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Incidence of ectoprotozoan parasites infecting *Mugil cephalus (*Linnaeus, 1758) and *Tilapia zillii (*Gervais, 1852) from Ain Ziana lagoon, Benghazi, Libya.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Zoology.

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Introduction

1. Introduction

All over the world, fishes are one of the main sources of protein supply to human, as it can serve to solve the malnutrition and deficiency of proteins in the world. Fishes contain proteins and fats, which are easily digested by humans. The economic importance of fishes were represented in the food resources for many countries , fishes have a major value for humans either to provide them with proteins or by acting as a definitive or intermediate hosts for several parasites and diseases which may infect fishes and human or other animals(Ahmed , 2010).

Fishes in nature are infected with a great variety of protozoan parasites, diseases resulting from these infections have not been reported very often, for several possible reasons such as certain stages of the parasites may be dispersed in a large volume of water and therefore fish are not heavily parasitized and parasites may do little harm or the most severely affected fish die.

Parasites are an important group of pathogen causes infection and diseases of fishes in freshwater and marine environments. With the increasing interests in aquaculture, parasitic infestations are becoming threats for fish health management and aquatic crop production in the world. It is therefore an essential area for proper attention to be given by the scientists for sustainable aquaculture production.

Many fish disasters, both in open water and fish farms were caused by different ectoparasitic protozoa parasites, which have direct life cycle and facilitate translocation from host to host making huge damages to fish health. Parasitic protozoa infecting fishes may be found in all body tissues, but they are particularly common on the skin and gills (Areerat *et al.,* 1981).

Many reports from all over the world indicated great losses in fish culture caused by protozoan parasites. Obligate parasites such as the ciliate *Ichthyophthirius* and certain species of the cnidosporidians are responsible for many of these losses. Many species, which are considered as commensally protozoans, may become pathogenic under certain conditions.

The most important reasons for studying fish parasites is to study relationships between these parasites and fishes, human and animals and to understand some human health problems and losses cultures fishes. (Williams and Jones, 1976 and 1994).

Main harm or protozoan parasites to the fish host are mechanical damage, secretion of toxic substance, occlusion of the blood vessels obtaining nutrition at the expense of the fish host, and rendering the host more susceptible to secondary infections. Some of the most common clinical signs are changes in swimming habits, such as loss of equilibrium, flushing or scraping, loss of appetite, abnormal coloration, tissue erosion, excess mucous production, hemorrhage and swollen body or distended eyes (Yokokawa, 1982).

Aims of the study:

The aim of the present study is :-

1- To identify the ectoprotozoan parasites of *Mugil cephalus* and *Tilapia zillii* from Ain Ziana lagoon.

2- Determine the incidence rate of these ectoprotozoan parasites.

3- Determine relationship between incidence and sex, season, length and weight.

Literature Review

2. Literature Review

Mugil cephalus:

The mullets or grey mullets (family: Mugilidae) of ray-finned fish found worldwide in coastal temperate and tropical waters, and some species in fresh water also. In Europe, the word "mullet" is usually qualified, the "grey mullets" being Mugilidae and the "red mullets" or "surmullets" being Mullidae, notably members of the genus Mullus, the red mullets. Outside Europe, the Mullidae are often called "goatfish". The family includes about eight species belong to seventeen genera. *Mugil cephalus* are distinguished by the presence of two separate dorsal fins, small triangular mouth, and absence of a lateral line organ. They feed on detritus, and most species have unusually muscular stomachs and a complex pharynx to help indigestion. *Mugil cephalus* mainly diurnal feed on zooplankton as larvae, detritus, micro-algae and benthic organisms as juvenile and adult fish. Reproduction takes place in the sea, at various times of the year depending on the location. Does not have an obligatory freshwater phase in its life cycle. Females spawn 0.8 to 2.6 million eggs. Sexually mature at 3 to 4 years (Gosline ,1961; Johnson and Gill ,1998; Froese and Pauly , 2009) .

***Mugil cephalus*:**

Kingdom: Animalia

Phylum Chordata

Class: Actinopterygii

Order: Mugiliformes

Family: Mugilidae

Genus: *Mugil* (gray mullets)

Species: *Mugil cephalus* Linnaeus, 1758

(Striped mullet) Plate(1)

Tilapia zillii:

The tilapia is the common name for nearly a hundred species of cichlid fish from the tilapian cichlid tribe. Tilapias inhabit a variety of fresh water habitats including shallow streams, ponds, rivers, lakes and estuaries. Most tilapia is omnivorous with a preference for aquatic vegetation and detritus. Historically they have been of major importance in artisanal fishing in Africa and are of increasing importance in aquaculture. Tilapia can become problematic invasive species in new warm-water habitats, whether deliberately or accidentally introduced but generally not in temperate climates due to their inability to survive in cool waters, (generally below 60 °F (16 °C). The tilapias serve as a natural, biological control for most aquatic plant problems, and rarely compete with other "pond" fish for food. Tilapia consume plants and nutrients unused by other fish species and substantially reduce oxygen depleting detritus, adding tilapia often increases the population, size and health of other fishes(Baker ,1988; Chapman ,1992).

Tilapia zillii :

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Cichlidae

Genus : *Tilapia*

Species: *Tilapia zillii* Gervais, 1852

(Green Tilapia)

Ectoparasitic protozoan parasites of fishes :

1-*Ciliates :*

Ciliates are highly organized protists with the cell covered by cilia arranged in rows or kineties. In some groups, the number of cilia is reduced or absent. Ciliates show nuclear dualism, having as a rule generative diploid micronuclei and vegetative polyploid macronuclei. The buccal apparatus serves for ingestion of particular food. Only several groups of ciliates became secondarily astome, feeding by pinocytosis. They divide by transverse binary fission and sexual process is conjugation. Ciliates living in and/or on fishes are range from completely harmless ectocommensals (e.g., *Erastophrya*) to most noxious parasites (e.g., *Ichthyophthirius* or *Tetrahymena*). Gill and skin tissue destruction is then necessarily reflected by the debilitated or moribund condition of the fish. Ciliates are common inhabitants of pond-reared fishes. In aquaria, which are usually closed system, ciliates should be eliminated. Uncontrollable or recurrent infestations with ciliated protozoan are indicative of husbandry problem. Many of the parasites proliferate in organic debris accumulated in the bottom of a tank .Ciliates are easily transmitted from tank to tank by nets, hosts , or caretakers wet hands. Symptoms cussed by ciliates include skin and gill irritation displayed by flashing, rubbing, and rapid breathing (Lom and Dyková ,1991; Klinger and Floyd ,2009).

1.1. *Trichodina* sp. *:*

The trichodinids are commensals or parasites on many aquatic animals. In the commensal form , they feed on waterborne particles, bacteria and detritus particles from fish surface. Reproduction of these ciliates is mainly by binary fission (conjugation and autogamy is rarely reported) the optimum temperature for reproduction of *Trichodina* is 20--28°C. The size of these parasites is usually range between 20-100µm .Transmission is direct by swimming individuals from fish to another when they are in close proximity. Trichodina was described free-swimming individuals move quite rapidly, always with the broad aboral end foremost, when swimming freely after leaving its host, the parasite spins like a wheel with its aboral end forward. The aboral end is concave, its most apparent structure is denticulating ring, and chitin striated ring. The aboral cilia are purely concerned with feeding , the aboral cilia are ring and the velum being used in locomotion. Fourteen species of protozoa parasites were recorded from New Zealand intertidal Zone fishes of 10 species. *T. Parabranchicola* from gills of eight fish species (Laird 1953; Lom , 1970 ; Kruger *et al.,* 1995. and Shaogi , 1985).

The life cycle of *Trichodina*, they stated that during binary fission, the buccal ciliature undergoes a rather intricate stomatogenic process .The horse-shoe shaped condenses before the division to ellipsoid shape, and after divided it assumes the original shape again. The adhesive disc separates in two semi-circles in daughter individuals. The old set of denticles is resorbed and the full number of new denticles is formed from thickenings on the radial pins, centripetally to the ends of blades of denticles; at first the central conical parts originate, then the blades and the thorns. New radial pins arise, each between a pair of the old ones, and thus the original size of the disc and the number of its constituents are achieved again. Genus *Trichodina* protozoan which are especially harmful to yang fishes. The species parasitize marine fish by attaching themselves mainly to the gills. *Trichodina* also causes problems to crowded fish reared in cages. Clinical signs of trichodinosis include excess mucous production, flushing, debility and hyperplasia and necrosis of the epidermis. The fin may become badly frayed in heavily infected fish and this may be accompanied by sluggishness and loss of appetite. Excessive numbers on the gills of infected fish interfered with respiration. (Yokokawa ,1982 ;Lom and Dykova ,1992)

Fourteen trichodinid species collected in this study can be divided into four categories, based on their host association and possible region of origin. The first category includes trichodinids with an African origin, *T. centrostrigeata*, *T. heterodentata* , *T. compacta* and possibly *T. canton* n. sp. The second category is species with an Eurasian origin and includes *T. acuta*, *T. modesta*, *T. mutabilis*, , *T. nigra* and *T. nobilis*. The third category comprises one species, *T. maritinkae*, with an Afro-Asian distribution. The last category is composed of new species that are most likely native to Asia: these are *T. matsu* n. sp., *T. mandarin* n. sp., *T. wulai* n. sp. and *T. pagoda* n. sp. (Basson and Van As ,1994)

A new species of *Trichodina*, *T.hippoglossi n.* sp. collected from skin and fins of *Hippoglossus hippoglossus.* from farmed Atlantic halibut larvae. Four species of trichodinid ectoparasites from cultured Tilapia fishes. These species are: *T. centrostrigeate* ,*T. comacta*, *T. magna* , and *paratrichodina Africana* (Nilsen , 1995 ;Abdel Ghaffer *et al.* , 1996).

During screening for the presence of *Trichodina* sp. among *Tilapia* sp. , he found the highest prevalence was observed in the spring and winter followed by autumn and summer. Statistically significant differences were existed for the trichodinid species among sites on the fish, among length classes and between the sexes of fish hosts. In general, the intensity of infection was low except in cases where outbreaks of trichodiniasis is endangered the survival of fish in some ponds. In light infestation, *Trichodina* is usually present on gills, fins and skin of apparently healthy fish. Investigated the epizootic *Trichodina*, the results revealed the presence of three species of Trichodinid ciliates. *T. clariformis* sp. n., *T. jadranica* and *T. raabei* . *T. clariformis* the first recorded of a peritrichous mobilina from Atlantic cod of the Baltic sea. In contrast to their economical importance and abundance in the aquatic environments the exact identification of trichodinid ciliates often remains unclear. (Hassan,1999; Dobberstein and palm, 2000; Özer ,2003).

The recording for the first time three species of the trichodinid ciliates in India, from some freshwater fish, these are species *T. diaptomi* , *T. heterodantate* , and *T. oligocotti* . He found five species of the genus *Trichodina* from the gills parasites in freshwater fishes in west Bengal .

. Four of them described as new: *T. ahmedi sp .n.* from *Chanda nama*, *T. hafizuddini sp.n.* from *Amblypharyngodon mola*, *T.mossmbicusisp.n.* from

Oreochromis mossambicus and *T. heterospina sp.n.* from *sardinella fimbriata.* In addition, *T. martinkae* from *clarias batrchus* was recorded for the first time in India. Four new species of *Trichodina* from fresh water estuarine fishes. These are: *T. colisae* from *Polyacanthus fasciatus*. *T. cirratusi* from an estuarine fish. *T. Glossogobius* from anther estuarine fish, Glossogobius giuris, and *T.oreochromisi sp.n.* from *Oreochromis mossambicus* (Asmat ,2004; Asmat , 2005; Asmat and Sultana , 2005).

Trichodina sp. From southern occupied Palestine (Red Sea) unusual case of hyperparasitism of trichodinid ciliates the trichodinids heavily co infested the host fish gills. The case is most likely a result of accidental hyperparasitism, brought about by perturbed environmental conditions. *Trichodina magna* described from the Nile tilapia *Oreochromis niloticus* collected from three regions of Santa Catarina State, Brazil. They found out of 146 examined fishes, 36 were parasitized on the skin, 14 in the gills and 33 on the skin and gills, simultaneously. possibility treatment of using garlic *(Allium sativum)* and Indian almond *(Terminalia catappa)* to treat fish ectoparasite . *Trichodina sp.* instead of treatment with formalin seems at present to be infective possibly leaves toxic residues in fish and harmful to consumers. (Chitmanat *et al.* ,2005; Colorni and Diamant ,2005; Martins and Ghiraldelli ,2008)

1.2. *Ichthyophthirius multifiliis:*

I. multifiliis was described as ectoparasitic ciliates and it is one of the major protozoa causing diseases. This disease cause mortality in freshwater fishes and all developmental stages are susceptible to the infection with fingerlings. The optimum water temperature for the proliferation of this parasite is 15--25°C. So early winter and late spring are the prevalent seasons. The fish in high-density-overwintering ponds is more susceptible to the disease. The life cycle of *I. multifiliis*, it comprises a small migratory stage in search of a host, the theront; the feeding and growing stage in the skin or gills of fish, is the trophont ,with a characteristic, large horse shoe-shaped macronucleus. After it has reached a certain size it escapes from the host and encysts on a convenient substrate as a tomont. Within its cyst, it divides by a series of 10 to 11 divisions to produce small tomites. They break through the cyst wall to become theronts again. The duration of the life cycle stages, their size and number depend on the ambient temperature. Due to the growing trophont volume the microcirculatory disorders and associated necrotic changes are enhanced. *I. multiphiliis* feeds on the cell debris, forms small cavities beneath the epithelial layer. Both in gills and in skin the regressive changes of the epithelium are the most deleterial.(Shaogi ,1985; Lom and Dyková ,1991)

I . multifiliis was described as white spot disease. The most common symptom is the presence of pinhead size white spots on the skin, fins and gills. It causes simple hyperplasia of the epidermal cells around the site of infection forming blisters. *I . multifiliis* is a ciliate protozoan parasite characterized by its relatively larger and horseshoe shaped nucleus in adults and large trophozoites. Incidence of large-scale mortality due to this infection is common in nursery and rearing ponds. causes the fish to have a large amounts of mucus sloughing off of fish skin. Infections of *I . multifiliis* occur during the spring and fall when the water temperatures between 18 and 25C. Fish with *I . multifiliis* may be observed making quick rubbing or scratching movements on objects or on the pond bottom. This behavior is sometimes called "flashing" because of the quick and sudden exposure of the fish's light-colored belly as it rolls during erratic movements. After burrowing, *I . multifiliis* is very hard to treat because of the protective layer of mucus and host cells covering the parasite. the parasitic phase of *I .multifiliis* can encyst in the skin or gills and the damage to the skin and gills results in osmoregulatory and respiratory distress. Mortality may be caused by the parasite when the gills are too damaged to function. Breaching the protective barrier of the skin by *I .multifiliis* may also allow opportunistic bacteria or fungi access to underlying tissues resulting in death from secondary infections. Outbreaks of *I .multifiliis* occur when conditions are favorable for rapid multiplication of the parasite. This includes a suitable environment and susceptible fish ,higher temperature of water, dense stock of fish for several weeks and total weakening of the fish by malnutrition or starvation.(Kumar ,1992 ;Durborow *et al* .,1998;Guillen , 2003)

The spontaneous disease occurrence in the course of grass carp culture. During the culture of grass carp (*Ctenopharyngodon idella*) some disease problems occurred causing a high level of mortality ,the ectoparasites were *Trichodina* sp., and *Chilodonella* sp. The mortality rate reached 70% in two-month old fry. Following transportation to indoor conditions an ectoparasite identified as *I. multifiliis* caused a high mortality rate of 80% , in one-year-old grass carp . The investigated freshwater and marine fishes in Bangladesh A total of 290 species of parasites have so far been recorded. Ectoparasites protozoans are recorded mainly from cultured fish species of farms. Commonly occurring parasitic diseases are agrulos is (fish louse), Ichthyophthirias is (white spot) and Myxobolias. (Uzbülek and Yildiz, 2002; Chandra ,2006) .

I. multifiliis it is easily introduced into a fish pond, tank, or home aquarium by new fish or equipment which has been moved from one fish-holding unit to another. Quarantine is an effective way of preventing this disease. Once the organism gets into a large fish culture facility, it is difficult to control due to its fast reproductive cycle and its unique life stages. If not controlled, 100% mortality of fish can be expected. With careful treatment, the disease can be controlled, but the cost will be high, both in terms of lost fish, labor, and the cost of chemicals. . *I . multifiliis* is an obligate parasite and capable of causing massive mortality within a short time. Because the encysted stage is resistant to chemicals, a single treatment is not sufficient to treat *I . multifiliis* Repeating the selected treatment every other day for three to five treatments will disrupt the life cycle and control the outbreak. Daily cleaning of the tank or vat helps to remove encysted forms from the environment.(Francis-Floyda and Reed ,2009; Klinger and Francis-Floyda , 2009).

1.3.*Chilodonella* sp. *:*

Chilodonella sp. described as ciliated protozoan parasites is an oval, flat or a large, heart-shaped protozoan with parallel rows of cilia along the long axis of the organism a notched anterior end . Serious cosmopolitan ectoparasites .It swims over the surface of the skin and gills of fish, feeding on bacteria, necrotic skin cells and other organic matter. It has been seen only infrequently on host skin and only on fish weakened by bacterial diseases. It is often found in association with other parasitic and fungal infection, causes secrete excessive mucus. Chilodonellas seem to be of a great ecological adaptability, proliferating both in cold and warm water. Under conditions which favour their proliferation, chilodonellas may cover the body surface in a contiguous layer. They disintegrate the surface tissue by means of their oral cytoskeletal armament and feed on the cell debris. Pathological manifestations may vary depending on the intensity of infection. Gills disintegration and necrosis renders the gills nonfunctional, fish lose osmotic balance and die.(Areerat *et al.* ,1981 ;Lom and Dyková ,1991)

That ectoparasitic ciliate, *C. piscicola* , became epizootic and caused chronic mortalities in juvenile masu salmon *Oncorhynchus masou* and pink salmon *O. gorbuscha* being reared at the Nemuro Hatchery, Japan . Experimental transmission in masu salmon fry confirmed that *C. piscicola* could cause severe proliferation of the gill epithelium in the absence of other stressors. The marked epithelial hyperplasia was followed by intense fusion of adjacent gill lamellae and filaments. Respiratory failure due to gill epithelial hyperplasia may have been a primary cause of the deaths. Signs of infection .excessive mucous production. During heavy infections the parasites can cover almost the entire surface of the fish. They break down the surface of the skin and gills, feeding on the debris. Heavily infected fish increase mucus production and have a grey. The gills lamellae can fuse, reducing the area over which respiration can occur. In severe cases the gills eventually become necrotic, causing the fish to suffocate *Chilodonella piscicola* may cause mortalities among cultured salmonids. (Urawa and Yamao,1992; Arthur and Te ,2006)

Indicated in the Czech Republic, the disease affects tench mostly in spring after overwintering and when rearing the early fry stage in warm water. *C. hexasticha* is found on skin and gills of tench of all ages. Transmission is horizontal and direct, with the gills being the main target organ. The time course of disease is likely to be rapid under stressful crowded conditions and a temperatures that are within the range for each *Chilodonella* species. Epizootics caused by *C. hexasticha* in Australia occurred during the winter months, and at the optimum temperatures for *C. cyprinid.* The disease chilodonellosis and consequent losses found in spring after wintering. Weakening of the fish by malnutrition, long wintering and unfavourable oxygen conditions are conditioning factors for outbreak of the disease. However, Fingerlings in four salmoind farms in north Finland were found to be more susceptible to *Chilodonella* infection than older fish, and mortality varied in the range 2–10%. Most mortality cases were caused by *C. hexasticha*, occurring mainly on the gills of the fish. *C. piscicola* was most often found in salmon and occurred at lower water temperatures than *C. hexasticha*. (Svobodova and Kolarova ,2004 ;Diggles and Hine , 2005; Rintamaki *et al.* ,2007)

Chilodonella can be treatments with chemotherapeutants in the water or feed in a recalculating system present special considerations; the main one is whether the biofilter will be treated and how the chemicals could affect its function. *Chilodonella* can be controlled with any chemical, and one treatment is usually adequate. *Chilodonella* has been eliminated in tanks using recalculating water systems by maintaining 0.02% salt solution . (Noble and Summerfelt ,1996; Klinger and Francis-Floyda ,2009)

That parasitic fauna recorded of ornamental fish from Florianópolis. Out of 189 examined fishes, 65 (34%) were parasitized: with 2.6% *Chilodonella* sp. The largest mean intensities of infection were 335 and 205, respectively to *X. maculatus* and *X. helleri*. While when exanimate of ectoparasitological for fish samples collected from four Croatian rivers. Revealed that infections by the ciliate parasites *T. nigra* (29%), *Epistylis* sp. (9%), *C.cyprinid* (58%), *I. multifiliis* (81%) and dinoflagellate *Piscioodinium pillulare* (45%). (Piazza *et al.* ,2006; Zrncic *et al* . ,2009)

1.4. *Tetrahymena* sp.:

T. corlissi, describe as a free-living protozoan in many types of tropical and cold water fish are susceptible to infection fresh water can alternate between free living, commensal and parasitic modes of survival. It is the causative agent of Guppy Disease. *Tetrahymena* spp. is a teardrop-shaped ciliate that moves along the surface of the external host. *Tetrahymena* cells have a striking variety of highly complex and specialized cell structures, unicellular organisms that divide by binary fission apparently However, they can also conjugate and exchange their genetic material prior to cell division, caused the death of large numbers of guppies (*Poecilia reticulatus*) and occasionally other fishes, in aquaria and hatcheries at several locations. Apparently the disease occurs when both the fish and protozoan populations are at a high level of density. Commonly found living in organic debris at the bottom of an aquarium and commonly found on dead material and is associated with high organic loads. The signs include white spots and epidermal damage. (Hoffman *et al.* , 1975; Klinger and Francis-Floyda ,2009)

2-Flagellates:

Flagellates was described as a protozoan parasite , trophozoites stage move by means of one or many flagella. They have one nucleus, or exceptionally more monomorphic nuclei. Phylum includes free-living and many parasitic organisms of different groups (six classes), autotrophic forms with chloroplasts and heterotrophs without chloroplasts. They reproduce by binary fission. (Lom and Dyková ,1991)

2.1. *Ichthyobodo necator:*

I. necator is a common and important flagellate parasite that infests the skin and gills of many freshwater and marine fish. *Ichthyobodo* infestations are often fatal and cause significant aquaculture losses worldwide. Recently it has been demonstrated that *Ichthyobodo* is a multispecies complex with differing host preferences. *I. necator*, formerly known as *Costia* , is a commonly encountered external flagellate. The *I. necator* parasite is a protozoan that has two life stages. One is a free-swimming stage, in the stage the parasite swims until it locates the host. When a suitable host is found, such as a fish, it attaches. It fast moving and can be seen flitting across the field of vision in jerky movements as it propels it's kidney-shaped or small sickles body with the flagella. (Callahan *et al.*,2005 , Klinger and Francis-Floyda ,2009)

Ichthyobodo sp. is a small flagellate external protozoan parasite of skin and gills causes considerable damage in fry and small fingerlings. Some species of fish are more susceptible to *Ichthyobodo* infection than others. *I. necator* as a dangerous ectoparasite of practically all freshwater fish causing mortalities in young fish and fish with lowered resistance. The feeding of attached form, fixed onto the epithelial cells, is highly modified. The cell furls in a pyriform shape, the flagella pointed off the fish surface. This produces severe irritation with excessive mucous secretion causing patches over the body. Symptoms of *I. necator* in fish is characterized by flat gray or white patches on the fish's body. It looks different from fungus, which usually forms wool-like tufts. Infection with *Ichthyobodo* is smooth in appearance. Once the parasite attaches to the fish it is now in the parasitic stage. During this stage it feeds off the fish's skin. It also multiplies on the fish's skin by cell division. (Lom and Dyková ,1991; Kumar ,1992)

That *Ichthyobodo* parasite being a cold-water parasite it is particularly dangerous in the spring when the immune system is weaker after the winter , does not cause distinctive lesions on the fish but do block the flow of oxygen when heavily loaded on the gills. As with most protozoa, environmental degradation and crowded conditions cause them to become more damaging. However, prevention measures such as reducing stocking densities and lowering feeding rates may make fish production unprofitable. *Ichthyobodo* occurs on the skin and gills. The organism is notorious for affecting fish in crowded environments such as cages and can cause significant mortalities when infestations are heavy (Durborow ,2003)

3- *Myxosporidia (Phylum Myxozoa):*

Large number of *myxosporeans* spp. that causes many serious pathogens of commercially important fishes. *Myxosporidia* are characterized by spores composed of several cell transfigured into 2 to 7 spore shell valves, 1 to 2 amoeboid infective germs (sporoplasms) and 2 to 7 polar capsules.

Myxosporean spores may be of various shape and structure. The trophic (or vegetative) stages, or trophozoites, vary greatly in dimensions and shape. Since vegetative stages offer mostly no features important for classification, the taxonomy is based solely on the shape and structure of the spore. Presently, there are about 1100 myxosporean species described from fishes. *Myxobolus cerebralis* (syn. Myxosoma cerebralis) is the agent of the "whirling disease" of salmonid fry affecting head and vertebral column cartilage. Spores are extremely variable, oval to circular in front view. Head and gill arch cartilage are sites of predilection. (Lom and Dyková ,1991)

3.1. *Myxobolus* spp.:

Many species belong to the order of Myxospore ; the class, of sporoza. Such parasites have the ability to parasitize any organs or tissues of all kinds of fish. Some species will cause harmful epidemic. *Myxosporidia* parasitize host generally in the form of cytocyst. Myxosporidian and microsporidian parasitic infections are very frequent in major carp species. Reports of large-scale mortalities of fry and fingerlings of carp species are common due to such infections. Several species of Myxosporidia have been found to infect all the carp species and form cysts on the body surface, fins, gills and internal organs such as the kidney and spleen . However, when large numbers of oocysts are present on the gills, breathing of the fish is adversely affected. The most common symptoms of the disease are weakness, emaciation, scale protrusion, loss of scales, abnormal pigmentation .Spores released from the infected and dead fishes remain viable for quite a long period in the pond bottom before they infect new hosts. Infected fish should immediately be removed and, if possible, the pond should immediately be dried and disinfected. In undrainable ponds where drying is not at all possible, the pond should be disinfected with chlorinated lime.(Shaogi , 1985; Kumar , 1992)

The traditionally classified with the protozoa due to their small size, it is now recognised that myxozoans are degenrate metazoans ,transmission is via an oligochaete alternate host. The Myxosporea are a class of microscopic parasites, belonging to the Myxozoa. They have a complex life cycle which comprises vegetative forms in two hosts, an aquatic invertebrate (generally an annelid) and an ectothermic vertebrate, usually a fish. Each host releases a different type of spore. The two forms of spore are so different that until recently they were treated as belonging to different classes within the Myxozoa (Svobodova and Kolarova , 2004).

Whirling disease, caused by the myxosporean *M. cerebralis*, occurs worldwide and in all trout and salmon species. Rainbow trout are particularly vulnerable and it is most severe in trout less than 6 months old. Myxozoa parasites are a 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. Myxozoans are parasites affecting a wide range of tissues. Clinical signs vary, depending on the target organ.Elimination of the affected fish and disinfection of the environment is the best control of myxozoans. There are no established remedies for fish. Spores can survive over a year, so disinfection is mandatory for eradication.(Durborow ,2003; Klinger and Francis-Floyda ,2009).

Materials and method

3. Materials and method

3.1. Study area:

Benghazi city (23°10 N /20°06 E) has a Mediterranean climate of moderate wet winters and worm, dry summers. During the winter the temperature may drop to less than 5C° at night whereas it rises up to 38C° at mid-day during the summer and high humidity.

3.1.1. Ain Ziana lagoon:

Ain Ziana lagoon is a brackish body of water lies about 15 Km east of Benghazi city centre (Located at 23°12□55.12″ N 20°09□ 15.23″ E). Its open water surface covers about 50 ha. In addition, there are large adjoining marsh areas on the southeastern and northwestern sides that total several hundred hectares in extent. Numerous underground springs charge the lagoon with freshwater, but there are also saltwater incursions from the sea. The open water area of Ain Ziana lagoon is host for a small mullet, eel and bream fishery (Fig 1).

3.2. Materials:

3.2.1. Fish sampling:

The fishes were sampled with the help of fishermen from Ain Ziana lagoon. Fishes were collected by netting, then brought alive to the laboratory in zoology department, Faculty of Science, Benghazi University. Fishes were studied immediately in the living condition; the collected fishes were put in aerated tanks.

Fig(1): Ain Ziana lagoon (source :Google earth)

Two hundred twenty four fishes (one hundred and four of Mullet and one hundred twenty of Tilapia, were collected randomly from Aian Ziana lagoon , during the period October 2008 to December 2009). Identification of fish species was and according to Froese and Pauly (2009).

3.3. Methods:

The fishes were checked for clinical signs, according to the method described by Amlacher (1970). The total length was merged, with and without the tail , the total length with the tail was approved (Plate 1). Body weight of examined fishes was determined. (plate 2) The specimen was then defected to determine the fish six.

Scraping the skin of fish with distilled water and then isolated the gills and cleaning with distilled water. Then make wet smears of skin and gills and examined under microscope. Positive slides were dried and fixed in absolute methanol then staining with Giemsa stain for 5-10 minute (Alvarez , 2008) . Then parasites were photographed using a photomicroscope-camera (Zeiss, Germany).

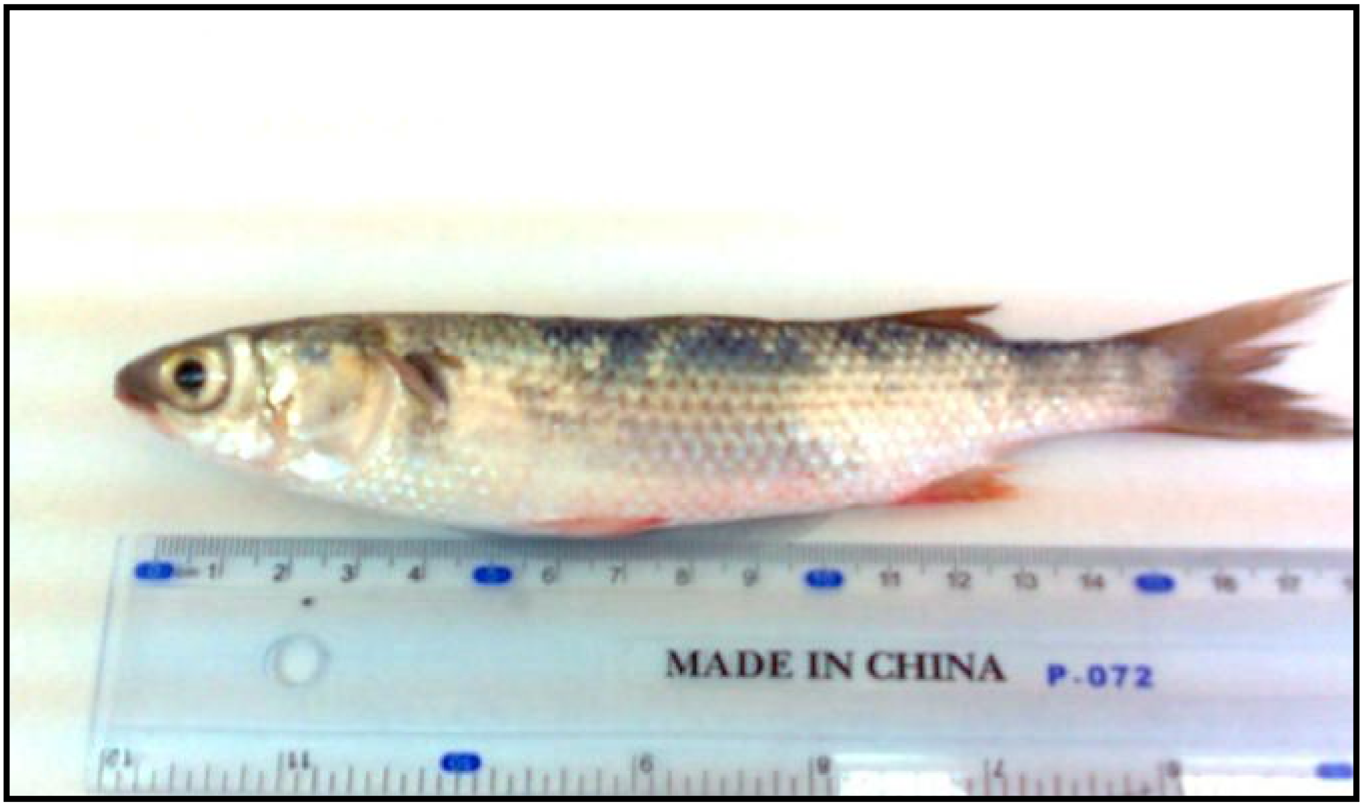


Plate (1): *Mugil cephalous* (Striped mullet)

Plate (2): *Tilapia zillii* (Green Tilapia)

3.3.1. Laboratory examination:

3.3.1.1. Detection of *Trichodina*:

Smears of skin were taken from living or freshly killed fishes and gill-scrapings were taken from the first gill arch, these were carefully searched for *Trichodina* sp.

Prepared smears were examined for the presence of *Trichodinid* ciliates. By using a compound research microscope, positive slides were investigated by using Klein´s silver –impregnation technique to demonstrate the component of the adhesive disc and the aboral ciliary spiral as described by Lom, 1958.

Dried slides then impregnated with 2% aqueous solution of silver nitrate (AgNO3) for 6-8min , rinsing in distill water, then placed in white clean Petri dish, covered with distal water and exposed to Ultra violet light for two hours ,then rinsed in distill water, dried in air, mounted in D.P.X. All morphometeric measurements were carried out by oil-immersion light microscope. (El-Tantawy and El-Sherbiny 2010).

3.3.1.2. Detection of other ectoparasites:

For detecting other ectoprotozoan parasites. Dry smears were fixed in absolute methanol (5-10) stained with Giemsa stain (to study the nuclear apparatus and cytoplamic compounds.) for 5-10 min then rinsed in distal water, dried in air, mounted in D.P.X. (El-Tantawy and El-Sherbiny 2010)

3.3.2. Statistical analysis:

Statistical analyses were carried out to determine the incidence and significance of the data. The logistic regression (Dowdy *et al*., 2004) used to find the relationships between the length, body weight, sex and the parasitic infection.

Results

4. Results

Mugil cephalus (gray mullet) and *Tilapia zillii* (green tilapia) are very common fishes in the study area.

4.1. Incidence:

Fifty (22.3%) out of two hundred twenty four collected *Mugil cephalus* and *Tilapia zillii* were found infected with ectoprotozoan parasites .The result revealed that thirty one of *M. cephalus* were infected with ectoprotozoan parasites with incidence rate 29.8%, and showed that out of one hundred and twenty *T .zillii* examined for ecto protozoan parasites nineteen fish were infected at incidence rate 15.8% (Table 1 and Fig. 2).The results revealed that six species of ectoprotozoan parasites of *M. cephalus* and *T. zillii* were detected during the examination of skin and gills contents of these fishes. Theses parasites were *Trichodina sp. , Tetrahymena sp. , Chilodonella sp. , Myxobolus spp. , Ichthyophthirius multifiliis , Ichthyobodo necator* .

The result-showed that, the most common protozoan parasite with highest infection rate was *Myxobolus spp*. 13.8% (31/224).followed by *Ichthyophthirius multifiliis* 6.5% (15/224) *Chilodonella sp* 4.5% (10/224). *Trichodina sp.* 2.7% (6/224), *Tetrahymena sp.* 1.3% (3/224) and *Ichthyobodo necator* 0.9% (2/224) (Table 2 and Fig. 3).

Table (1) :Overall incidence of ectoparasitic protozoan parasites infection in infected fishes .

Type fishes	No. examined	No. Infected	Percent%
M. cephalus	104	31	29.80%
T. zillii	120	19	15.8%

Fig. (2) :Overall incidence of ectoparasitic protozoan parasites infection in infected fishes .

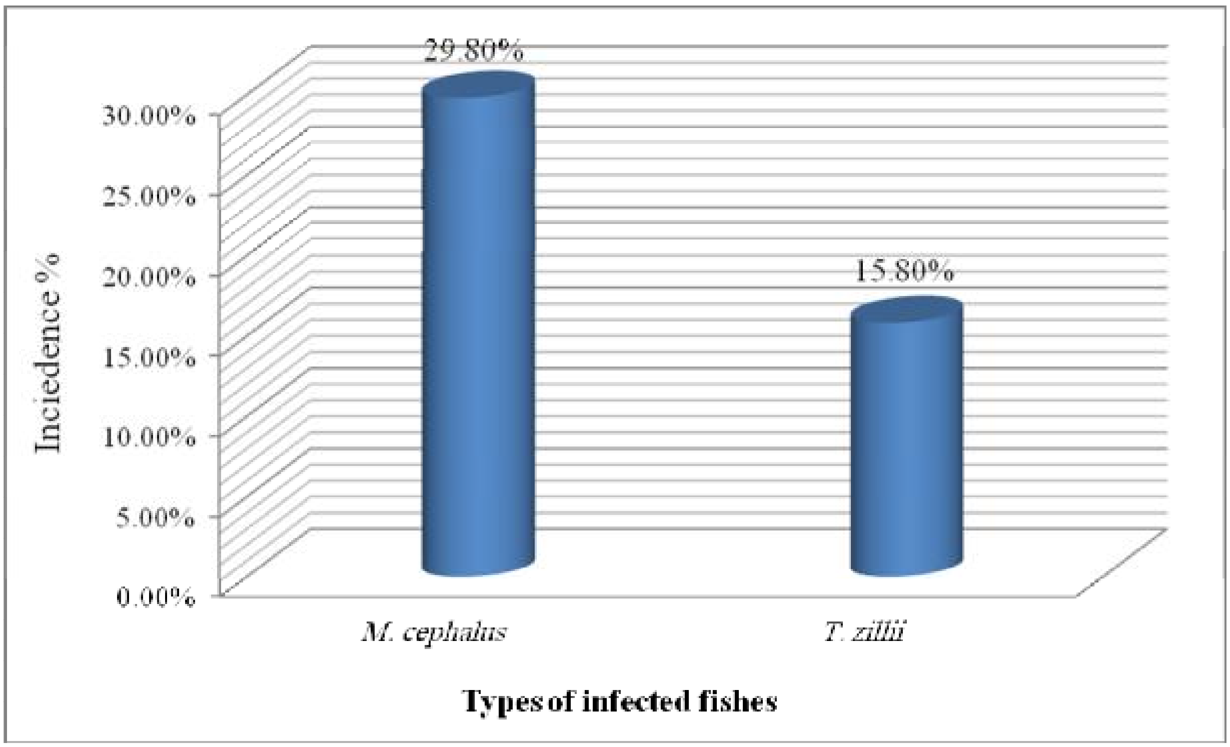
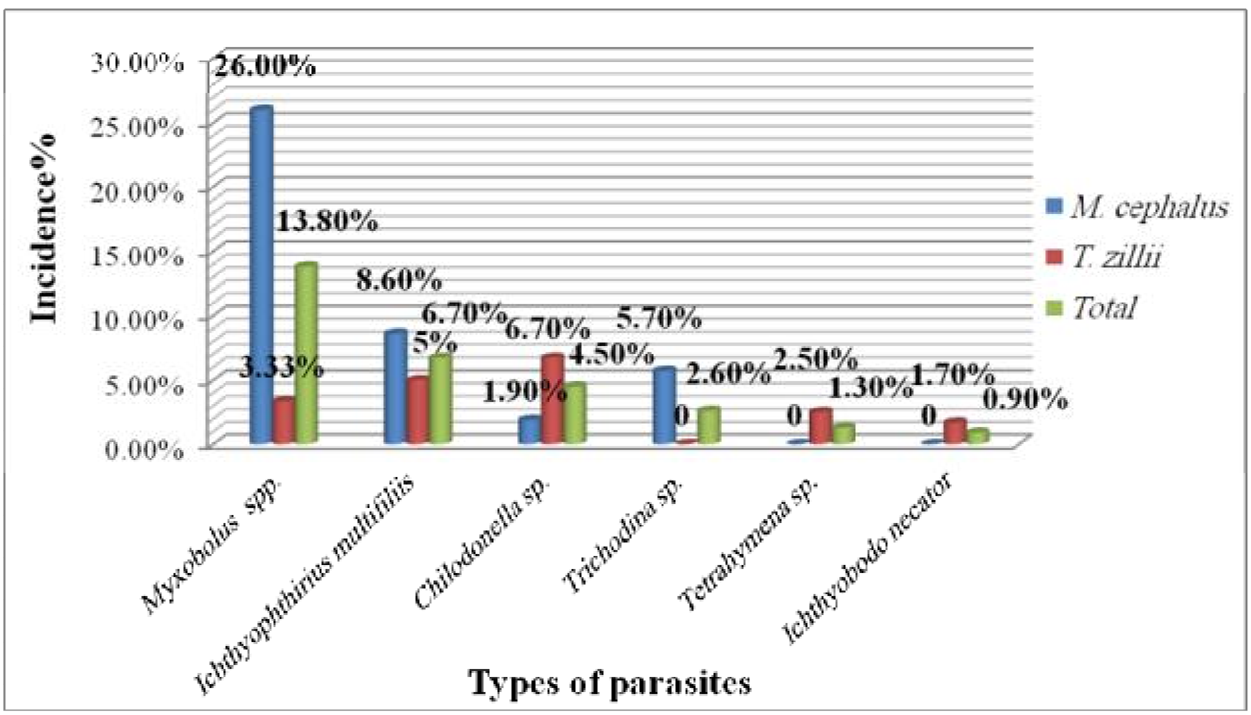


Table (2) :Overall incidence of ectoprotozoan parasites types in infected fishes.

Parasites type	*M. cephalus* (N=104)		*T. zillii* (N=120)		Total (N=224)	
	No. infected	(%)	No. infected	(%)	No. infected	(%)
Myxobolus spp.	27	26.0%	4	3.33%	31	13.8%
Ichthyophthirius multifiliis	9	8.60%	6	5%	15	6.7%
Chilodonella sp.	2	1.90%	8	6.70%	10	4.5%
Trichodina sp.	6	5.7%	–	–	6	2.6%
Tetrahymena sp.	–	–	3	2.50%	3	1.3%
Ichthyobodo necator	–	–	2	1.70%	2	0.9%

Fig . (3): Overall incidence of ectoprotozoan parasites types in infected fishes.



4.2 . *Myxobolus* spp. *:*

***Myxobolus* spp.** was the most common protozoan parasite recovered from the skin and gills of *Mugil cephalus* 12.1% (27/224) and *Tilapia zillii* 1.8% (4/224) samples from the study area .

The result revealed that 4 species. of *Myxobolus* spp. ,described spore of *Myxobolus* spp. is formed by two Chitin shells of same size and thickness. character of plasmodium have line that two shells joins is called sutural line. The sutural line, because of thickness, shows the ridge texture, which called sutural ridge. The side with sutural ridge is called lateral side. The side without sutural ridge is called front side. The spore contains two polar capsules and sporoplasm . Each polar capsule has a polar filament that curls spirally. The number of nuclei inside the sporoplasm may differ in different developmental stages of the spore.

Overall incidence was (13.8%) three species recovered from the gills , and one species was recovered from the skin, and one species recovered from both gills and skin .Different incidence rates for each species was at: sp.1 (5%) , sp.2 (6.2%) ,sp.3 (4.4%), and sp4. (9.3%) (Table 3).

Classification :

Kingdom: Protista

Sub-Kingdom : Protozoa

Phylum: Myxozoa

Class: Myxosporea

Order : Bivalvulida

Family : Myxobolidae

Genus : *Myxobolus* sp.1,sp.2,sp.3, and sp.4. Bütschli, 1882.

Table (3) : Incidence rates of *Myxobolus spp*. in infected fishes (N:224).

Type of *Myxozoa spp*.	**No. infected**	**Incidence**
Myxobolus sp.1	11	5%
Myxobolus sp.2	14	6.2%
Myxobolus sp.3	10	4.4%
Myxobolus sp.4	21	9.3%

Description :

***Myxobolus* sp.1:**

This species recovered from skin and gills of examined *M. cephalus,* at incidence rates 3.8% (4/104) , and 9.6% (10/104) respectively, while *T. zillii* infection was detected only in skin at incidence rate 0.8% (1/120). This species (*Myxobolus* sp.) was rounded in shape and with two layers and two big equal size polar capsules extend to level of beyond the middle with contact wall, and small sporoplasm, measured 3.6µm(3.0 -5.0µm) in length and 2.0µm (1.0µm – 3.0 µm)in width (Plate 3).

***Myxobolus* sp.2:**

This species was isolated from the gills of examined M. *cephalus* only at incidence rate 13.4% (14/104). It was ovoid in shape with polar capsules nearby wall , extend to the level of the middle with one polar larger than other , and big sporoplasm, measured 4.6µm (4.0µm-5.0µm) in length and 3.3µm (3.0-4.0µm) in width (Plate 4).

***Myxobolus* sp.3:**

This species was recovered from the gills of examined M. *cephalus,* only at incidence rate 9.6% (10/104) . It was round in shape, have small equal size polar capsules extend to the level of beyond the middle with nearby wall, and small sporoplasm and measured about 2.4µm (2.0 – 3.0µm) in length and 1.0 µm (1.0-1.1 µm) in width (Plate 5).

***Myxobolus* sp.4:**

This species was detected in the skin of ***M. cephalus*** and ***T. zillii*** , at incidence rate 21/224 (9.3%).In examined ***M. cephalus*** incidence rate was 18/ 104(17.3%) while in examined ***T. zillii*** incidence rate was 3/120(2.5%). It was ovoid in shape, have small equal size polar capsules extend to the level of the end of anterior third , measured about 4.5µm (3.2-5.5µm) in length and 3.3µm (3.0µm-4.0µm) in width (Plate 6)

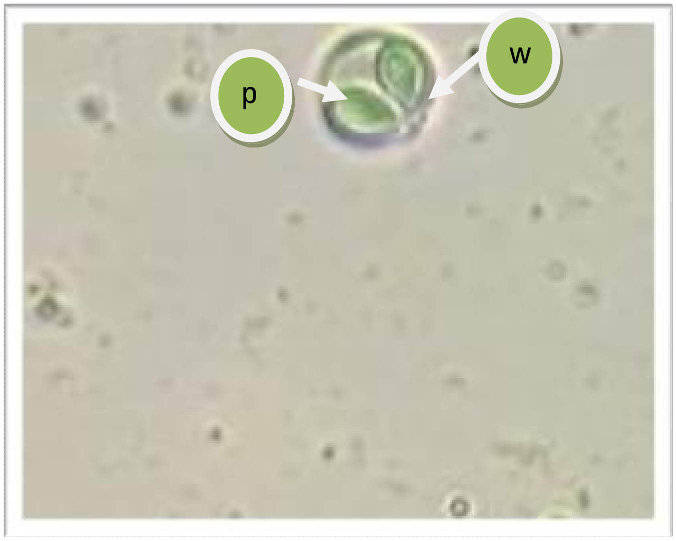


Plate (3): *Myxobolus* sp.1 from the gills of *M. cephalus* (p: polar filament .w: wall.).(x 40).

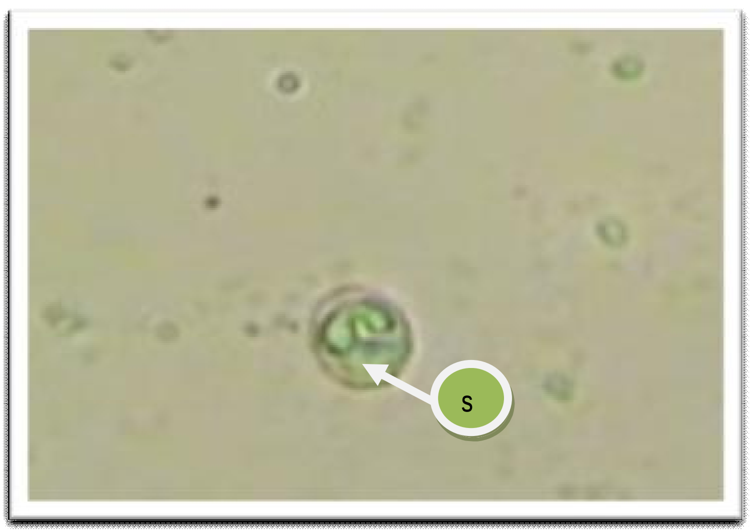


Plate (4): *Myxobolus* sp.2 from the gills of *M. cephalus* (S: sporoplasm) .(x 40)

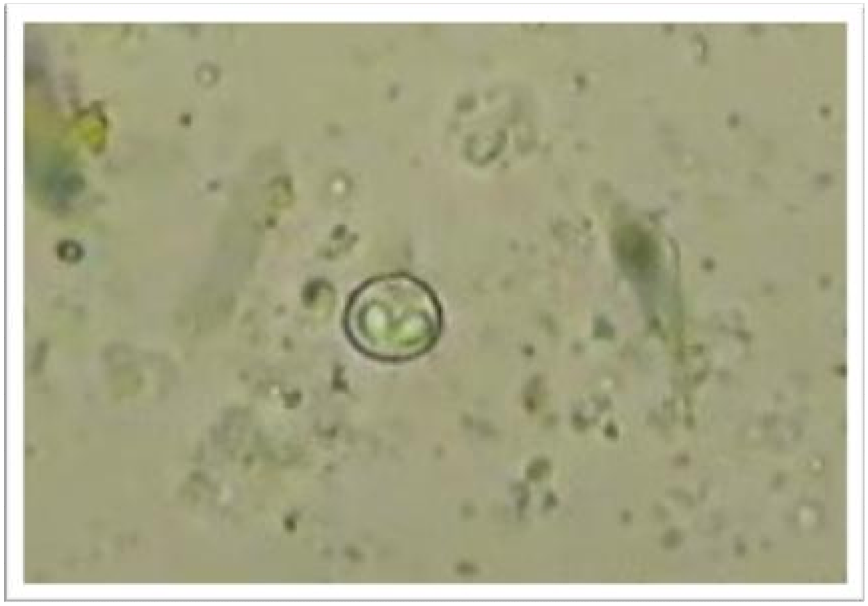


Plate (5): *Myxobolus* sp.3 from the gills of *M. cephalus*.(x 40)

Plate (6) : *Myxobolus* sp.4 from the skin of *M. cephalus* .(x 40)

4.3. *Ichthyophthirius multifiliis:*

This species was isolated from of the skin of *M. cephalus* and *T. zillii* fishes. The overall incidence rate was 6.7% (15/224). The incidence rate for each fish species was 8.6% (9/104) in examined *M. cephalus* and 5% (6/ 120) in examined *T. zillii* .

Classification:

Kingdom: Protista

Sub-Kingdom : Protozoa

Phylum: Ciliophora

Class: Oligohymenophorea

Order : Hymenostomatida

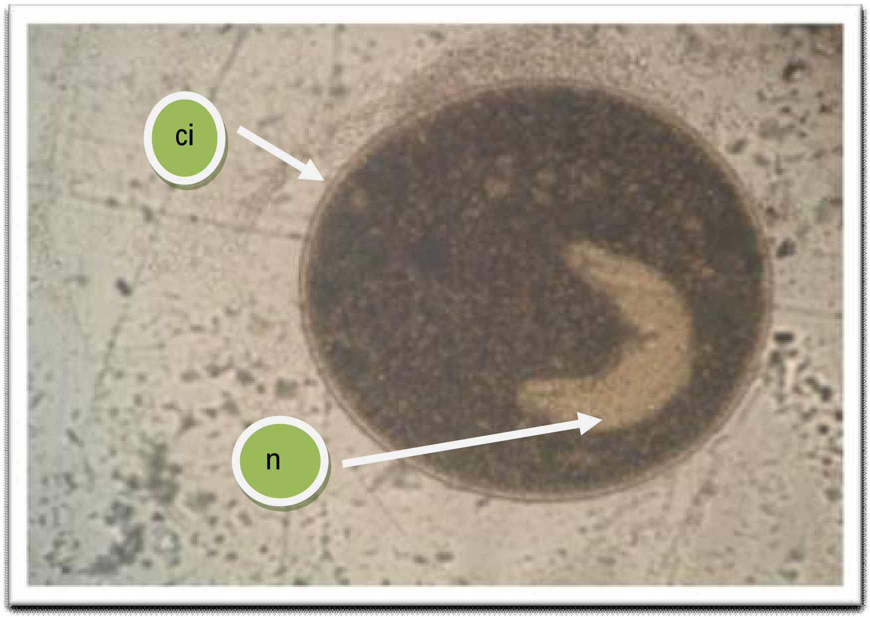
Family : Ichythyophthiriidae

Genus: *Ichthyophthirius*

Species : *Ichthyophthirius multifiliis* Fouquet, 1876

Description :

I. multifiliis was sub-spherical in shape and measured about 20µm(19 – 22 µm) in length and 15 µm (10 – 20 µm) in width . have two layers with large size horseshoe shaped nucleus and vacuole , their movement was slowly spiral motion and has caudal cilium.(Plates 7).



Plate(7): ***I. multifiliis*** from Web site .(n : nuclei, ci: cilia,) .

4.4. *Chilodonella* sp. :

This species was recovered from the skin of both *M. cephalus* and *T. zillii* fishes. The overall incidence rate was 4.4% (10/224). The incidence rate for each species was 1.9% (2/104) in examined *M. cephalus* and 6.6% (8/120) in examined *T. zillii*.

Classification:

Kingdom: Protista

Sub-Kingdom : Protozoa

Phylum: Ciliophora

Class: Oligohymenophorea

Order : Cyrtophorida

Family : Chilodnellidae

Genus: *Chilodnella*

Species : *Chilodnella* sp. Strand ,1926.

Description:

Their body was oval or heart-shaped, with vacuole in their cytoplasm , has caudal cilium and measure about 25µm (17- 33µm) in length and 15 µm (6-24µm) in width (Plate 8).

Plate (8): *Chilodonella* sp. from the skin of *M. cephalus* and *T. zillii*.

(ci: cilia) (x 40).

4.5. *Trichodina* sp.:

Trichodina sp. Was isolated from the gills of *M. cephalus* fishes. No *Trichodina* was detected in *T. zillii*. The incidence rate was 12% (6/224).

Classification:

Kingdom: Protista

Sub-Kingdom : Protozoa

Phylum: Ciliophora

Class: Oligohymenophorea

Order : Mobilina

Family : Trichodinidae

Genus: *Trichodina*

Species : *Trichodina* sp. Raabe,1959

Description :

Trichodina sp. described as flying saucer or scrubbing bubbles shape , rounded with surrounded cilium. Adhesive disc consists of ring of hollow conical elements with flat lateral projections known as denticeles and Adoral ciliary spiral. It is composed of about 21 denticeles. The centrifugal projections of denticeles mostly semicircular. Measured about 37 µm (32 -44 µm) diameter, their movement was fast motion. (Plates 9 a, b).

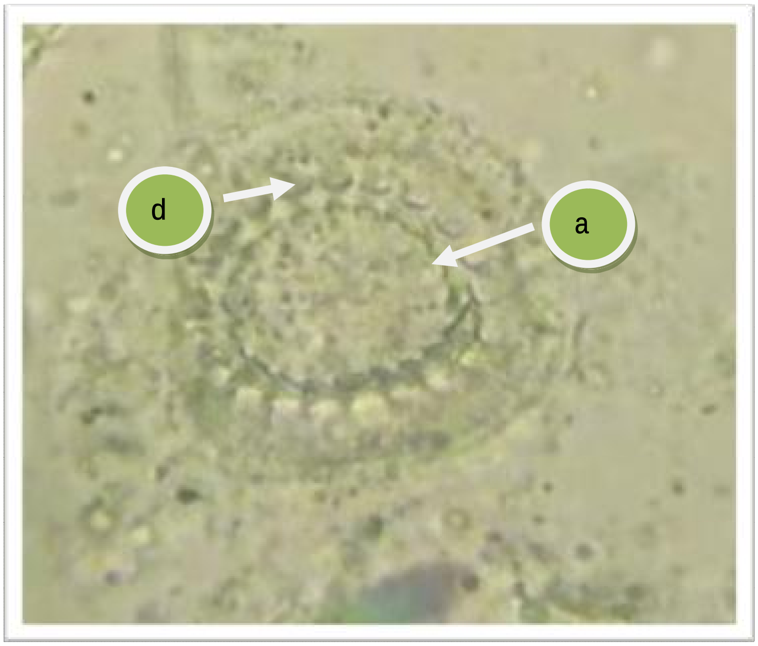


Plate (9 a): *Trichodina* sp. from the gills of *M. cephalus*.(a: adhesive disk, d :denticle) .(x 40)

Plate (9 b): *Trichodina* sp. from the gills of *M. cephalus*. (ci : cilia).(x 40)

4.6 *Tetrahymenina* sp.:

This species was isolated from the skin of ***T. zillii*** fish. No *Tetrahymena* was recovered from ***M. cephalus***. The overall incidence rate was 1.3% (3/224)

Classification:

Kingdom: Protista

Sub-Kingdom : Protozoa

Phylum: Ciliophora

Class: Oligohymenophorea

Order : Hymenostomatida

Family : Tetrahymenidae

Genus : *Tetrahymena*

Species : *Tetrahymena* sp. Furgason, 1940

Description :

Tetrahymena sp. recovered from skin and it was ovoid or tear-shaped, measured about 50 µm (40 - 60µm) in length and 25µm(15 -35µm) in width , with a caudal cilium and many vacuoles ,has apical loop and peroral suture (Plate 10).

Table (10): *Tetrahymenina* sp. from the skin of *T. zillii* .(v : vacuole. w: wall , ci: cilia). (x 40)

4.7. ***Ichthyobodo necator*:**

This species was detected in the skin of *T. zillii* . No *Ichthyobodo* was detected in *M. cephalus* . The overall incidence rate was *0.8*% (2/224).

Classification:

Kingdom: Protista

Sub-Kingdom : Protozoa

Phylum: Mastigophora

Class: Dinoflagellida

Order : Blastodini

Family : Bodonidae

Genus : *Ichthyobodo*

Species : *Ichthyobodo necator* (Henneguy , 1883)

Description :

I. necator was isolated from the skin . Very fast randomly spiral motion. Flat, oval body, it's strongly convex dorsally and slightly concave ventrally , two unequal flagella extend from flagellar pocket , an oval. centrally located nucleus , measured about 7.6µm(6.1 -9.1µm)in length and 4.1 µm(2.0 -6.2µm) in width .(Plate 11).

Plate (11): *Ichthyobodo necator* from the skin of *T. zillii*. (w: wall. n: nuclei. f: flagella). (x 40)

4.8.1 Incidence and sex:

Infection of ectoprotozoan parasite was detected in both males and females of *M. cephalus* and *T. zillii*. The relationship between incidence of ectoparasitic protozoan parasites and sex is presented in Table (4) and Fig.(4) .

Out of the total *M. cephalus* males constituted 22(24.4%) and female 4(28.6%) , and *T. zillii* males constituted 16(21.3%) and female 3(6.70%)There was significant difference was detected between incidence and sex (χ^2 =18.147,df =1, p>0.05, p-value=0.000*).

4.8.2. Incidence and seasons:

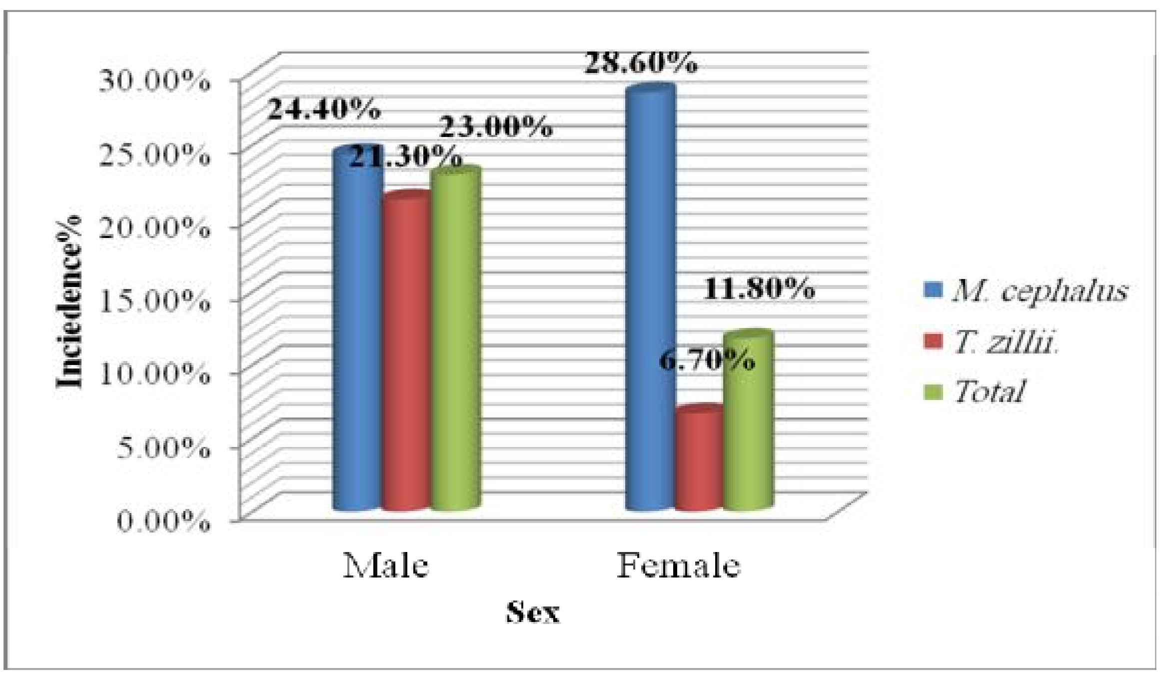
The incidence of ectoprotozoan parasites of *T. zillii* was higher in Autumn (77.3%) rather than *M. cephalus* was (43.8%), but *M. cephalus* was higher in Winter (42.9%) rather than *T. zillii* was (28.6%). In Summer infection in *M. cephalus* was (13.8%) but no infection was detected in *T. zillii.* (Table 5 and Fig 5) . (χ^2 =17.196,df=3, p>0.05, p-value=0.001*)

Table (4): Relationship between infection ectoprotozoan parasites of examined fish and sex.

Fishes types	Sex			
	Male		Female	
	No. examined	No. infected	No. examined	No. infected
M. cephalus	90	22(24.4%)	14	4(28.6%)
T. zillii.	75	16(21.3%)	45	3(6.70 %)
Total	165	38(23.0%)	59	7(11.8%)

χ^2 =18.147,df =1, p>0.05, p-value=0.000*

Fig (4): Relationship between infection ectoprotozoan parasites of of examined fish and sex.



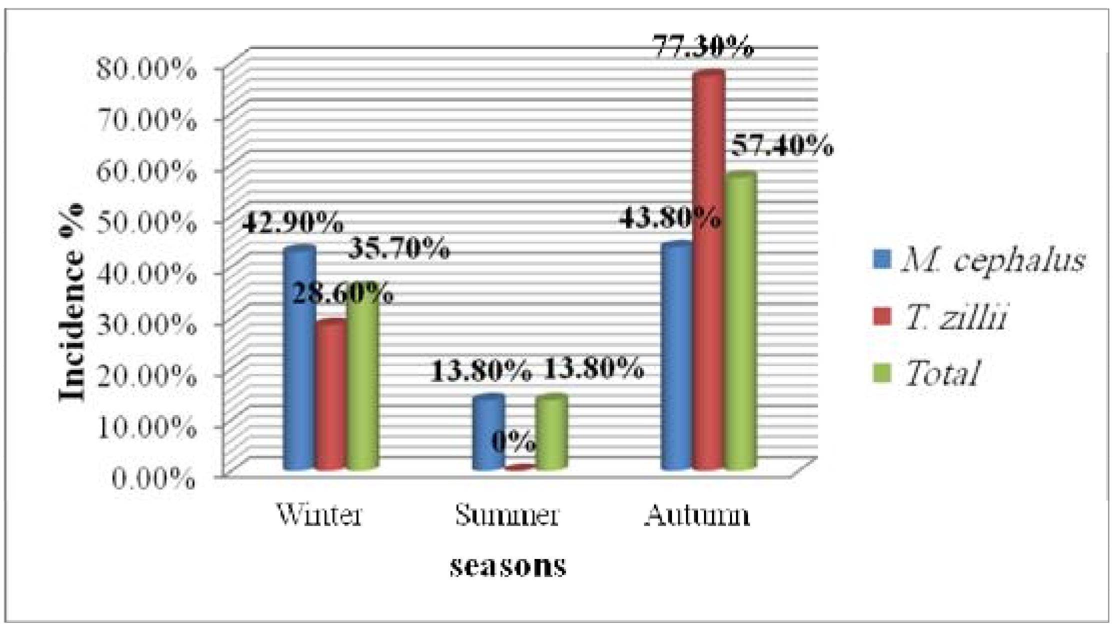
χ^2 =18.147,df =1, p>0.05, p-value=0.000*

Table (5): Relationship between infection ectoprotozoan parasites of examined fish and season.

Fishes type	Seasons					
	Winter		Summer		Autumn	
	No. examined	No .infected	No. examined	No .infected	No. examined	No .infected
M. cephalus	7	3(42.9%)	65	9(13.8%)	32	14(43.8%)
T. zillii	7	2(28.6%)	91	(0%)	22	17(77.3%)
Total	14	5(35.7%)	156	9(13.8%)	54	31(57.4%)

χ^2 =17.196,df=3, p>0.05, p-value=0.001*

Fig .(5): Relationship between infection ectoprotozoan parasites of examined fish and season.



χ^2 =17.196,df=3, p>0.05, p-value=0.001*

4.8.3. Incidence and body length :

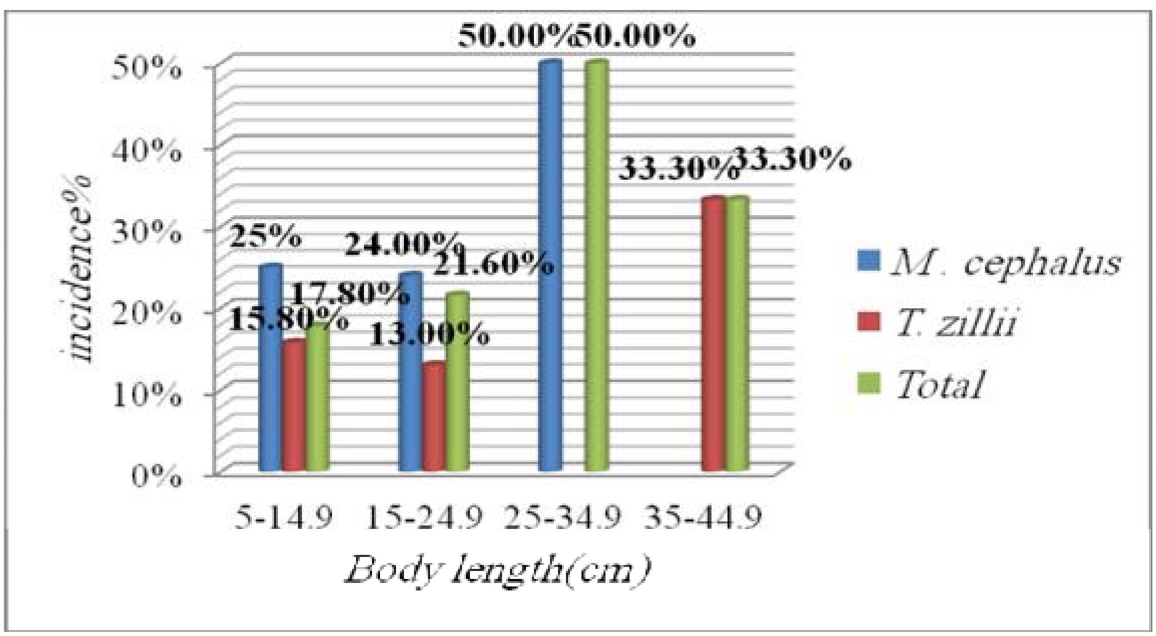
The incidence of infection with ectoprotozoan parasite on skin and body length of *M. Cephalus* and *T. zillii* presented in Table (6) and Fig. (6). Significant difference between incidence and body length was detected (χ^2 =86.539,df= 3 , p>0.05, p-value=0.000*).

Table (6) :Relationship between infection ectoprotozoan parasites of examined fish and body length.

Fishes types	Body length (cm)							
	5-14.9		15-24.9		25-34.9		35-44.9	
	No. examined	No .infected	No. examined	No .infected	No. examined	No .infected	No. examined	No .infected
M . cephalus	28	7(25%)	72	17(24.0%)	4	2(50%)	—	—
T. zillii	101	16(15.8%)	16	2(13.0%)	—	—	3	1 (33.3%)
Total	129	23(17.8%)	88	19(21.6%)	4	2(50%)	3	1(33.3%)

χ^2 =86.539,df= 3 , p>0.05, p-value=0.000*

Fig. (6) : Relationship between infection ectoprotozoan parasites of examined fish and body length.



χ^2 =86.539,df= 3 , p>0.05, p-value=0.000*

4.9.1. Single and mixed of infection on skin:

Ectoprotozoan parasites of skin was detected in 45(90%) of total 50 infected fishes ,and incidence was 26(57.8%) in *M . cephalus* and 19 (42.2%) in *T. zillii* .Twenty one (20.20%) of infected *M . cephalus* had single infection (with one species of parasites) and five (4.80%) had mixed infection (infected with more than one species of parasites).while sixteen (13.30%) of *T. zillii* had single infection and three (2.50%) had mixed infection. The result showed that there was a significant differences were detected between incidence and type of infection (χ^2 =17.531,df=6, p>0.05, p-value=0.008*) (Table 7 and Fig. 7).

The infection with *Myxobolus* sp.4 15(14.4%) showed the highest infection of ectoprotozoan parasite on skin of *M . cephalus*, followed by *I. multifiliis* 4 (3.8%), and *Myxobolus* sp.1 2 (1.9%). while highest infection of ectoprotozoan parasite on skin of *T. zillii* was detected in *Chilodonella* sp 5 (4.2%), followed by *Tetrahymenina* sp and *I. multifiliis* 3 (2.5%) each, *Ichthyobodo necator* and *Myxobolus* sp.4 2 (1.7%) each , and *Myxobolus* sp.1 1 (0.8%).

Mixed infections with two species of ectoprotozoan parasite in *M. cephalus* were *Chilodonella* sp + *I. multifiliis* at incidence rate 2 (1.9%) followed by *Myxobolus* sp.4+ *I. multifiliis* 1 (0.9%). while mixed infection with two species of parasite in *T. zillii* was *Chilodonella* sp + *I. multifiliis* 2 (1.7%).

Mixed infections with three species of ectoprotozoan parasites of *M. Cephalus* were *Myxobolus* sp4+ sp1+ *I. multifiliis* at incidence rate 2 (1.9%). While in *T. zillii* was *Myxobolus* sp4+ *Chilodonella+ I. multifiliis* at incidence rate 1 (0.8%) (Table 8).

Table (7): Single and mixed infection of ectoprotozoan parasites on skin of examined fishes.

Types of infection	Type	
	M. cephalus	*T. zillii*
Single infection	21(20.20 %)	16(13.30%)
Mixed infection	5(4.80%)	3(2.50%)

Fig. (7): Single and mixed infection of ecto protozoan parasites on skin of examined fishes.

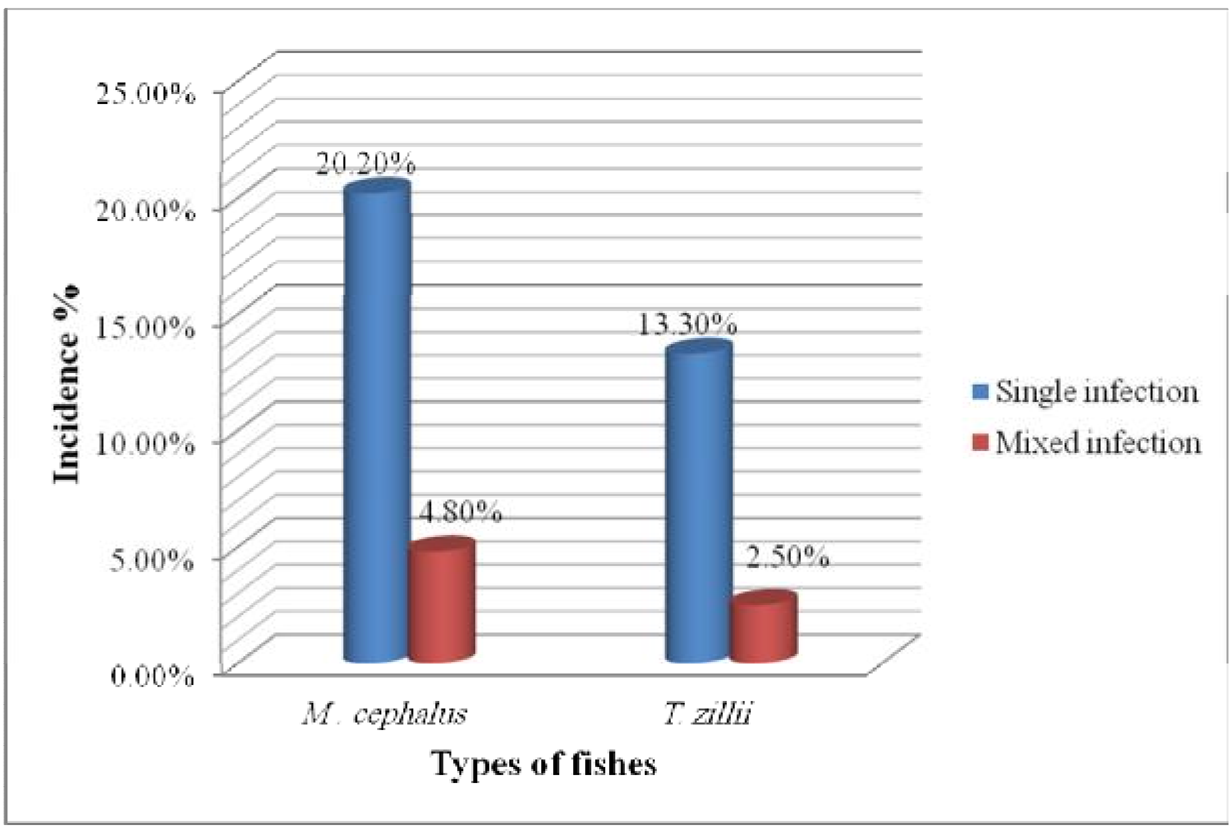


Table (8): Single and mixed infection of ectoprotozoan parasites on skin of examined fishes.

Type of infection		Fish type	
		M . cephalus	*T. zillii*
Single infection	*Myxobolus* sp.1	2(1.9%)	1(0.8%)
	Myxobolus sp.4	15(14.4%)	2(1.7%)
	I. multifiliis	4(3.8%)	3(2.5%)
	Chilodonella sp.	—	5(4.2%)
	Ichthyobodo necator	—	2(1.7%)
	Tetrahymenina sp	—	3(2.5%)
Mixed infection	*Myxobolus* sp.4+ *I. multifiliis*	1(0.9%)	—
	Chilodonella sp. + *I. multifiliis*	2(1.9%)	2(1.7%)
	Myxobolus sp4+sp1+ *I. multifiliis*	2(1.9%)	—
	Myxobolus sp4,+ *Chilodonella*+ *I. multifiliis*	—	1(0.8%)

χ^2 =17.531,df=6, p>0.05, p-value=0.008*

4.9.2. Single and mixed of infections on gills :

The result showed that gills infection was detected only in *M. cephalus***.** However no gills infection was detected in *T.zillii.* The overall incidence of ectoprotozoan parasites on gills was 20(19.2%)**.** Ten (9.6%) of infected *M. cephalus* had a single infection and ten (9.6%) had mixed infection. (Table 9).

The highest single infection of ectoprotozoan parasite on gill in *M. Cephalus* was detected in *Myxobolus* sp.1 6(5.8%), followed by *Myxobolus* sp.2 2(1.9%).

Mixed infection of two species of parasites was *Myxobolu* sp.2+ *Myxobolus* sp.3 and *Myxobolus* sp.2+ *Trichodina* sp 2(1.9%).While mixed infection of three parasitic species was observed in *Myxobolus* sp.2+sp.3 *Trichodina* sp. 4(3.8%) and *Myxobolus* sp.1+sp.2+sp.3 4(3.8%) (Table 10).

Relationship between infection of ectoprotozoan parasites on gills and length , weight , and seasons showed no -significant differences .

Table (9): Single and mixed infection of ectoprotozoan parasites on gills of examined *M . cephalus* .

Types of infection	*M . cephalus*
Single infection	10(9.60 %)
Mixed infection	10(9.60 %)

Table (10): Single and mixed infections of ectoprotozoan parasites on gills of infected *M . cephalus* .

Types of infections		*M . cephalus*
Single infection	*Myxobolus* sp. 1	6(5.8%)
	Myxobolus sp.2	2(1.9%)
Mixed infection	*Myxobolus* sp.2+ *Myxobolus* sp.3	2(1.9%)
	Myxobolus sp.2+ *Trichodina* sp.	2(1.9%)
	Myxobolus sp.1+sp.2+sp.3	4(3.8%)
	Myxobolus sp.2+sp.3 *Trichodina* sp.	4(3.8%)

Discussion

5. Discussion

5.1. Incidence:

The study of *Mugil cephalus* and *Tilapia zillii* is of a great importance in fisheries, especially in terms of commercial and aquaculture, the commonest fishes in fish markets of Benghazi. These fishes are characterized with its good quality of taste and cheap price. They are found in large number in the coastal seawaters of Benghazi and Ain Ziana lagoon. This study was carried out during the period from October 2008 to December 2009.

So far no study on ectoprotozoan parasites was done on fishes in Libya. But there are with the exception of a few studies had been done of intestinal helminthes marine. (El-Marimi , 2002 ; Al-Bassel and Ouhida , 2008; Al-Bassel *et al.* ,2009; and Ahmed , 2010).

The present study revealed that the incidence rate of ectoprotozoan parasites was 22.3% in examined of fishes. Such incidence lower than those reported by other authors (Heckmann and Farley ,1973 "51%" ; Urawa ,1992 "61.3% " ; Piazza *et al*. 2006 " 34%" ; Kayis *et al*. 2009 " 30%"). However a higher incidence was reported by Thilakaratne *et al*. ,2003 (18.4%). Such variation in the obtained data could be due to fish health condition , affected by environmental ,geographical distribution, water temperatures, type of water supply, crowding, transport, and management practices such as handling.(Urawa ,1992; Subasinghe ,1997;Wildgoose ,1998; Thilakaratne *et al* . ,2003; Piazza *et al* ., 2006 and Kayis *et al* ,2009).

The present task reveled the infestation of *Mugil cephalus* and *Tilapia zillii* by six ectoprotozoan parasites .They are (*Trichodina sp.* , *Tetrahymena sp.* , *Chilodonella sp.* , *Myxobolus* spp. , *Ichthyophthirius multifiliis* and *Ichthyobodo necator.*) . The same parasite species were recorded from different fishes : (Heckmann and Farley , 1973; Urawa , 1992; Thilakaratne , 2003 ; Athanassopoulou *et al* . , 2004 ; Piazza *et al* . , 2006 ; Garcia *et al* ., 2009 and Bichi and Yelwa , 2010).

Morphology:-

Identification of the detected parasites in the present study was based on keys and \or description given by Lom and Dykova, 1992; Bahri and Marques, 1996; Gbankoto *et al.*, 2001; Durborow,1998 ; Al-Bassel *et al* 2007 and Al-Bassel *et al.* , 2009.

Myxobolus spp.:

The detected incidence of *Myxobolus* spp. obtained in the present task was 13.8% . Such incidence was lower than those reported by other authors (Bahri and Marques ,1996 " 48.05 %" ; Al-Bassel *et al*. ,2009 " 27.3%"). However, a higher incidence was reported by Gbankoto *et al.* ,2001 was(11.76%).

Ichthyophthirius multifiliis:

The detected incidence of *I. multifiliis* reveled in the present study was (6.7%). Such incidence was higher than those reported by Piazza *et al*. ,2006 “3.7%”. However such incidence was lower than those reported by other authors (Heckmann and Farley ,1973 " 9.1%" ; Navratll ,1991" 9.1%" ;

Kim *et al.* ,2002 "54.5%" ; Ogut *et al.* ,2005 " 100%"; Zrncˇ ic´ *et al* . ,2009 " 81% " ; Maceda-Veiga *et al* ., 2009 " 21%") .

Chilodonella sp.:

The incidence of *Chilodonella* sp. obtained in the present task was (4.4%) . Such incidence was higher than those reported by Piazza *et al.* ,2006 "0.5% " . However incidence was lower than those reported by other authors (Urawa and Yamao , 1992 "20%" ; Zrncˇ ic *et al.*,2009 "58%").

Trichodina sp.:

The detected incidence of *Trichodina* sp. reveled in the present study was (3.1%), Such incidence was slightly lower than those reported by other authors (Piazza *et al*.,2006 " 4.7% " ; Mitra and Haldar ,2005 " 5.3%") . However incidence was lower than those reported by other authors (Heckmann and Farley ,1973 " 26.1% " ; Nilsen ,1995 "21.7%" ; Hassan ,1999 "49.6%" ; Asmat , 2005 " 13.3%"; Al-Bassel ,2009 " 95.5%").

Tetrahymena sp.:

The detected incidence of *Tetrahymena* sp. obtained in the present task was (1.3%), Such incidence was lower than those reported reported by other authors (Kim *et al.* ,2002 " 7.2%" ; Thilakaratne *et al.* ,2003 " 4.1 %").

Ichthyobodo necator:

The incidence rate of *I. necator* was (0.8%) . Such incidence was lower than those reported reported by other (Navratll ,1991 " 30%"; Urawa , 1992 " 15.2 %" ; Isaksen and Einar ,2003 " 38% "; Thilakaratne *et al.*, 2003 " 1.7%").

Relationship:

The incidence of detected of ectoprotozoan parasites in examined fishes was significant high in male (24.3%) than females (11.8%). This agreed with Gbankoto *et al.* (2001). This was in accordance with Ramadan (1991); Bichi and Yelwa (2010). Such variation in the obtained data could be due to male are known to be usually more sensitive to parasites than females due to testosterone synthesis which may exert a cost , decreasing immune competency (Poulin ,1996).

Concerning seasonal detected the present task revealed that significant the incidence to the detected parasites higher in Autumn (57.4%) followed by winter (35.7%) and lowest in summer (13.8%) .This agreed with Ramadan (1991) and Paperna (1996). Such variation in the obtained data could be due to combination of fluctuations in salinity, temperature and pH and geographical distribution (Gbankoto *et al.* 2003).

With regard to effected of length, the present task revealed that these was a significant increase in incidence of the detected parasites with the increase in fish length. This was in accordance with Ramadan, (1991); Bichi and Ibrahim (2009) . May increase infection with increases large body size fishes in the present study return to increase loaded ectoprotozoan with time.

The present study for examined fishes revealed that overall incidence on skin 2.5% and 13.3% of infection were mixed and single infection respectively. Single infection of ectoprotozoan parasite recorded in many studies (Koura *et al*., 1998; Gbankoto *et al*., 2001; Mitra and Haldar ,2005). Mixed infection on skin with, *I.multifiliis* and *Chilodonella* sp. (Piazza *et al*., 2006), *I. multifiliis and Myxobolus* sp. (Heckmann and Farley, 1973), *Tetrahymena sp., I.multifiliis and Ichthyobodo necator* (Thilakaratne *et al*.,2003). Concerning incidence on *M. cephalus* gills was 9.6 % and 9.6 % of infection were mixed and single infection respectively. Mixed infection on with *Trichodina sp.* and *Myxobolus* sp. from *M. cephalus* (Al-Bassel *et al.*, 2009).

Summary

6.Summary

1- Both fishes tilapia and mullet (*Mugil cephalus* and *Tilapia zillii)* are the common fishes in the study area (Ain Ziana lagoon).

2- Two hundred and twenty-four fishes were collected from the area of Ain Ziana lagoon, during the period from October 2008 to December 2009.

3- The results showed that the overall incidence of the ectoprotozoan parasites in fishes at incidence rate 22.3% , *Tilapia zillii* was infected with the ectoprotozoan parasites at incidence rate 15.8% , while *Mugil cephalus* was infected with the ectoprotozoan parasite at incidence rate 29.8%.

4- The results showed that there was six species of ectoprotozoan parasite were detected after examining the skin and gills of fishes .

5- Detected ectoprotozoan parasite is the: *Trichodina sp.* ,*Tetrahymena sp.* , *Chilodonella sp.*, *Myxobolus spp.* , *Ichthyophthirius multifiliis* and *Ichthyobodo necator* .

6- The results showed that the highest incidence was : *Myxobolus spp.* (13.80%) followed by *Ichthyophthirius multifiliis* (6.5%) , *Chilodonella sp.* (4.5%) , *Trichodina sp* . (2.7%), *Tetrahymena sp.*(1.3%), *Ichthyobodo necator* (0.9%) .

7- Noted that there were significant differences between the incidence of infection and sex (χ^2 =18.147,df =1, p>0.05, p-value=0.000*) .

8- Noted that there were significant differences between the incidence of infection and seasons of the year (χ^2 =17.196,df=3, p>0.05, p-value=0.001*)

9-Noted that there were significant differences between the incidence of infection and length . (χ^2 =86.539,df= 3 , p>0.05, p-value=0.000*).

10- Not observed that there were significant differences between the incidence of infection and weight (χ^2 =2.086 ,df=3, $p>0.05$, p-value=0.555).

11- It was noted that there are sixteen (13.33%) in *Tilapia zillii*, and twenty-one (20.2%)in *Mugil cephalus* single infection (one parasite) ectoprotozoan parasite, and three(2.5%) infection in *Tilapia* ,six infection (4.8%) in *Mugil cephalous* mixed infections in the skin (a lot of the parasite).

12- Observed a significant difference between the incidence and type of infection (both individual and mixed) in skin .

(χ^2 =17.531,df=6, $p>0.05$, p-value=0.008*)

13- It was noted that ten (9.6 %) single infection (one parasite), ten (9.6 %) mixed infections (a lot of the parasite) in the gills of *Mugil cephalus*.

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الخلاصة

1- تعتبر كلاً من سمكتي البوري و البلطي الأخضر (*Mugil cephalus* and *Tilapia zillii*)

من الأسماك الشائعة الانتشار في منطقة الدراسة (عين زيانه).

2- تم جمع مائتان وأربعة وعشرون سمكة من منطقة عين زيانه , خلال الفترة من أكتوبر2008 إلي ديسمبر 2009 .

3- أظهرت النتائج أن الإصابة الكلية 22.3% من الأسماك كانت مصابة بالطفيليات الأولية, *Tilapia zillii*15.8% كانت مصابة بالأوليات الطفيلية الخارجية, وإن *Mugil cephalus* 29.8% كانت مصابة بالأوليات الطفيلية الخارجية.

4- أظهرت النتائج وجود ستة أنواع من الأوليات الطفيلية الخارجية, بعد فحص الجلد والخياشيم.

5 - الطفيليات الخارجية هي: *Chilodonella sp.* و *Tetrahymena sp.* و *Trichodina sp.* و *Ichthyobodo necator* و *Ichthyophthirius multifiliis* و *Myxobolus spp.* .

6- أظهرت النتائج أن أعلي معدل انتشار لطفيل *Myxobolus spp.*بنسبة (13.8%) يليه طفيل *Ichthyophthirius multifiliis*بنسبة (6.5%) و *Chilodonella sp.* بنسبة(4.5%)و

*Ichthyobodo necator*بنسبة (0.9%) و

*Trichodina sp .*بنسبة (2.7%) و *Tetrahymena sp.* بنسبة (1.3%)

7 - لوحظ وجود فروق معنوية بين معدل حدوث الإصابة والجنس

$(\chi^2 = 18.147, df = 1, p>0.05, p\text{-value}=0.000^*)$

8- لوحظ وجود فروق معنوية بين معدل حدوث الإصابة وفصول السنة

$(\chi^2\ value=0.009^*17.196, df=3, p>0.05, p\text{-value}=0.001^*)$

9- لوحظ وجود فروق معنوية بين معدل حدوث الإصابة و اختلاف في طول السمكة

(χ^2 =86.539,df= 3 , p>0.05, p-value=0.000*)

10- لم يلاحظ وجود فروق معنوية بين معدل حدوث الإصابة واختلاف في وزن السمكة

(χ^2 =2.086 ,df=3, p>0.05, p-value=0.555).

11- لوحظ وجود ستة عشر(13.33%) في البلطي و واحد وعشرون(% 20.2)في البوري إصابة مفردة (طفيل واحد) بالطفيليات الخارجية, و (2.5%) 3 إصابات في البلطي و 6(4.8%) في البوري إصابات مختلطة في الجلد (أ كثر من طفيل) .

12- لوحظ وجود فروق معنوية بين معدل حدوث الإصابة ونوع الإصابة (فردية و مختلطة) في الجلد .

(χ^2 =17.531,df=6, p>0.05, p-value=0.008*)

13- لوحظ وجود عشرة إصابات(% 9.6) مفرده (طفيل واحد) , وعشرة إصابات(% 9.6) مختلطة (أكثر من طفيل) في خياشيم البوري.

جامـــعة بنغازي

كـلية العـلوم

قـسم عـلم الحـيوان

معدل حدوث الإصابة بالأوليات الطفيلية الخارجية التي تصيب أسماك البوري *"Mugil cephalus"* و البلطي *"Tilapia zillii"* في بحيرة عين زيانة ، بنغازي ، ليبيا.

أطروحة مقدمة أستكمالاً لمتطلبات درجة الإجازة العليا (الماجستير)

في علم الحيوان

مقدمة من قبل

ندا محمود عبد السلام

تحت إشراف

الأستاذ الدكتور حامد إحميدة قاسم

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