared in lactophenol and mounted in glycerol gelatin (Meyer & Olsen, 1992). The prevalence of parasites was estimated according to the following formula:

$$\text{Prevalence} = \frac{\text{No. of infected fish}}{\text{No. of examined fish}} \times 100$$

(Margolis *et al.*, 1982).

### Identification of isolated parasites

Isolated parasites were collected separately and provisionally identified at the species level based on morphological details. Their dimensions were measured, and whether they occurred singly or in groups was noted. They were directly photographed without staining (Elsheikha & El-Shazly, 2008; El-Shahawy *et al.*, 2017). Helminths were identified according to Yamaguti (1961) and Cheng (1973). Identification of cestodes and trematodes depended on the morphological details of Oros *et al.* (2010).

### Histopathological study

Autopsy samples were taken from fish liver, intestine and muscles and fixed in 10% formalin. Washing was done in tap water, then serial dilutions of alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin wax. Paraffin wax tissue blocks were prepared for sectioning at 4 micra by slide microtome. The prepared sections were collected on glass slides, deparaffinized, stained with hematoxylin and eosin stain, examined under the electric light microscope and photographed (Banchroft *et al.*, 1996).

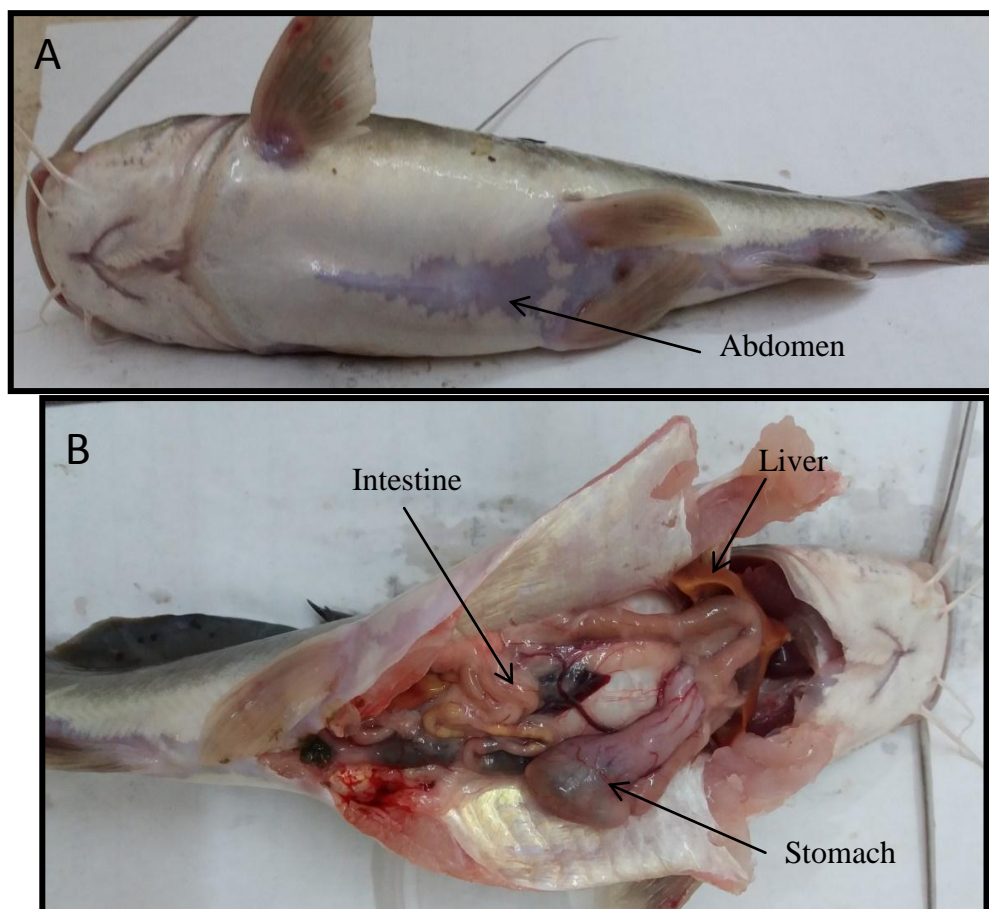
## RESULTS

### Physico-chemical characters

The maximum average value of surface water temperature was recorded during summer (29.25°C) and the lowest (24.13°C) during spring. Concerning hydrogen ion concentrations (pH), the highest average value was recorded during summer (7.84) and the lowest (7.34) during spring. On the other hand, the highest average value of total ammonia (0.82mg/ L) was recorded during summer, and the lowest (0.56mg/ L) was observed during spring. Nitrate concentration fluctuated between 0.28mg/ L during spring and 0.39mg/ L during summer. However, nitrite concentration ranged between 0.01mg/ L during spring and 0.04mg/ L during summer. The maximal value of total alkalinity was recorded during summer (239.66mg/ L) and the minimal value (178.24mg/ L) during spring. In contrast, the highest dissolved oxygen value was recorded during spring (7.14mg/ L) and the lowest (6.62mg/ L) during summer. Moreover, the maximum value of total hardness was recorded during summer (285.97mg/ L) and the minimum value (214.33mg/ L) during spring. However, the highest value of total dissolved solids was recorded during spring 0.163mg/ L and the lowest (0.151mg/ L) during summer.

### Clinical signs and postmortem lesions

Clinical signs and postmortem lesions in the infected fish showed skin darkness, erosion of the skin, enlarged abdominal region and enlarged general viscera (Fig. 2).



**Fig. 2.** Photomicrographs of dissected *B. bajad* showing: enlarged abdominal region (A) and general viscera, liver, stomach and intestine (B).

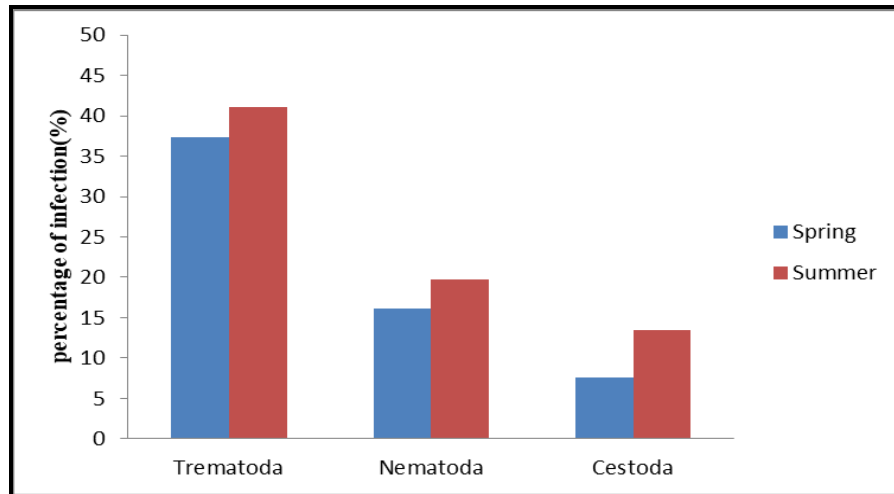
#### **Seasonal variation of prevalence of endoparasites in *B. bajad***

Table (1) and Fig. (3) reveal that the maximum prevalence of trematodes in *B. bajad* was recorded during summer (41.01%), and the minimum (37.29 %) was observed during spring. The maximal prevalence of parasitic cestodes was recorded during summer (13.48%); it dwindled by about two folds during spring (7.63%). Concerning nematodes; however, the highest value (19.66 %) was recorded during summer and the lowest (16.10 %) during spring. At all parasitic infections and the two seasons, the highest parasitic infection in *B. bajad* was recorded in trematodes (41.01%) during summer; and the lowest in cestodes (7.63%) during spring. Moreover, the endoparasites in this fish can be ordered as follows: Trematoda > Nematoda > Cestoda.

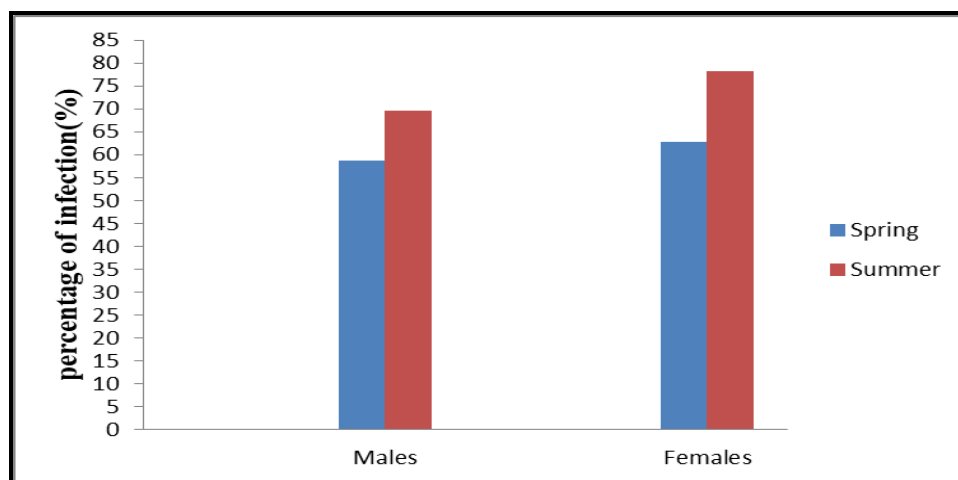
Results showed that the prevalence of parasitic infection is according to sex groups in *B. bajad*, the highest value of parasitic infection was recorded during summer, and the lowest value was observed during spring, with values of 69.51% and 58.82%, respectively, in male groups and 78.13% and 62.69% in female ones (Table 2 & Fig. 4).

**Table 1.** Seasonal variations of parasitic infection in *B. bajad* according to the type of parasites

Type of parasite	Seasons	Spring (118)	Summer (178)
		<b>No. of infected fish</b>	44
Trematoda	<b>Percentage of infection (%)</b>	37.29	41.01
	<b>No. of infected fish</b>	19	35
Nematoda	<b>Percentage of infection (%)</b>	16.10	19.66
	<b>No. of infected fish</b>	9	24
Cestoda	<b>Percentage of infection (%)</b>	7.63	13.48

**Fig. 3.** Prevalence of parasitic infection in *B. bajad* according to the type of parasite**Table 2.** Seasonal variations of parasitic infection in *B. bajad* according to sex groups

Sex	Seasons	Spring	Summer
		<b>No. of examined fish</b>	51
Males	<b>No. of infected fish</b>	30	57
	<b>Percentage of infection (%)</b>	58.82	69.51
	<b>No. of examined fish</b>	67	96
Females	<b>No. of infected fish</b>	42	75
	<b>Percentage of infection (%)</b>	62.69	78.13



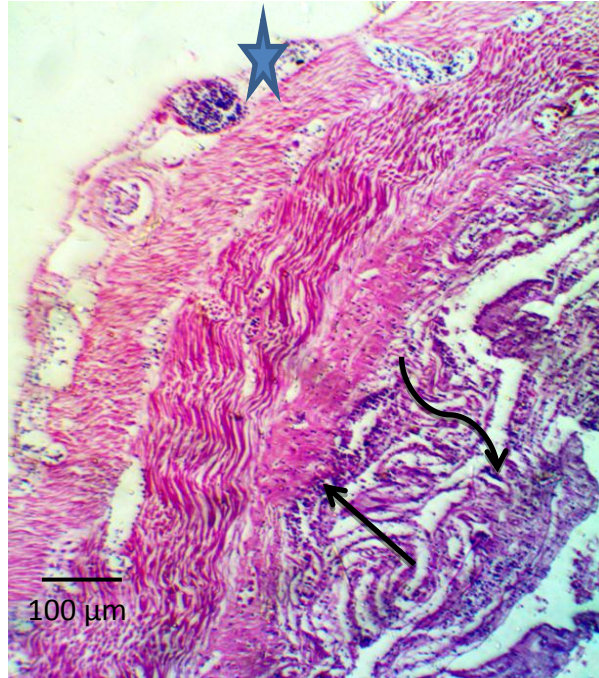
**Fig. 4.** Prevalence of parasitic infection in *B. bajad* according to sex groups

### Histopathological changes in the target organs of the infected *B. bajad*

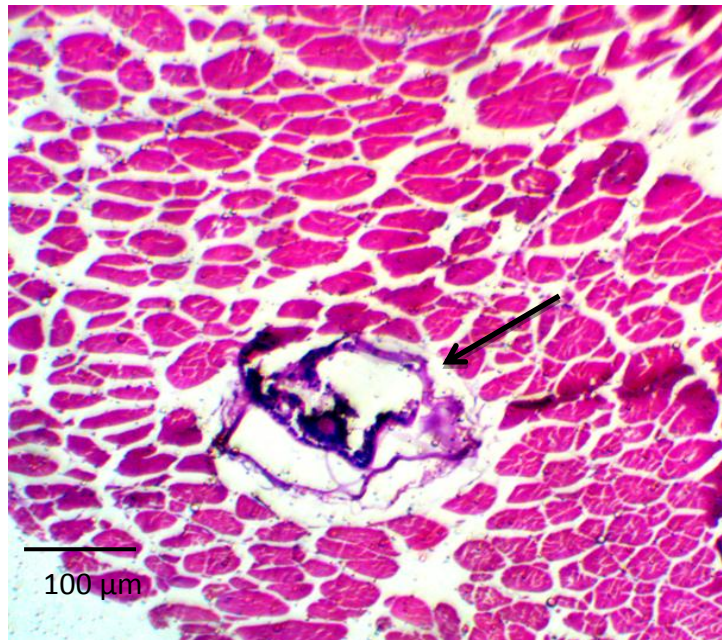
Results revealed that the liver of the infected *B. bajad* (Fig. 5) showing focal areas of congested necrosis was represented by the loss of cellular detail and maintained tissue architectures, with pyknotic nuclei and more eosinophilic cytoplasm. Most hepatocytes revealed degenerative changes, mostly vacuolations. Congested blood vessels with thrombi formations were also detected. The intestine showed necrotic enteritis manifested by desquamated and necrotic villus epithelium, inflammatory cells infiltrating lamina propria and submucosa. Additionally, cross-sections of enteric parasites were seen within intestinal lumina (Fig. 6). Muscles showed chronic inflammatory reactions around the remnant of parasites. Moreover, the destruction and degeneration of myofibers with focal areas of inflammatory cell aggregates, mainly eosinophils and lymphocytes, were distinctly observed (Fig. 7).



**Fig. 5.** Section of liver of infected *B. bajad* showing: focal areas of coagulative necrosis (arrow) with degenerative changes in most hepatic parenchyma. H&E (Scale bar=100  $\mu$ m).



**Fig. 6.** Section of the intestine of infected *B. bajad* showing: necrotic enteritis with desquamated and necrotic villus epithelium (curved arrow), inflammatory cells infiltrations within lamina propria and submucosa (arrow) and cross sections of enteric parasites within intestinal lumina (star). H&E (Scale bar=100 μm).

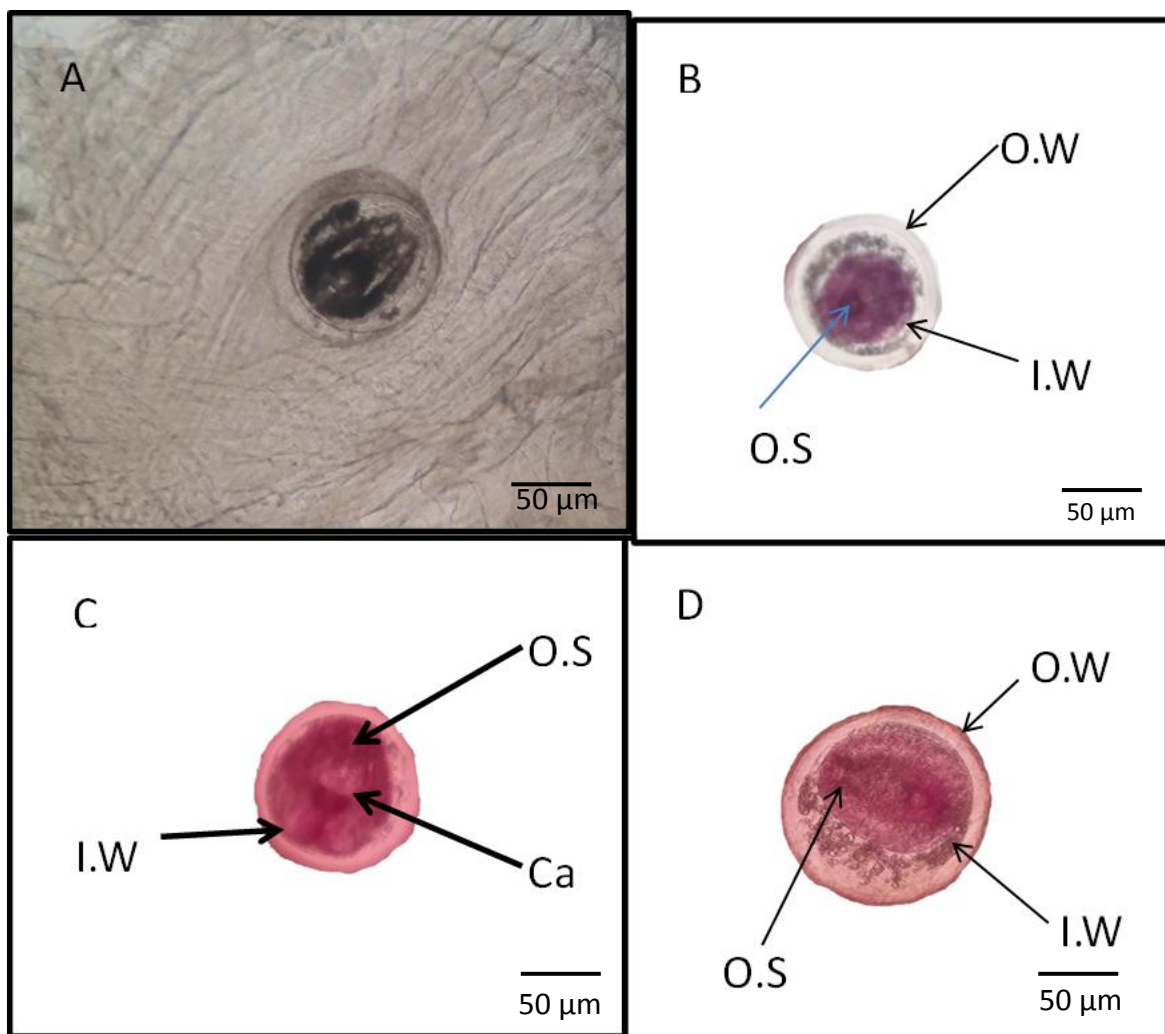


**Fig. 7.** Photomicrograph of the skeletal muscles of infected *B. bajad* showing: chronic inflammatory reaction around the remnant of parasite. H&E (Scale bar=100 μm).

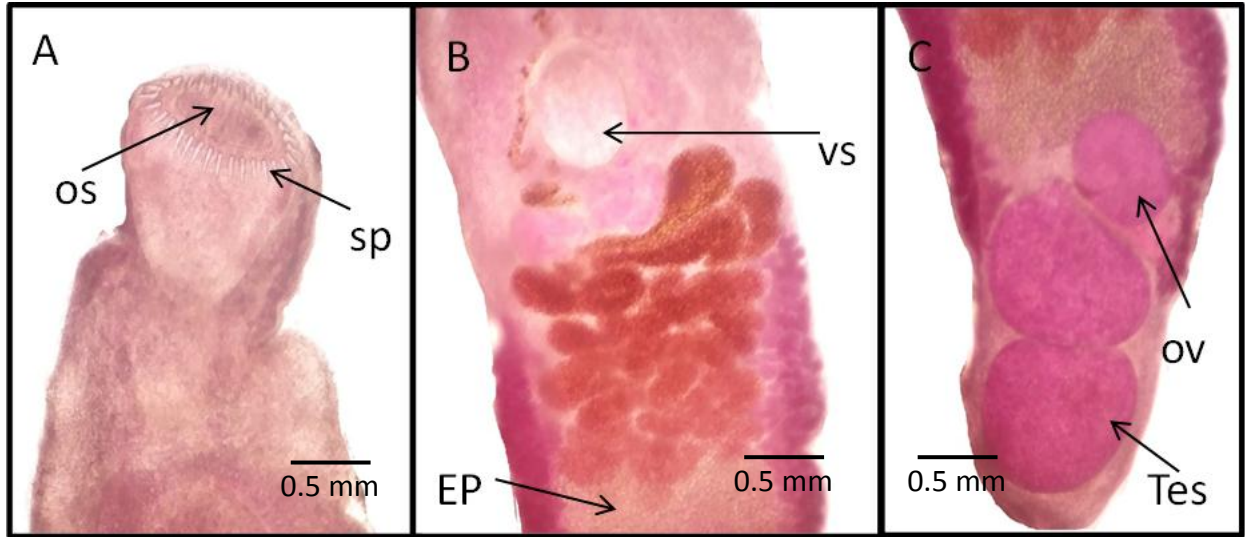


**Identification of parasites isolated from the examined fishes**

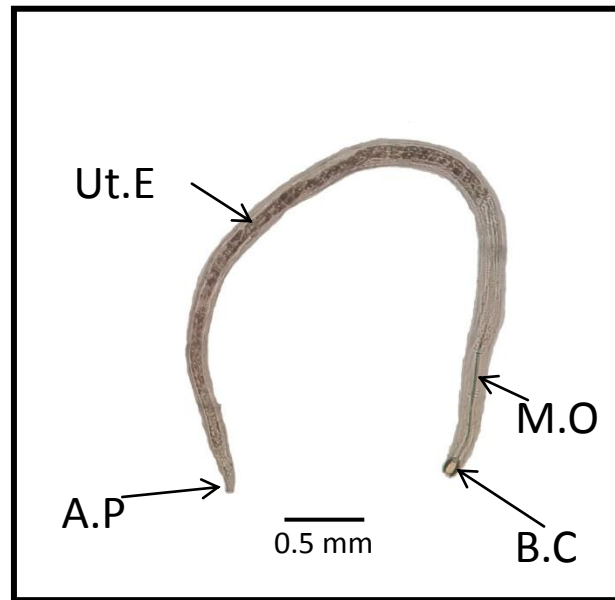
The collected parasites were identified as encysted metacercaria of digenetic Trematodes (*Diplostomum* sp., *Haplorichis* sp., *Heterphyes* sp., *Prohemostomum* sp.); adult digenetic Trematodes (*Acanthostomum* sp., *Orientocreadium batrachoides*); Cestoda (*Polyonchobothrium clarias*) larva and adult Nematoda (*Procamallanus laevionchus*) (Figs. 8- 11)



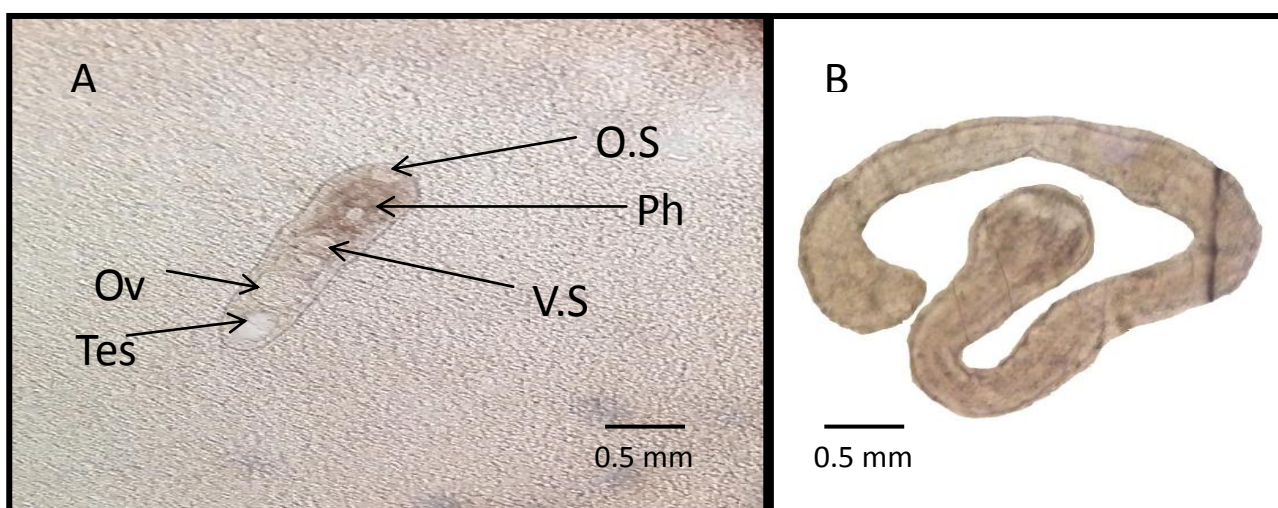
**Fig. 8.** Photomicrographs of encysted metacercaria (Scale bar=50μm). Fresh *Diplostomum* sp. (A) and *Heterphyes* sp. (B), *Haplorichis* sp, (C) and *Prohemistomum* sp. (D) were isolated from the infected *B. bajad* and stained with acetic acid alum carmine showing: oral sucker (O.S), inner wall (I.W) and outer wall (O.W).



**Fig. 9.** Photomicrographs of the anterior(A), middle (B) and posterior part (C) of adult *Acanthostomum* sp. isolated from the infected *B. bajad* and stained with acetic acid alum carmine (Scale bar= 0.5 mm) showing: oral sucker (os), spines (sp), ventral sucker (vs), excretory pore (EP), ovary (ov) and testes (tes).



**Fig. 10.** Photomicrographs of adult *Procammallanus laevionchus* isolated from the alimentary canal of infected *B. bajad* (Scale bar= 0.5mm) showing: buccal capsule (B.C), muscular esophagus (M.O), uterus with eggs (Ut.E) and anal processes (A.P).



**Fig. 11.** Photomicrographs of unstained *Orientocreadium batrachoides* adult (A) and *Polyonchobothrium clarias* larva (B) (Scale bar= 0.5mm), isolated from the infected *B. bajad* showing: oral sucker (O.S), pharynx (Ph), ventral sucker (V.S), ovary (Ov) and testes (Tes).

## DISCUSSION

Water quality is one of the most important factors affecting living organisms and their biological activities (Shehata *et al.*, 1996 a, b; Khalaf-Allah, 2009). Results revealed that the maximum average surface water temperature in Ismailia Canal was recorded during summer (29.25°C) and the lowest (24.13°C) during spring. The present findings agree with those of Bichi and Ibrahim (2009), Noor El Deen and Zaki (2010) and Mdegela *et al.* (2011). For hydrogen ion concentration (pH), it ranged between 7.34 and 7.84 during spring and summer, respectively. In general, overall mean pH values were significantly higher in pond waters, possibly due to the increase in photosynthetic rate and the depletion of carbon dioxide. The present results coincide with those of Boyd (1990), Ali (2007) and Bichi and Ibrahim (2009). On the other hand, the dissolved oxygen showed significant differences during different seasons. The lowest concentration (6.62mg/ l) was recorded during summer; this may be due to the presence of organic matter loaded by higher amounts of drainage water discharged in these areas, while the highest concentration (7.14mg/ l) was observed during spring, which may be attributed to the abundance of phytoplankton communities. Similar findings were observed by many authors, including El- Nagaawy (2000), Abd El-Halim *et al.* (2013), Goher *et al.* (2014) and Eissa (2014). The present study recorded significant differences in total hardness concentrations during different seasons. The lowest concentration (214.33mg/ l) was recorded during spring, while the highest concentration (285.97mg/ l) was detected during summer. An identical observation was reported in the study of Eissa (2014). The highest value of total alkalinity (carbonate and bicarbonate) was observed during summer (239.66mg/ l) and the lowest (178.24mg/ l) during spring. This may be related to the

organic fertilization and the presence of bacteria generating Co<sub>2</sub> from feed metabolism, manure decomposition and dissolved calcium and magnesium carbonate in the pond sediment (Boyd, 1990). The current results are nearly in accordance with that observed by Ezzat *et al.* (2012). In the present study, total un-ionized ammonia concentrations ranged between 0.56mg/ l during spring and 0.82mg/ l during summer. Higher ammonia concentrations may be due to the decomposition of organic matter in water ponds. Ammonia formation depends mainly on water pH and temperature; however, free toxic ammonia is released to critical levels at high pH and water temperature. Similar observations are those of Meade (1985), Boyd (1990) and Konsowa (2007). Results showed that the total prevalence of parasitic infestation rate in *B. bajad* was 68.92%. The infestation rate of this fish could be attributed to its feeding behavior as a carnivorous fish (bottom feeder) that assists in the transmission of more enteric parasites through its feeding on aquatic animals that harbor the infective stages of these parasites, or even young infested fish. In addition, those fish are scaleless; this may permit the penetration of the infective stages into the external body surface. The present observations concur with those of Mdegela *et al.* (2011), Omeji *et al.* (2013) and Abdel-Gaber *et al.* (2015). Results revealed that the highest rate of parasitic infestation in *B. bajad* was recorded during summer and the lowest rate during spring. This may be attributed to the availability of intermediate hosts of these parasites during these seasons and increasing feeding activity in warm temperatures. Several authors determined similar observations, such as Negm El-Din *et al.* (1988) and Shager (1999). The present study exhibited that the prevalence of parasitic infestation rate was relatively higher in most females than that of males. This finding matches those of Ibrahim (2005), Abdel-Gaber *et al.* (2015) and Radwan (2022), who recorded a higher prevalence rate in fish females.

Moreover, the highest prevalence was noticed by trematodes. This result is partially in agreement with those reported in the studies of Taha (2018), Eissa *et al.* (2020) and Mansour *et al.* (2021). However, the current finding differs from that of Okpasuo *et al.* (2016), who did not find digenetic trematodes *B. bajad*.

The morphological characteristics of *Diplostomum* sp., *Heterphyes* sp., *Haplorichis* sp., *Prohemistomum* sp., *Acanthostomum* sp., *Orientocreadium batrachoides*, *Procamallanus laevionchus* and *Polyonchobothrium clarias* in the present study are similar to previous descriptions reported in the studies of Hamouda (2014), El-Shahawy *et al.* (2017), Gamal and Ibraheem (2019) and Radwan (2022). In addition, the encysted metacercaria of digenetic Trematodes (*Diplostomum* sp., *Haplorichis* sp., *Heterphyes* sp., *Prohemistomum* sp.), recorded in the present work match those observed by Hamouda (2014), El-Shahawy *et al.* (2017) and Radwan (2022). Regarding *Acanthostomum* sp. and *Orientocreadium batrachoides* found in the present study, they coincide with those of El-Shahawy *et al.* (2017), Taha (2018), Gamal and Ibraheem (2019) and Radwan (2022). Moreover, the *Procamallanus laevionchus* observed are

similar to those described in the works of **Taha (2018)** and **Radwan (2022)**. The *Polyonchobothrium clarias* observed match the descriptions of **Taha (2018)** and **Radwan (2022)**.

Histopathological studies of the liver, muscles and intestine of the infected fish revealed that, most internal organs are affected by infection caused by parasites. Many authors detected similar observations, viz., **Mahdy et al. (1995)** and **Hamouda and Bazh (2019)**. Additionally, the study showed that there was damage in liver of the infected fish. Aggregation of lymphocytes, vacuolization and dilatation of most hepatocytes, edema, disappearance of pancreatic acini and focally ruptured hepatocytes were observed. These findings agree with those recorded in the investigations of **Liebel et al. (2013)** and **Ali et al. (2015)**.

## CONCLUSION

Water quality is one of the most important factors affecting living organisms; the presence of parasitic infection in this study is enough to cause pathological effects in fishes by reducing their growth, development and even the market.

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### تأثير العدوى الطفيلية وجودة المياه على أسماك بياض الماء العذب القاطنة لمياه ترعة الإسماعيلية ، مصر

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تمت دراسة تأثير العدوى الطفيلية والخصائص الفيزيوكيميائية لمياه ترعة الإسماعيلية على أسماك البياض (*Bagrus bajad*) في الفترة من مارس ، 2021 إلى سبتمبر، 2021 م . أسفر الفحص الإكلينيكي للأسماك الحية المصابة على وجود بعض الأعراض المرضية مثل إنتفاخ البطن ونزف على أجزاء من البطن والخياشيم . أمكن حصر الطفيليات الداخلية المسجلة في كل من المجاميع التالية : الديدان المفطحة ثنائية العائل. وتضم اليرقات المتحوصلة لكل من (*Diplostomum sp.*, *Heterophyes sp.*, *Haplorichis sp.* , and *Prohemistomum*) والأطوار البالغة لكل من طفيلي *Orientocreadium batrachoides* و *Acanthostomum sp.* والديدان الشريطية (*Polyonchobothrium clarias*) ، الديدان الخيطية (*Procamallanus laevionchus*). أظهر فحص الأسماك المصابة ظهور بعض التغيرات الهيستوباثولوجية ؛ متمثلة في وجود الميتاسركاريا المتحوصلة بين الألياف العضلية والخلايا الكبدية محاطة بغلالة ليفية رقيقة. أدت الميتاسركاريا المتحوصلة إلى ضمور وتآكل ونخر موضعي في هذه الاعضاء وضمور انضغاطي في الحزم العضلية، وأيضا تلف وإنفصال موضعي في الطبقة الطلائية المبطنة للخملات المعوية.