

CHAPTER INTRODUCTION

Fish is important to human populace in trade and economy; it is of importance in the diet of different countries especially in the tropics and subtropics where malnutrition is a major problem (**Osuigwe and Obiekezie, 2007**). As the human population inevitably increases, the demand for fish as source of protein will grow (**Abolarin, 1996**). 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**).

Bagrus bayad is an important food fish, with flesh of good eating and of economic importance in Egypt. It has high growth rate and attains maximum weight of 12.5 kg but it has not been cultured due to limited information on their breeding under captivity, like other catfishes, are not able to exhibit their natural spawning behavior in artificial ponds (**Lamai SL, 1993**).

Parasitic infection and diseases are some of the factors hindering high productivity in fish farming (**Kayis et al., 2009**). Parasites are the most diverse and common pathogens the aquaculturist may likely encounter and parasitic diseases are very common in fish all over the world and are of particular importance in the tropics (**Roberts and Janovy, 2000**) and Protozoan among other parasites cause immeasurable damage to the fishing industry.

Fish parasites are numerous and many phyla in the animal kingdom have representative that are parasitic to fish. There are by far more parasite species that infect fish than any other group of infectious disease (**Blazer, 1996**).

Fish parasites result in huge economic losses as they increase mortality; increase farm inputs via increased treatment expenses and cause reduction in growth rate and possibly weight loss during and after the period of parasitic disease

outbreak ,all these militate against expansion of aquaculture (**Kayis et al., 2009**). The most commonly encountered fish parasites are protozoa (**Klinger and Francis-Floyd, 2000**).

The topic of fish disease is so complex that it would need a whole book to discuss it thoroughly. Fortunately, most of the proprietary medicines available are broad spectrum, killing most disease organism. It there for does not usually matter if you are unable to identify the precise disease affecting your fish, as the treatment will usually help (**Brian, 1996**).

Parasite infection in fish refers to a diseased condition in fish resulting from organism living in or on the fish (**Basseyy, 2011**).

Parasites from temporary host become independent soonest. Reservoir host serves as a source of parasite for other host. Protozoan and Metazoans are examples of fish parasites. Protozoan is a unicellular organism while metazoans are multi cellular organisms (**Basseyy, 2011**).

Even moderate infection of these organisms on small fish may prove a fatal disease, since the infection may cause the fish to stop feeding, Fish parasitic protozoa gain a lot of attention (**Abdel- Ghaffar et al., 2008**).

Objective:

1. To conduct a general survey of external (skin and gill) and internal protozoan parasites in intestine, liver, kidney, spleen and gonads on *Bagrus bayad* collected from Jebal Aulia Dam.

CHAPTER TWO

LITERATURE REVIEW

2.1. Protozoa

Most of the commonly encountered fish parasites are protozoan. With practice, these can be among the easiest to identify, and are usually among the easiest to control. Protozoans 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) (Organization of Wikimedia Foundation, Inc2012).

A Review of some ecto-and endo protozoan parasites infection in *Tilapia zillii* from Damietta Branch of River Nile, Egypt, found fish species revealed that, fishes were infected with eleven parasitic protozoan species belonging to eight genera. These species were: *Apiosoma piscicolum*, *A. conica*, *Scopulata epibranchialis*, *Vorticella sp.*, *Ambiphrya ameiuri*, *Amphileptus sp.*, *Chilodonella hexasticha*, *Tetrahymena corlissi*, *Trypanosoma mansouri*, *T. syanophilum* and *Trypanosoma sp.* Among the obtained parasites, the following were recovered for the first time in Egypt. *Apiosoma conica*, *Vorticella sp.*, *Ambiphrya ameiuri*, *Amphileptus sp.*, *Tetrahymena corlissi* and *Trypanosoma sp* (**Akinsanya and Otubanjo ,2006**).

The external fish protozoan parasites such as *costia* , *Ichthyophthirius* and *Trichodina* are among the genera which may externally infected fish seriously enough to cause discomfort and occasionally be responsible for mortalities in fish population(**Anderson,1974**).

Also the internal protozoan parasites like *myxosporidians* which are one of the most serious internal fish pathogen exist lodged in the fish tissue and either kill the host or persist for long period of time (**Anderson, 1974**).

2.2. External protozoa:

2.2.1. Ciliates:

Most of the protozoan's identified by aquarists well be ciliates. These organisms have tiny hair-like structures called cilia that are used for locomotion and/or feeding. Ciliates have a direct life cycle and many are common inhabitants of pond-reared fish (**Organization of Wikimedia Foundation, Inc, 2012**).

2.2.1.1. *Ichthyophthirius multifiliis*:

The disease called "Ich" or "white spot disease" has been a problem to aquarists for generations. Fish infected with this organism typically develop small blister-like raised lesions along the body wall and/or fins (**Moeller, 2013**)

Omeji et al., (2011) reported that *Ichthyophthirius multifiliis* was the most common protozoan parasites found in *C. gariepinus* from the wild (River Benue) and cultured (pond) environments. Also Bigger fishes of total length (25–48 cm) had more parasite load than the smaller ones (19–24 cm).

Infection by *Ichthyophthirius multifiliis* have reported from *Tilapia sp.* in Uganda and South Africa (**Paperna, 1980**). Attaches itself to epithelial cells and through an inserted protrusion consumes their contents, whereas *Chilodonella spp.* browse the epithelial surface (**Paperna & Van As, 1983**).

Histopathological changes in the integument following infection by *Chilodonella spp.* and *I. necator* are an outcome of two counteracting cellular processes — hyperplasia of the epithelial cells, including mucus cells and chloride cells, versus a progressive cellular destruction. Cellular destruction primarily occurs due to direct action of the parasites, and later by enhanced abrasion of the peripheral cells after the depletion of mucus forming cells.

The production of mucus cells is limited. Accelerated mucus cell production stimulated by the infection apparently exhausts resources for mucus production, and the infected fish become “dry”. Some parasites seem to yield cytotoxins or proteolytic enzymes which could be the cause of spongiosis, which affects both the proliferated and unchanged epithelial layer (**Paperna & Van As, 1983**). Secondary cellular damage due to degeneration, necrosis and desquamation results in the degradation and disintegration of the epithelial layer.

2.2.1.2 *Chilodonella sp*

Chilodonella is a ciliated protozoan that causes infected fish to secrete excessive mucus. Infected fish may flash and show similar signs of irritation (**Organization of Wikimedia of Free Encyclopedia (2012)**).

2.2.1.3 *Tetrahymenasp*

Tetrahymena is a protozoan commonly found living in organic debris at the bottom of an aquarium or vat. *Tetrahymena* is a teardrop-shaped ciliate that moves along the outside of the host.. Identification of *Tetrahymena* internally is a significant but untreatable problem. A common site of internal infection is the eye (**Organization of Wikimedia of Free Encyclopedia (2012)**).

2.2.1.4 Trichodinas:

Trichodina sp is one of the most common ciliates present on the skin and gills of pond-reared fish. Low numbers (less than five organisms per low power field) are not harmful, but when fish are crowded or stressed, and water quality deteriorates, the parasite multiplies rapidly and causes serious damage (**Organization of Wikimedia of Free Encyclopidia (2012)**). Although there are a number of reports on poor condition and mortalities, particularly of fry, coinciding with massive infestation of *trichodinids*, *Trichodinella epizootica* in particular and the sessilians *Apiosoma*, *Ambyphrya* and *Scopulata* (**Paperna 1991**), histopathological changes in events of massive infections by these ectoprotazoans are hardly evident, if occurring at all (**Fitzgerald et al., 1982**). *Trichodinella epizootica* in carp and *Tripartiella cichlidarum* in cichlids (**Paperna, 1991**) cause some erosion of the gill epithelium. However, food vacuoles of *trichodinids* revealed bacteria rather than sloughed cells. Ultrastructural observation on attached *Apiosoma* did not reveal any interference with the host cell serving as substrate (**Fitzgerald et al., 1982**) or peripheral tissue response. Thus, mortalities following massive colonisation of gills by sessilians could result from the dense cover of sessilians disrupting gas exchange through the respiratory epithelium. The only exception among these infections are the colonies of the stalked sessilia *Heteropolaria* (*Epistilis*) which cause lesions (“red sore”) at the stalk attachment to the fish skin, these inflamed haemorrhagic lesions are also contaminated with the bacterium *Aeromonas hydrophila*. Reported localised infection above the opercular bone (in cultured tilapia in Israel) resulted in aggravation of the lesion into a wide (6 mm in diam.) perforation of the bone (**Paperna, 1991**).

Suctorians (*Trichophrya spp.*) in certain instances cause cytological damage to the gill lamellae cells in direct contact with the parasites and subsequent hyperplasia and haemorrhages of the gill tissue (**Heckmann & Carroll, 1985**).

Tilapia from dam reservoir at port Elizabeth in south Africa were found heavily infected with integumental protozoans : *Trichodina sp.* *Costia* and *childonella* (paperna , 1980).

2.2.1.5. Ambiphyra:

Ambiphyra , previously called *Scyphidia* , is a sedentary ciliate that is found on the skin, fins, or gills of host fish. Its cylindrical shape, row of oral cilia, and middle bank of cilia identify *Ambiphyra*. It is common on pond-reared fish, and when present in low numbers (less than five organisms per low power field), it is not a problem (**Organization of Wikimedia of Free Encyclopedia (2012)**).

2.2.1.6. Apiosoma:

Apiosoma , formerly known as *Glossatella* , is another sedentary ciliate common on pond-reared fish. *Apiosoma* can cause disease if their numbers become excessive (**Organization of Wikimedia the free encyclopedia, 2009**).

2.2.1.7. Epistylis:

Epistylis is a stalked ciliate that attaches to the skin or fins of the host. *Epistylis sp.* is of greater concern than many of the ciliates because it is believed to secrete proteolytic ("protein-eating") enzymes that create a wound, suitable for bacterial invasion, at the attachment site (**Organization of Wikimedia the free encyclopedia (2009)**).

2.2.1.8 Capriniana:

Capriniana , historically called *Trichophyra* , is a sessile ciliate that attaches to the host's gills with a sucker. They have characteristic cilia attached to an amorphous-shaped body (**Organization of Wikimedia the free Encyclopedia (2009)**).

2.3. Internal protozoa

2.3.1. Flagellates:

Flagellated protozoans are small parasites that can infect fish externally and internally. They are characterized by one or more flagella that cause the parasite to move in a whip-like or jerky motion (**Organization Wikimedia the free encyclopidia (2009)**).

Saly et al., (2005) examined 1088 fish (*Oreochromis niloticus*, *O. aureus*, *Tilapia zillii*, *Clarias gariepinus* and *Cyprinus carpio*) from Abassa farms, Egypt, for internal protozoa during 2001-2003. Their study revealed that 67% of fish were infected with enteric protozoa.

2.3.1. Hexamita / Spirotrunculus:

Hexamita is a small (3 -- 18 μ m) intestinal parasite commonly found in the intestinal tract of freshwater fish (Figure 10). Sick fish are extremely thin and the abdomen may be distended. The intestines may contain a yellow mucoid (mucus-like) material. **organization of Wikimedia foundation, Inc(2012)**.

Hexamitosis (octomitosis) in aquarium held South American cichlids, notably *Symphysodon discus* and *Pterophyllum scalare*, often coincides with poor conditions and mortalities. In recent years infection has frequently been diagnosed in tilapia hybrids cultured in Israel. Massive numbers often congest the posterior digestive tract and coincide with food retention. It is, however, not yet certain if *Hexamita* is a primary pathogen or a synergist in other clinical conditions and bacterial contaminations. Heavy infections in grass carp or South American cichlids cause haemorrhagic enteritis, with injuries to the mucosal epithelium, some necrotic changes in the liver and sometimes haemorrhagic dropsy (ascitis) (**Molnar, 1974**).

S.Aly Worldfish Centre, Fish Health **Abbassa,(2005)** reported That infection rate with *hexamita* among *O. niloticus*, *O. aureus*, *T. zillii*, *C. gariepinus* and *C. carpio* was 62, 57, 80, 58 and 50%, respectively. The highest infection rate was seen in spring (81%), followed by summer (72%), autumn (60%) and winter (48%).

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2.3.2. Piscinoodinium:

Piscinoodinium is a sedentary flagellate that attaches to the skin, fin, and gills of fish. The common name for *Piscinoodinium* infection is "Gold Dust" or "Velvet" Disease. The parasite has an amber pigment, visible on heavily infected fish **Organization of Wikimedia Foundation, (2012).**

2.3.3. Cryptobia:

Cryptobia is a flagellated protozoan common in cichlids. They are often mistaken for *Hexamita* as they are similar in appearance. However, *Cryptobia* are more drop-shaped, with two flagella, one on each end **Organization of Wikimedia Foundation, (2012).**

Woo (1987) reported the development of *cryptobia* in leech does resemble the sequential development of trypanosomes.

Heavy intestinal infection by *Cryptobia iubilans* in the South American cichlids *Herichthys cyanogutatus* and *Cichlasoma meekei* caused severe inflammation in the entire digestive tract, though the epithelial layer remained by and large intact. Oedema, atrophy and necrosis occurred in the lamina muscularis; lesions extended to the liver and the spleen (**Dykova & Lom, 1985**). Fish stopped feeding, developed dropsy, and gradually died (Bejerano, pers. comm.).

Mortality of aquarium reared, South American cichlids (*Symphysodon aequifasciata*) coincided with heavy infection by opalinids (*Protoopalina symphysodontis*), resulting in congestion of the digestive tract (**Foissner et al., 1979**).

2.3.4. Trypanosome:

Trypanosome sp microscopic, one-celled protozoan of the genus *Trypanosoma*, typically living as an active parasite in the bloodstream of a vertebrate; hundreds of species are known. A trypanosome is long and pointed and possesses a flagellum. (**Encyclopedia.com 2012**).

Shammat (1989) found *trypanosome* in *Tilapia sp.* in White Nile and pond with 56% in White Nile and 19% in pond.

During 2001 and 2002, blood smears from 37 of 120 fishes belonging to 10 species captured in the Okavango Delta region of Botswana, were found to harbour *trypanosomes*, and ranged in total length from 29.5 to 80.8 microm. Mixed populations of the smaller and larger *trypanosomes* were found in most fish examined. identified as *Trypanosoma mukasai* Hoare, 1932, likely adding another 9 new hosts to those known for this parasite.com, (**Encyclopedia 2012**).

Paperna (1996) reported prevalence of infection by *trypanosome* in cat fish , *C.gariepinus* in lake Victoria was about 50%. **Nico etal (2004)** also reported a high infection rate of *trypanosome* with 43% prevalence in *C.gariepinus* .

2.3.5. Myxozoa:

Myxozoa are parasites that are widely dispersed in native and pond-reared fish populations. Most infections in fish create minimal problems, but heavy infestations can become serious, especially in young fish. **organization of Wikimedia foundation,Inc(2012)**

Shammat (1989) reported that presence of spores in white Nile and fish pond in *Tilapia sp.* in different internal organs of the infected fish and high infection density was observed in spleen and kidney.

The internal protozoa parasite like myxosporidians which are one of the most serious internal fish pathogen exist lodged in the fish tissue and either kill the host or persist for along period of time (**Anderson1974**).

Paperna 1996 noted that the protozon parasite, myxosporea are basically parasite found in the tissues where they cause histozoic infection and in the internal cavities e.g in gall and urinary bladder where the cause coelozoic infections. The cysts can found in the gills skin and digestive tract of their host fish.

Clarias alone recorded infections in blood by myxospora with a prevalence of 1.09%, although there has been a reported case of infection by myxosporidia in *C.gariepinus* by **landsberg (1986)**, the infection in *C.gariepinus* in Lekki lagoon may be due to the host specificity exhibited by different species of myxospora as reported by **Paperna (1996)**.

Several *myxosporidian* infections of cultured fish were reported to be pathogenic. Most notorious is the whirling disease of trout, manifested by skeletal deformities, which is also claimed to have been introduced with rainbow trout into South Africa. In farmed carp, *Myxobolus spp.* caused locomotory disturbances coupled with emaciation, **(Bauer et al., 1991)** and sunken eyes in brain infections, anaemia and haemorrhagic dropsy and mortality in a heavy cardiac infection and circulatory disfunctions in infections at the bases of the gill lamellae. Heavy infection of carp gills with *M. koi* caused fusion through epithelial hypertrophy, and congestion; rupture of cysts caused inflammation. Damage to the gills by dense infestation resulted in respiratory problems; fish were swimming near the surface with distended operculi **(Rukyani, 1990)**.

Presporulating forms named Hofferellus, with a postulated identity with species of *Mitraspora* **(Molnar et al., 1986)**, cause Kidney Enlargement Disease by infecting the epithelial cells of the renal tubules. Proliferative stages of *Sphaerospora* cause proliferative hypertrophy of the kidney in salmonids and grey mullets **(Paperna, 1991)**. Proliferative, presporulating stages of *Sphaerospora renicola* were demonstrated to be the aetiological cause of swimbladder inflammation in carp **(Kovacs-Geyer, 1986)**.

Myxosporan infections become pathogenic to their host and cause clinical conditions (examples: gill infections of American catfish with *Henneguya exilis* - *Channa punctata* with *Henneguya* and carp with *Myxobolus koi* - **Rukyani, 1990**).

Myxobolus cysts occur in the pharyngo-branchial cavity (of *Ctenopoma spp.*), the interior organs, muscles and viscera. Such infections are best known from cichlids, but also occur in fish from other families (Sphaerospora occur in kidneys of *Clarias lazera* and Grey mullets (**Paperna, 1991**).

The only reported coelozoic infection in freshwater African fish is that of *Myxidium clariae* from *C. lazera* gall bladder (**Landsberg, 1986**).

2.3.6. Microsporidia:

Microsporidians are intracellular parasites that require host tissue for reproduction. Fish acquire the parasite by ingesting infective spores from infected fish or food. **Organization of Wikimedia foundation, Inc(2012)**.

Microsporidian infections were reported from lake George, East Africa (swim bladder of *Halochromis spp.*) (**Paperna,1996**) and in the republic of Benin (in gills and viscera of tilapia).

In *Plistophora* infected *Haplochromis angustifrons* and *H. elegans*, the swimbladder walls were thick and white. Microscopic examination revealed most wall tissue to be loaded with inclusions (pansporoblasts) containing up to 48 spores. Prevalence of infection in L. George was very low (below 1%, out of 302 fish examined from both species) (**Paperna, 1996**).

Infections by *Nosemoides tilapia* in *Tilapia zillii*, *T. guinensis* and *Sarotherodon melanotheron* were common (13–30% prevalence in Lake Nakoue and Porto Novo lagoon), with some fish demonstrating numerous xenomata on the gills (some reaching 560 × 800 µm in size) as well as in the mesenteries, the gut wall and in the liver (up to 40–100 µm in size), but without apparent clinical effect on the fish .Prevalences of infection with *Plistophora* in glass eels and elvers in Transkei rivers were 6.8–9.5% and 2.6% respectively. *Microsporidial* infection of kidneys in *Oreochromis spp.* are rare and do not induce any detectable pathological changes.

2.3.7. Coccidia:

The economic damage done by *coccidiosis* to warm water pisciculture has apparently been grossly underestimated. Since *coccidiosis* in fish usually manifests itself as a chronic infection, mortality is gradual and is overlooked in most farms. Losses only become evident when yields are checked at the end of the growth period.

Cyprinids and cichlids contract intestinal coccidiosis as soon as they hatch. Infection (of *G. carpelli*) has been identified in 8-day-old goldfish, with mortality occurring 30–45 days later. *G. carpelli* seems to be more pathogenic to goldfish than to carp. In cichlids (cultured *Oreochromis hybrids*), intestinal infection (of *E. vanasi*) was detected in fry by the end of nursing in their parents' mouth, losses became evident when infection reached maximum levels by two or three weeks after hatching (**Kim, 1992**).

Heavy infections in carp fingerlings (25–50 mm long) have been found to coincide with severe emaciation. Emaciation also occurred in infected cichlid fry. Surviving fish demonstrated spontaneous recovery, infection was low or absent in carp and goldfish or cichlids older than 2 months. Nonspecific defence response parameters (leucocytosis, eosinophilia, activation of phagocytes and elevation of natural antibody titer and of coaguloplasmin) were detected in carp infected with *G. subepithelialis* (**Studnicka & Swicki, 1990**).

Damage caused by intestinal *coccidiosis* occurs principally by the rupture of the epithelium by the escaping merozoites and oocysts. In the intestine of cichlid fry infected with *E. vanasi*, most damage is caused to the mucosal cells by the developing intracytoplasmic parasites (**Paperna, 1980**).

Epicytoplasmic infections seem to have less effect on the gut epithelial layer and the damage induced through consumption of the nuclei by the intranuclear generations has not yet been evaluated. Inflammatory changes in intestinal coccidiosis only occur following disintegration of the mucosal layer and in response to accumulated cellular debris.

In nodular *coccidiosis*, caused in carp by *G. subepithelialis*, the accumulation of oocysts in the lamina propria induces inflammation with intense leucocyte infiltration (enteritis) (Molnar, 1984).

The *G. cichlidarum* epicytoplasmic infection leads to an intense desquamation of the swim bladder epithelial lining in cichlids (Paperna, 1996). Proliferation of the mucosal epithelial cells, observed in intestinal (Molnar, 1984) and in swim bladder infections, blocks the release of oocysts from the epithelium, resulting in a condition similar to that observed in nodular *coccidiosis*.

Swim bladders of cichlids with late infections turn opaque white. The pathogenicity of the swim bladder coccidiosis to juvenile cichlids still needs to be critically evaluated.

Coccidia of *Clarias gariiepinus* have not been studied. They form yellow bodies similar to those of *G. carpelli*. Data on the pathological effect of *Epieimeria anguillae* infections are available from cultured New Zealand eels (*Anguilla australis* and *A. diffebachii*) Oocysts which aggregate within or below the gut mucosa (in the lamina propria) induce inflammatory infiltration. In more severe infections, the condition is reminiscent of nodular coccidiosis, the basal membrane breaks and, following the aggregation of oocysts, the sub-mucosal tissue degrades and the loosened epithelial mucosa is sloughed off. Mature oocysts and sporocysts are passed out with necrotic tissue, eels become severely emaciated and die. Infected *A. mossambica* in South Africa passed free sporulated oocysts, and their intestines seemed to be normal.

Host response to oocyst aggregation in the livers of fish with visceral coccidiosis (*Calyptospora funduli* in American killifish, *Fundulus spp.*), even when replacing up to 85% of the organ's volume, was limited to formation of a thin fibrotic capsule sometimes with collagen, or melanin (**Molnar, 1991**).

Mortality of aquarium reared, South American cichlids (*Symphysodon aequifasciata*) coincided with heavy infection by opalinids (*Protoopalina symphysodontis*), resulting in congestion of the digestive tract (**Foissner et al., 1979**).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study area

This study was conducted at Department of Fisheries Science and Wildlife, College of Animal Production Science and Technology, Sudan University of Science and technology during the period May to June 2016.

3.2. Materials

Slides , Distel water, Methanol , Giemsa staim, Dropper , Scissor , Hand lens, Filter paper.

3.3. Source of fish

A total of 16 fish samples of Bagrus bayad were collected from Jebel Aulia dam during the summer season. The total weight (g) and standard lengths of each fish were measured in centimeters (cm) using meter ruler. The sex of fish was ascertained by both morphological examination and observation of the presence of testis and ovary by dissection of the fish to expose the gonad.

3.4. Method

3.4.1. External examination

External examination of each of the fish for parasites was carried out from the gills and skin, using hand lens.

Firstly the fishes were brought out of water and the total lengths were taken. Then a sample was taken from gill and skin with hand lens in clean slide then left to air to dry.

3.4.2. Internal examination

Internal examination of each of the fish for parasites was carried out from the blood, liver, kidney, gonad, spleen.

3.4.3. Blood smear

Firstly the fishes were brought out of water and the total lengths were taken. The tails was cut with sharp (scissor) to obtain blood from caudal vein or artery with pressure and placed on clean slides and left to air dry.

3.4.4. Spleen, kidney, liver and Gounod

First the fishes were brought out of water and the total lengths were mustered.

The smears were taken (by clean slide) from organs and were left to air dry.

3.4.5. Fixation

After all that smears dried methanol was added for 10 minutes.

3.4.6. Staining

One ml of Gimsa stain was mixed with 9 mL of distilled water after that one drop of the stain was added on the surface of smear for 10 minutes and then left to dry by the air.

3.4.7. Microscope examination

The smears were placed under light microscope (the lens x40 for out content and x100) for the identification of the protozoa parasites.

3.5. Statistical analysis

All the results were analysis by SPSS version (16) by using descriptive statistics to determine the percentage and prevalence of parasite represent by tables.

CHAPTER FOUR

RESULTS

The result obtained from this study taples (4. 1),(4. 2) and(4 .3) showed that (*Bagrus bayad*) fish do not show any significant external lesion or abnormality. The result also revealed *Trichodina spp*, *Cryptobia spp*, *Hemoggarine spp*, *Chilodonella spp* and *Ichthyobodo sp* from mentioned species.

Table(4.1): External Protozoan Parasites on *Bagrus bayad*.

Parameters	Skin		Gill	
Effect	Positive	Negative	Positive	Negative
Number Count	4	4	6	2
Percentage	10.26	23.26	15.38	11.76

Table (4.2): Internal parasites Protozoan Parasites in *Bagrus bayad*

parameters	intestine		Kidney		Spleen		Liver		Gonad	
Effect	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Number.	6	2	7	1	3	5	8	0	5	3
Count										
Percentage	15.38	11.76	17.94	5.88	7.69	29.41	20.51	0.0	12.84	17.64

Whereas:

-ve = negative, +ve = positive, No. count = number of parasites counted in fish

Table (4.3): Protozoan parasites species found in density and prevalence

Number Samples	Parasites	Number	Percentage %
1	<i>Trichodina sp</i>	16	41.03
2	<i>Cryptobia sp</i>	14	35.90
3	<i>Chilodonella sp</i>	4	10.25
4	<i>Hemoggarine sp</i>	3	7.69
5	<i>Icthyobodo sp</i>	2	5.13
	Total	39	100%

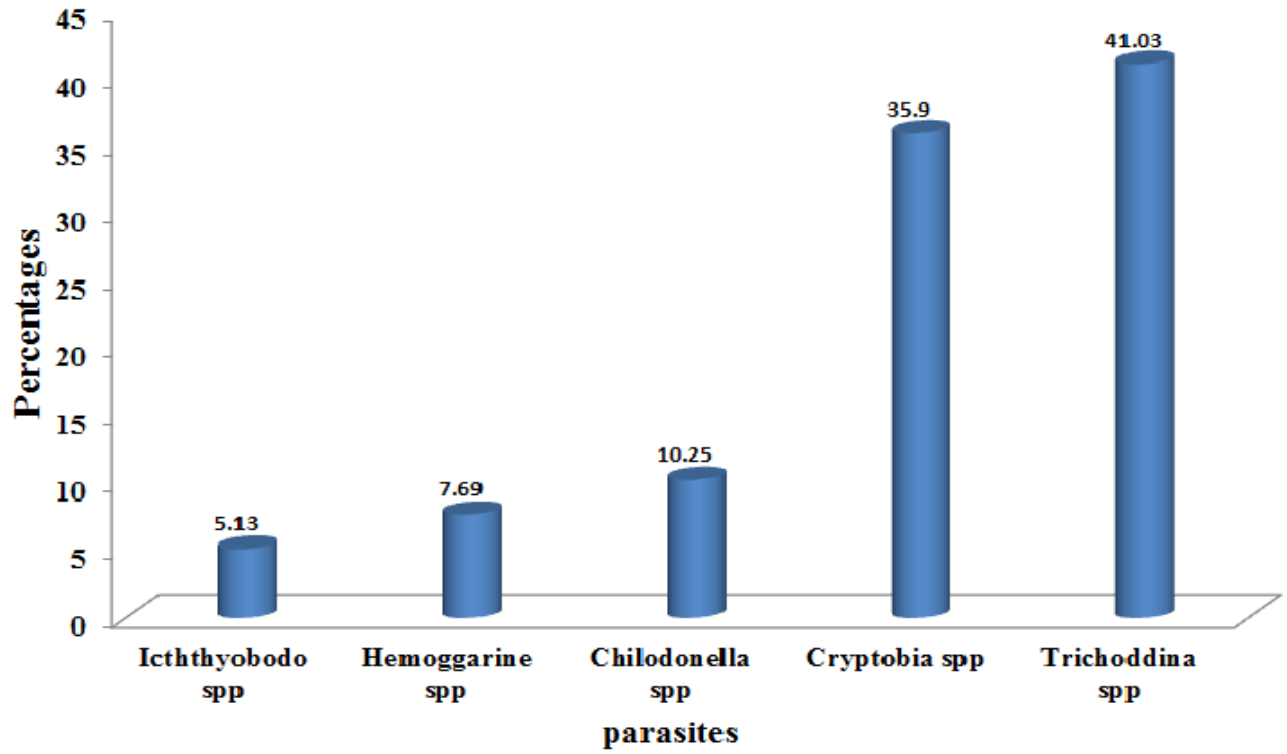


Figure (1): Parasites prevalence and density on *Bagrus bayad*.

CHAPTER FIVE

DISCUSSION

The effect of protozoa parasites in skin of *Bagrus bayad* were equal infection positive and negative with its 10.26% and 23.26% with there no significance difference at level of ($p < 0.05$). At gill the rate of infection is huge of positive with their percentage 15.38% and negative 11.76,% the result agreed with the **(Organization of Wikimedia of Free Encyclopidia (20012). And also with Anderson, 1974** who said the external fish protozoan parasites such as *Ichthyophthirius* and *Trichodina* are among the genera which may externally infected fish seriously enough to cause discomfort and occasionally be responsible for mortalities in fish population.

In the internal infection also they were effect of parasites on the *Bagrus bayad* most were found high in negative case in intestine, kidney, spleen, liver and gonad that mean the internal organs have high rate of parasites infection compared to external organs. The results agree with **(Kim, 1992)**.

Most parasites which were found in high density and prevalence is *Trichodina* sp with their percentage 41.03% followed by *Cryptobia* sp 35.90%, and *Ichthyobodo*, *Hemoggarine*, *Chilodonella* is found fewer in most species these results is agree with some report from world **(paperna et al .1984)**.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study concluded that positive result protozoa infection in *Bagrus bayad* collected from Jebal Aulia Dam appearance in Skin, Gills, and intestine. These include (*Trichodina spp*, *Cryptobia spp*, *Hemoggarine spp*, *Chilodonella spp* and *Icthyobodo sp*). In the internal infection they effected of parasites on the *Bagrus bayad* most were found high in positive case in intestine, kidney, spleen, liver and gonad that mean the internal organs have high rate of parasites infection compared to external organs.

6.2. Recommendation:

Based on the results obtained from this study we recommended that:

- This work, on the parasites of one species of freshwater fishes is a step towards a more rational and practical utilization of Sudanese resources of freshwater fishes.
- Determine the parasites load and density in different freshwater species.
- Determine protozoan parasites in all season over year.
- Determine protozoa parasites in fish from different water resources.

REFERENCES

- Abdel-Ghaffar FA, Ali MA, Al Quraishy S, Al Rasheid K, Al Farraj S, Abdel-Baki AS, Bashtar AR (2008). Four new species of *Ceratomyxa telohan*, 1892 (Myxozoa: Myxosporea: Ceratomyxidae) infecting the gallbladder of some Red Sea fishes. *Parasitol. Res.* 103:559- 565.
- Abolarin MO (1996). A new species of *Henneguya* (myxosporida Protozoa) from West African cat fish, *Clarias lazera* (Vaal) with a review of the genus *henneguya* (Thelohan). *The Afr. J. Trop. Hydrobiol. Fish.*, 1: 93-105
- Akinsanya, B. and Otubanjo, O.A., 2006. Infection not other pathogenic use virosus, fungi and bacteria and the most dangerous parasitic group that probably cause more diseases in fish culture than any other type of animal parasite.
- Anderson(1974): Infication in cultured tilapia in Israil .*J.protozoal* 32;199-212
- Baker ,A; 1963. : Observation on anatomy on protozoa.*Journal of the Egyptian german Sociaty of Zoology and parasitology*,2:147-175.
- Bassey SE (2011). *A Concise Dictionary of Parasitology*. 1st Edn., Zetus Concepts, Port Harcourt, pp.115.
- Bauer, O.N., Voronin. V.N. and Yunchis O.N., 1991. Infection of the heart in carp caused by *Myxobolus dogieli* (Myxosporea, Myxobolidae). *Angew. Parasitol.*, 32: 42.
- Blazer VS (1996). *Major Infectious Diseases of Fish* (Online). <Http://www.afip.org/vetpath/POLA/fish diseases>.
- Brian W (1996). *Aquarium fish and other Survival Manual of London N 79BH* Printed in china.
- Dykova, I. and Lom, J., 1985. Fish *Coccidia*: critical notes on life cycles, classification and pathogenicity. *J. Fish Dis.*, 4: 487–505.

- Fitzgerald, M.E., Simco, B.A. and Coons, L.B., 1982. Ultrastructure of the peritrich ciliate *Ambyphrya ameiuri* and its attachment to the gills of the catfish *Ictalurus punctatus*. *J. Protozool.*, 29: 213–217.
- Heckmann, R.A. and Carroll, T., 1985. Host-parasite studies of *Trichophyra* infesting cutthroat trout (*Salmo clarki*) and longnose suckers (*Catostomus catastomus*) from Yellowstone lake Wyoming. *Great Basin Nat.*, 45: 255–265.
- Kayis S, Ozcelep T, Capkin E, Altinok I (2009). Protozoan and metazoan parasites of cultured fish in Turkey and their applied treatments. *The Israeli J. Aquac.-Bamidgeh*, 61: 93-102.
- Kim, Soo-Hyun and Paperna, I., 1992. Fine structure of epicytoplasmic stage of *Eimeria vanasi* from the gut of cichlid fish. *Dis. Aquat. Org.*, 12: 191–197.
- Klinger R, Francis-Floyd R (2000). *Introduction to Freshwater Fish Parasites*. Institute of Food and Agricultural Sciences (IFAS) University of Florida.
- Kovac-Geyer, E. and Molnar, K., 1986. Studies on the biology and pathology of the common carp parasite *Myxobolus basilemellaris* Lom & Molnar, 1983 (*Myxozoa: Myxosporea*) *Acta Vet. Hungar.*, 31: 91–102.
- Lamai SL (1993) Aspects of the applied biology of *Clarias gariepinus*, aquacultural techniques and Deldrin toxicity. Ph.D. Thesis. Dept. of Pure and Applied Zoology, University of Reading.
- Landsberg, J. H. and Paperna, I., 1981. Intestinal infection by *Eimeria* s.l. *vanasi* n. sp. (Eimeridae, Apicomplexa, Protozoa) in cichlid fish. *Ann. Parasitol. Hum. Comp.*, 62: 283–293.
- Moeller, Jr. 2013. *External Protozoan Diseases of Fish* by California Animal Health and Food Safety Laboratory System University of California.
- Molnar, K., 1974. Data on “octomitosis” (Spiro-nucleosis) of cyprinids and aquary fishes. *Acta Vet. Acad. Scient. Hungar.*, 24: 99–106.

- Omeji .S., 2011. A Comparative Study of the Common Protozoan Parasites of *Clarias gariepinus* from the Wild and Cultured Environments in Benue State, Nigeria. Journal of Parasitology Research Volume 2011 (2011), Article ID 916489, 8 pages.doi:10.1155/916489
- Organization of Wikimedia Foundation,Inc 2012. Protozoan parasites of fishes,Elsevier,london –new york:87-9
- Osuigwe DI, Obiekezie AI (2007). Assessment of the growth performance and feed utilization of fingerling *Heterobranchus ongifilis* fed raw and boiled jackbean (*Canavalia ensiformis*) seed meal as fishmeal substitute. J. Fish. Int., 2: 37-41.
- Paperna (1996): parasites ,infection and diseases of fishes in Africa update CIFA technical paper No.31,Rome,FAO.1996,220 er type of animal parasite
- paperna ,I; (1980).: parasites ,infection and diseases of fishe in Africa update CIFA technical paper No.31,Rome,FAO.1980,220 er type of animal parasite
- Paperna, I. & Van As J.G., 1983. Epizootiology and pathology of *Chilodonella hexasticha* (Kiernik, 1909) (Protozoa, Ciliata) infections in cultured cichlid fishes. J. Fish Biol., 23:441–450.
- Paperna, I., 1991. Diseases caused by parasites in the aquaculture of warm water fish. Annual Rev. Fish Dis. 1: 155–194.
- Roberts LS, Janovy J (2000). Gerald D. Schmidt and Larry S. Roberts' Foundations of parasitology, 6th Edn., International Editions, Boston.
- Rukyani, A., 1990. Histopathological changes in the gills of common carp (*Cyprinus carpio*) infected with the myxosporean parasite *Myxobolus koi*, Kudo, 1920. Asian Fish. Sci. 3: 337–341.
- Shammat,I. M., 1989. Protozoan and fungl parasites of the freshwater fishes *Oreochromis niloticus*.MSC thesis, Faculty of Science, U of K

- Smit NJ, Davies AJ, Van As JG (2000). "A trypanosome from the silver catfish (*Schilbe intermedius*) in the Okavango Delta, Batswana." *Bull.Eur. Assoc. Fish pathol.* 20 :116-119.
- Studnicka, M. and Siwicki, A., 1990. The nonspecific immunological response in carp (*Cyprinus carpio L.*) during natural infection with *Eimeria subepithelialis*. *Israel J. Aquacult. - Bamidgeh*, 42: 18–21.
- Wikimedia the free encyclopidia.,2012. The amarican Journal of the medical sciences Journal of plankton research43: 17–31.