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INVESTIGATION OF HAEMOGREGARINA IN SAWROW FISH (*TRACHURUS MEDITERRANEUS*) IN ZLITEN COASTAL AREA, LIBYA

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ABSTRACT: This study was conducted to estimate the prevalence, mean abundance, and mean intensity of Haemogregarina infection on Sawrow fish (*Trachurus mediterraneus*) from Zliten coast, Libya. A total of 12 specimens of Sawrow fish species were collected randomly, the length (19.23 ± 0.72cm) and the weight (110.25 ± 7.88 g). The fish were transported immediately alive to the laboratory in the Department of Fish Biology and Fish Culture, College of Marine Resources, Asmarya University, where they were maintained alive in well aerated glass aquaria (1x2x4 m). The gills, fins, and skin were examined for ectoparasitic protozoa using a light microscope. The results showed positive effect of haemogregarina in the Sawrow. Blood recorded the highest prevalence and abundance among all the other organs in Sawrow fish followed by kideny, liver and spleen respectively. The results provide a significant difference at ($p<0.01$), and the results were discussed with other studies in the haemogregarinidae.

KEYWORDS: Protozoa; Disease; *Haemogregarina* spp; Sawrow fish; *Trachurus mediterraneus*; Zliten

INTRODUCTION

Protozoa's are single-celled organisms, many of which are free-living in the aquatic environment. Typically, no intermediate host is required for the parasite to reproduce (direct life cycle) (Iwamoto, 2012; Marino *et al.*, 2015; Ryan *et al.*, 2019). Increased interest in fish culture has also increased awareness of and experience with parasites that affect fish health, growth, and survival. It is of importance in the diet of different countries especially in the tropics and subtropics where malnutrition is a major problem (Roberts *et al.*, 2001). As the human population inevitably increases, the demand for fish as source of protein also grows. In recent times, there has been tremendous increase in the development of fish farming and culture attributable to the increased need for affordable animal protein especially in the tropics (Davies *et al.*, 2006; Omeji *et al.*, 2011).

Parasite of fish can either be external or internal. Parasitic infections often give an indication of the quality of water, since parasites generally increase in abundance and diversity in more polluted waters (Poulin, 1992; Reynolds, 2017). Parasites are capable of causing harm to the fish, either through injury to the tissues or organs in the process of

burrowing or consuming food or the removal of digested food in the gut of the fish as well as the secretion of proteolytic enzymes.

Fish parasites result in economic losses not only mortality, but also from treatment expenses, growth reduction during and after outbreak of disease and this militate against expansion of aquaculture. Protozoan parasites cause serious losses in fishponds and wild in fish, and their lesions render the fish unmarketable. Fish carrying protozoa parasites are capable of passing on the infective disease to man after its consumption (Coupe *et al.*, 2018; Moratal *et al.*, 2020). Haemogregarines are apicomplexan protozoa, broadly distributed among vertebrate hosts, including fishes (Davies *et al.*, 1994). They are especially common in marine fishes, where they are recognized in circulating erythrocytes, but also in cells of the leukocytic series (Davies, 1995). The life cycle of *Haemogregarina bigemina* was studied by Davies and Johnston (1976) in the marine fish *Blennius pholis*, the subsequent stages living in the intestine of *Gnathia manllaris* (isopoda). Haemogregarina comprise several blood protozoan parasites (Diniz *et al.*, 2002, Eiras, 2013). They are commonly found in both erythrocytes and leukocytes of marine fishes (Davies, 1995). According to Davies *et al.* (2008), although most fish Haemogregarina life cycles are unknown, fishes are likely to act as intermediate hosts, while leeches are probably the definitive ones. Haemogregarina is characterized by the presence of an intra-erythrocyte maroon phase in fish host (Davies *et al.*, 2004). The only pathologically significant infections are the leucocytic hemogregarines which induce proliferative lesions, thus far reported only from cultured marine fish. Lesions may be comprised either entirely of encapsulated aggregates of merozoites (Paperna, 1979) or of infected macrophages embedded in granulomatous tissue (Furguson and Roberts, 1975). Protozoa are common tropical and subtropical marine water fish parasites that affect public health and cause losses to fishes, hence its choice for this study.to investigate hemogregarines on Sawrow fish (*Trachurus mediterraneus*) in the coastal area in Zliten city. The main aim of this study to investigate Haemogregarina infection on Sawrow fish (*Trachurus mediterraneus*) from Zliten coast, Libya.

MATERIAL AND METHODS

Samples collection: A total of 12 specimens of fish species namely Sawrow (*Trachurus mediterraneus*) (Fig. 1) were collected randomly. Fishes were collected by fishermen by using cast net and gill nets, during August 2016 from Zliten coastal area (Lat 32°30' N; Long. 14°43' E) Modern harbor facility 156 km east of Tripoli. The fish were transported immediately alive to the laboratory in the Department of Fish Biology and Fish Culture, College of Marine resources, Asmarya University, where they were maintained alive in well aerated glass aquaria.

Internal examination:

Blood smear: Firstly the fishes were brought out of water and the tails has been cut by sharp scissor to obtain blood from caudal vain or artery with pressure.

A drop of blood (0.5 ml) was placed on the edge of microscope slide and touched with another slide (spreader) at 45 angle and moved quickly forward to make smear, then the smear was left to dry.

Liver, kidney and spleen smear: The routine dissection method was adopted, as a ventral incision was made from the anus to the pectoral region and another vertical from

the anus to the lateral line. The side flap was lifted and the internal organ exposed. The operculum was removed to expose the gill (Bucke, 1980), then samples were taken from: liver, kidney and spleen and after that dried from blood with filter paper to absorb the fluids and blood. The impresser smear was done by press the tissue on different places on glass slide by forceps. The smears were dried, fixed in methanol for 10 min, and then stained with 5% Giemsa's solution in phosphate buffer (pH 7.3) for 30 min. Smears were then examined using light microscope fitted with an oil immersion lens.

Photography: Krussoptronic microscope fitted with camera (BEL, Eurkm 10.0) was used to photograph the parasites. The parasites were identified using images from websites such www.fishbase.se and by making their sketches as observed on the microscope and compared with the practical guide on fish parasites.

Fig. 1. Sawrow *Trachurus mediterraneus*

Data Analysis: The obtained data of the study were categorized and summarized in the Microsoft Excel sheets. Then SPSS statistics program, (Version 21.0) was used for the comparison. Statistical significance was set at ($p<0.01$). The number of *Haemogregarina* in Sawrow fish *Trachurus mediterraneus* species and the total number of *Haemogregarina* was calculated to determine the prevalence, mean abundance, and mean intensity, by using the mathematical calculations formulated by (Bush *et al.*, 1997).

$$\textbf{Prevalence (P \%)} = \frac{Number\ of\ infected\ with\ particular\ parasite\ species}{Total\ number\ of\ hostes\ examined} \times 100$$

$$\textbf{Mean abundance (MA)} = \frac{Total\ number\ of\ parasite\ species\ in\ host\ species}{Total\ number\ of\ examined\ hostes}$$

RESULTS AND DISCUSSION

A total of 12 fish of *Trachurus mediterraneus* were collected and inspected for parasites from Zliten coastal area. The present study showed the existence of *Haemogregarina* sp. in Sawrow fish *Trachuru smediterraneus* (Table 1. and Figs 2-5). The results showed that *Haemogregarina* sp. in blood, liver and spleen.

Table 1. *Haemogregarina* parasites species found in in Sawrow fish *Trachurus mediterraneus*

No.	Site of infection			
	Blood	**Liver**	**Spleen**	**Kideny**
1	+	+	+	+
2	+	+	-	+
3	-	-	-	+
4	+	+	-	+
5	+	+	-	-
6	+	+	+	-
7	+	-	-	+
8	-	+	-	+
9	+	-	-	+
10	+	-	-	-
11	+	-	+	-
12	-	+	-	+
Total	**9**	**7**	**3**	**8**
Abundance	**0.75**	**0.58**	**0.25**	**0.66**
Prevalance (%)	**75**	**58**	**25**	**66**

The results showed that *Haemogregarina* sp. in blood, liver and spleen. These groups comprise several blood protozoan parasites (Diniz, 2002; Eiras, 2013; Buchmann, 2015; Marino *et al.*, 2019; Moratal *et al.*, 2020). They are commonly found in both erythrocytes and leukocytes of marine fishes (Davies, 1995), fishes are likely to act as intermediate hosts, while leeches are probably the definitive ones. Haemogregarines have been most often recorded in fish erythrocytes (Jeffrey and Hendrickson, 1991; Laird and Bullock, 1969; Khan, 1986, Eiras *et al.*, 1995). Also *Haemogregarina* sp. were found in blood of *Siganus rivulatus* in the Red sea coast of Sudan (Ahmed, 2012) and the same result obtained by (Laird and Bullock, 1969) who reported that Haemogregarines have been most often recorded in fish erythrocytes. Different stages were encountered in the spleen and liver of *Trachurus mediterraneus* in Libya and this agree with finding of some researchers who declared piscine hemogregarines divide in the erythrocytes, while some (such as *H. simondi* in marine flat fish (*Solea solea*), (Kirmse, 1979) will also have a pre-erythrocytic merogony in circulating and tissue leucocytes (macrophages). Furthermore, large cyst-like bodies, containing numerous *Hemogregarina merozoites*, were reported in

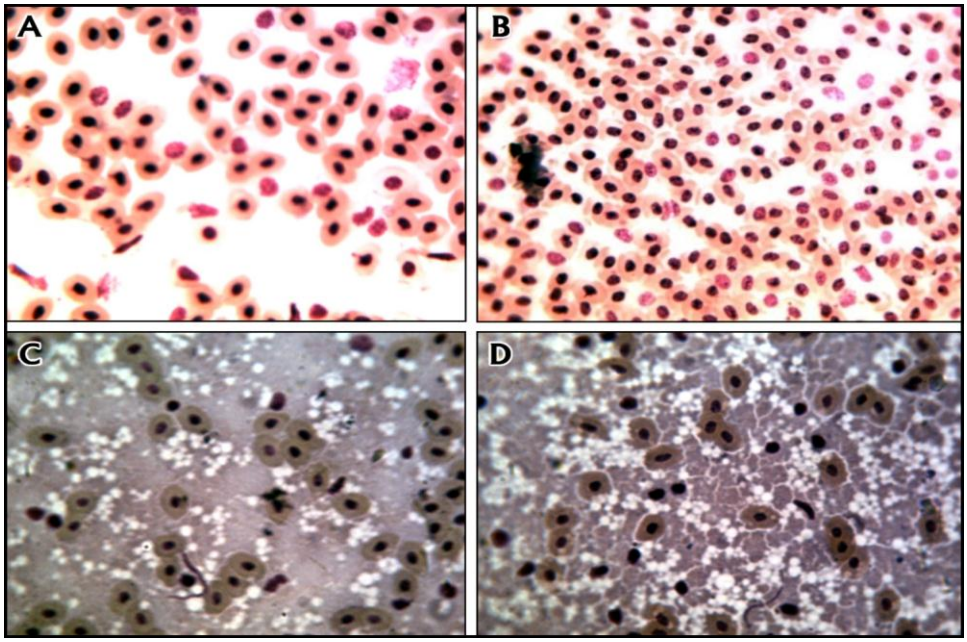

Fig. 2. *Haemogregarina* sp.: A & B, in blood; C & D, in liver.

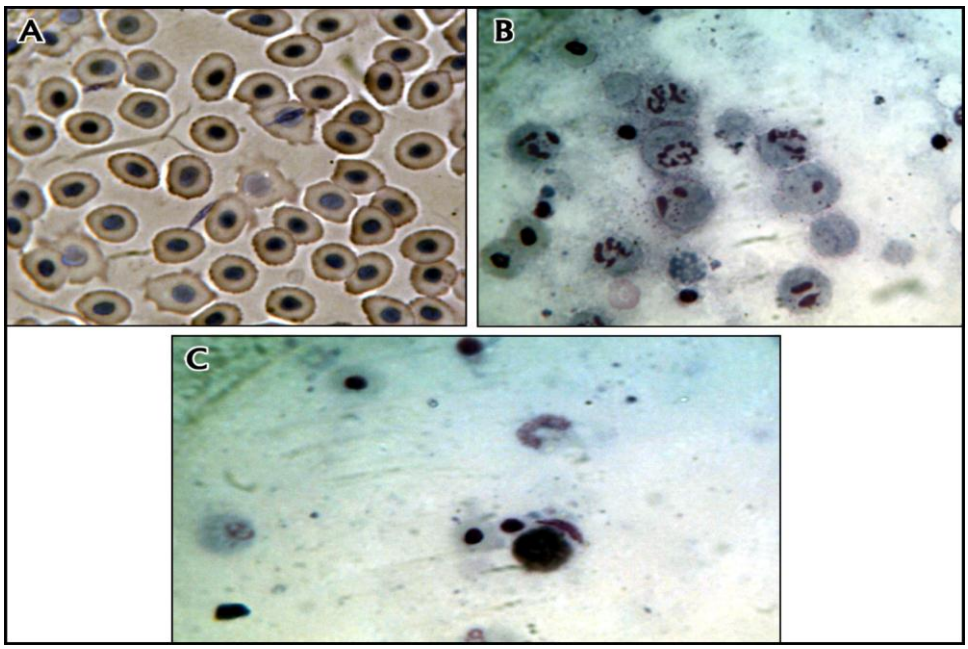

Fig. 3. A, *Heamogregarina* sp. in the blood; B, Merozoite of *Heamogregarina* sp. in the spleen; C, *Heamogregarina* sp. in gill.

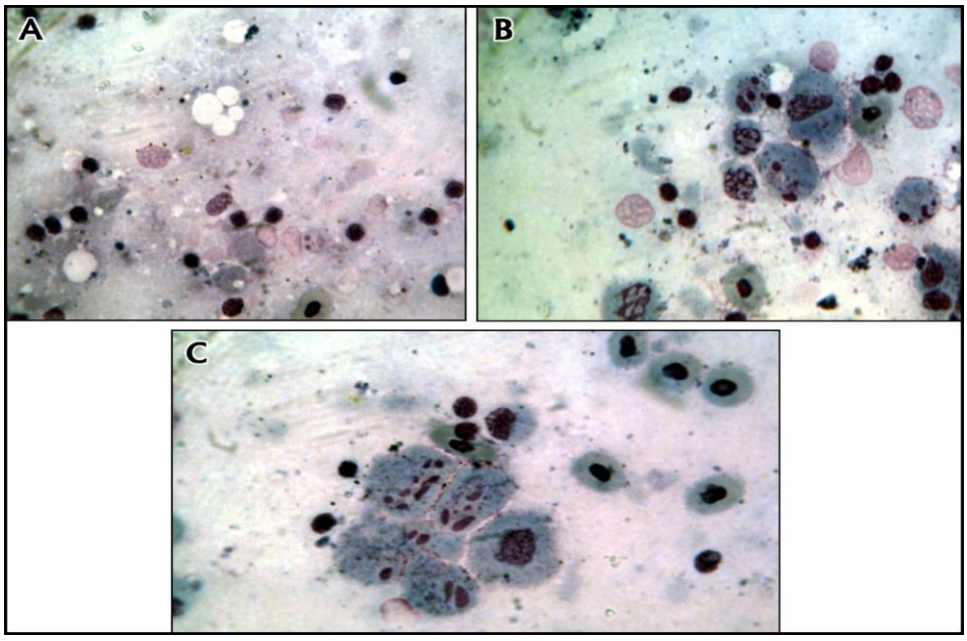

Fig. 4. A & B, Shizont of *Heamogregarina* sp. in spleen; C, merozoite of *Heamogregarina* sp.

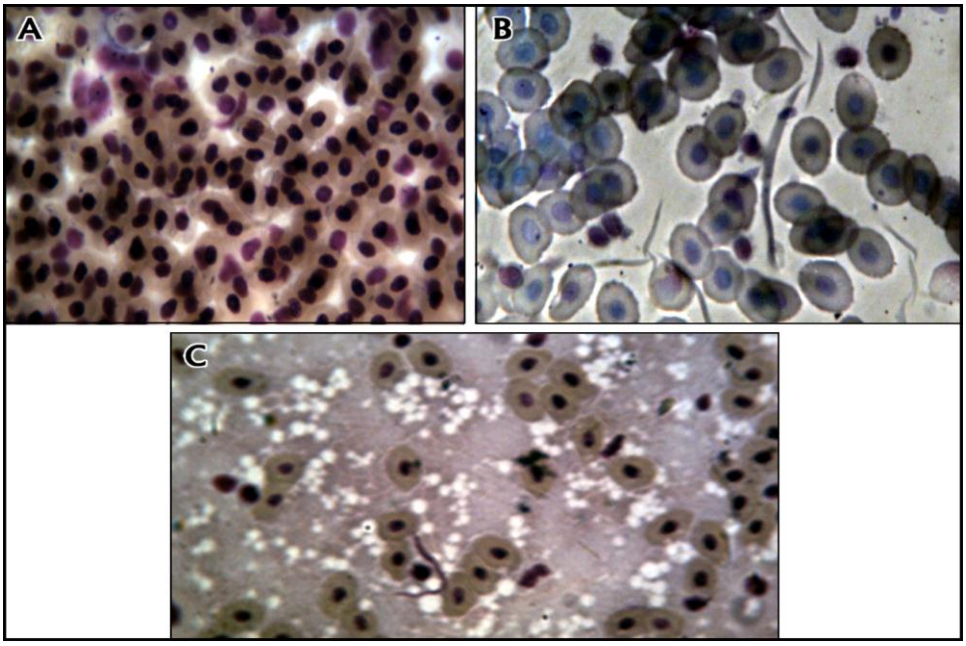

Fig. 5. A & B, *Trypansoma* sp. in the blood; C, *Trypansoma* sp. in the liver.

the visceral organs and muscles of several marine fish (Furguson and Roberts, 1975; Paperna, 1979; Paperna and Sabnai, 1982). The authors recommeded more studies in different location and also other fish species in Zliten coast, Libya.

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