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Reproductive Behaviour, Hematological Profile and Monogenean Microfauna of the Nest-Breeding, Nile Green Tilapia (*Tilapia zilli*) Gervais, 1848

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Abstract: *The aims of the present study were to throw lighton somebehaviouralaspectsof the nest-breeding Nile green tilapia, Tilapia zilli in one of its natural environments, namely Mansouria Canal, Nile Delta, Egypt, to clarify the relationship between these aspects and some hematological and biochemical parameters, and toestimate the infection variables of ectoparasiticmonogeneans on this cichlid host. During the spawning time, males and females of the green tilapia were observed to colour up, cooperatein diggingthe spawning pits, defend their eggs, and pay intensive care to theirfingerlings.Hematological testsshowed significantly higher levels of the thrombocytes (PLT), serum catalase (CAT) and malondialdenhde(MDA)in the breeding than non-breeding forms. However, the erythrocytes (RBCs),leucocytes (WBCs) and hematocrit (HCT) attained lower, but non-significant, count in the blood of the nest mates. Similarly, other hematological and biochemical indices showed no significant variation between the two forms of the green tilapia. There were no serum bactericidal activities against Vibrio cholera, Pseudomonas and Salmonella spp.Factors contributing to the spawning-induced stress and possible influences of the hormonal and physiological manifestations on themonogenean infestation level on the breeding forms of tilapia are discussed, with reference to the behavioural and physiological aspects of the conspecific, non-nesting forms of tilapia.*

Keywords: *Mansouria Canal, Tilapia zilli, Reproductive Behaviour, Hematological Profile, Monogenean Microfauna.*

I. Introduction

Recent environmental impactassessment projectsindicate that the hematological and biochemical parameters are an indicative tool for assayingmodifications in the normal physiology, and can provide invaluable data about the overall health of fish [1, 2, 3]. The stress is defined as a condition in which homeostasis (i.e. the affinity of an organism to adjust its internal environment) is alteredin response to the arrangementsof intrinsic or extrinsic circumstances [4]. According to [5, 3], fish undergo stress in captivity, a characteristic feature of the nest mates which devote their activities to the spawning ground. Hematological parameters of fish can reflect some aspects of stress [6, 7, 5]. [8] contributed the validity of the hematological variables, serum enzymes and hormones as bioindicators of the fitness of fish to the fact that fish are merely impregnated in the aquatic environment and their blood image (profile) can express an array of mechanisms regulating the body functions of these organisms. This procedure can provide an early sign prior to thepropagation of disease.

Physiological and immunological aspectsof fish are strongly affected by abiotic (non-living) and biotic (living) elements of the surrounding environment. Teleost fish exhibit similar immune responses to mammals, including non-specific (innate or natural) and specific (acquired or adaptive) mechanisms [9]. The reproduction of fish, and particularly the spawning course,exhibitscrucialinfluences on different biological mechanismsof the mating partners [10]. Imagingthe blood includes defining the total red blood cell count (RBCs), total white blood cell count (WBCs), hematocrit (HCT), hemoglobin concentration (HGB), in addition tothe erythrocyte indices (MCH, MCV, MCHC) [11]. Estimating the biochemical profile comprises a range of chemical compounds and enzymes in the blood that likely reflect theoverallcondition andgeneral health status of the organism. Modifications in the hematological indicesrely upon fish age,sex and sexual maturity cycle [12, 13, 14, 7, 15]. A complete profile of the hormonal and physiological manifestationsof the nest-breeding tilapiawould enrich our understanding of the interaction between the fish host andits environment, including physical, chemical and biological factors. [16] reviewedthe role of parasites and immunological resistance in host sexual selection based on physiological systems and tradeoff (i.e. be deprived of one aspect as an exchange for gaining another), with reference to the breeding, sexual coding and immune task. [17] studiedthe relationship betweensex hormones, attractive colourationand potency for immunologic response.[18] demonstrated the significance of sex hormones in regulating the allocation of resources between immune mechanisms and reproductive arrangements.[19] studied the relationship between sperm quality, ornamentation,

sex hormones and immunity.[16] investigated the interplay between stress, testosterone and allocation to different components of the immune system, and showed that sexual displays specifying parasite resistance in male vertebrate hosts are activated by testosterone, a seemingly immunosuppressive hormone.[20] suggested that different parasite life-strategies affect diverse features of the host physiological manifestations and trigger various immunity pathways. They found that fish with a poorer condition rank were infested more by monogenean parasites, which are the most abundant parasite group. Moreover, higher cestode burden seemed to stimulate phagocytosis [20]. *Tilapia zilli* is native to Africa and has been introduced to many geographical areas around the globe. It is an omnivore cichlid,feeding on a variety of food items of plant and animal origin, and is regarded in aquaculture systems as a profitable source of the animal protein (e.g. [21]).This cichlid fish contributes to about 71% of the world total tilapia production [11]. This cichlid is naturally distributed throughout the lake systems of the Northern and Western Africa [22, 23]. Of particular interest is the study of hematological and biochemical parameters of the nest-breeding tilapia that spawns in shallow, inshore water over a period of approximately five months (from early April to late August). Owing to their limited range of movement, nest matesin territories may become more susceptible to physicochemical and biological parameters which may alter the blood components and chemistry profile. To the best of our knowledge, no previous reports were made of the hematological profile of *T. zilli* in the freshwater streams of Egypt. The present investigation aimed atevaluating the impacts of the breeding environment on some hematological and biochemical parameters,and exploring the possible impacts of the reproductive behaviouron monogeneanmicrofauna of the breeding tilapia.

II. Materials And Methods

2.1. Study Area:

Mansouria canal (Figure 1) is one of the primary freshwater resourcesin the Nile Delta, Egypt. The canal is regarded as the life artery for millions of people and provides thousands of acres of cultivatedareaswith a predominant water supply in such amazing and highly populated geographical area of Egypt. In addition, Mansouria Canal is a nursing ground for a variety of fish kinds that meet the growing needs of the community for the animal protein. The canal is affected by many anthropogenic activities and bad practices (Figure 1B). The study area is locatedin the vicinity ofSalaka Village, MansouraCity (Figure 1A).

2.2. Field observations and Fishing:

Specimens of *Tilapia zilli* were caught monthly during the breeding season that extends from early April to late August in 2016. Immediately after capture with rod and line, the fish were kept alive in appropriate container provided with an air pump. Then, the fish were transported to the laboratory as soon as possible to minimize the stress of the captive fish. At the laboratory, the harvest was assorted into males and females. Observations were made of the nest selection, nest building, spawning pit digging, egg deposition and rearing, and parental care in *T. zilli* was inspected with the aid of video-camera systems. Close inspection of the reproductive behaviour was made only during daytime.

2.3. Hematological studies:

Hematological parameterssuch as hemoglobin content and the number of red blood cells (RBCs), white blood cells (WBCs) and blood platelets (PLT) were determined using standard hematological techniques. Using a hypodermic needle, a 3-ml syringe immersed in EDTA, few millimeters of blood were withdrawn from the caudal vein of freshly caught and physically fit tilapia.For proper collection of the blood, the needle was tilted to acquire an angle of 45º from the peduncle. Then, the sterilized needle was inserted carefully into the skin and between the scales. Immediately, the needle was pushed skillfully into the underlying tissues and straight down toward the core area of the peduncle, to reach the blood stream in the tail region. When the blood began to flow inside the needle, a pressure was maintained on the plunger, which was released once the target amount of blood was obtained.

Two sampling protocols were designed. The first blood sample was transferred to EDTA tube containing Sodium Thiosulphate as an anticoagulant factor. Each blood sample was allowed to coagulate for about 20 minutes.Immediately after settlement of the blood components, the blood was prepared for subsequent treatment. This blood sample was transferred assoon as possible to be imaged or analyzed by Hematology Analyzer (Mindray BC-3200) at the Nile Research Center. The second blood sample was collected in anticoagulant-free tubes and then centrifuged at 3000 rpm for 15 min.According to [24], the red blood cells were separated from plasma and the haemolysate (i.e. pale straw supernatant as a result of the lysis of erythrocytes) was produced. This procedure was repeated two or three times to obtain clear and transparent haemolysate. Following centrifugation, the supernatantwas isolated safely and kept in the cold freeze until analyzed later for serum enzymes, hormones and bactericidal activity.

The blood image comprised the red blood cells (RBCs), white blood cells (WBCs), hemoglobin content (HGB), platelets (PLT), hematocrit ratio (HCT), mean corpuscular volume (MCV), mean platelets volume (MPV), lymphocytes (LYM), red cell distribution width (RDWc) and platelet distribution width (PDWc). Estrogen and Testosterone to measure sex hormones. Levels of these hormones were determined by Vidas mini 30, an automated immunoassay tests utilizing ELFA (Enzyme Linked Fluorescent Assay) technology. The concentrations of Estradiol (E2) and Testosterone (T) were determined by radioimmunoassay using 1251 RIA kits (ICN pharmaceuticals) [25].The serum bactericidal activity was indicated by *Vibrio cholera, Salmonella* and *Pseudomonas* spp. The activity of the three species of bacteria was tested by the activity of serum antibody using Laminar Flow System.

A freshly dissected segment (0.32 gm) of the first gill arch (right gill set)was weighed by VIBR HT 220/0.0001 electronic balance andhomogenized in distilled water using a Heidolph Homogenizer (5000-26000 rpm speed).Then,the gill tissue homogenate was digested with 1 ml of 0.1 N NaOHfor an hour at 98°C. The digestate was then centrifuged in Sigma 1-14 Centrifuge and the supernatant was introduced into Implen P330 Nanophotometer to determine the levels of the Nitric oxide (NO), Catalase (CAT), Glutathione I (GSH), Glutathione II (GST) and Malondialclenhyde (MDA). Catalase activity,measured in U units, was measured at 540 nm. The measuring unit of catalase is defined as the quantity of catalase required to decompose one µmol of hydrogen peroxide (H_2O_2) per minute at 25 ºC and pH=7 [26].

2.4. Statistical analysis:

All data were recorded as (Mean ±SD). Two parametric tests were chosen on the SPSS package (version 20), namely One-Way ANOVA and Student's *t*-Test. The parametric One-Way ANOVA Test was utilized to explore variations of the hematological parameters between the four forms of tilapia. The Student's *t*-Test was employed to explore variations of the blood parameter (count of corpuscles, hemoglobin, hematocrit, hormones and antioxidants) between males and females of the two forms, between males of the breeding and their corresponding genders of the non-breeding forms, as well as between females of the breeding and their corresponding genders of the non-breeding forms. Probability values were selected as follows:$P \leq 0.05$, significant; $P \leq 0.01$, highly significant and $P \leq 0.001$, very highly significant.However, P-values> 0.05 were considered as non-significant.

III. Results

3.1. Reproductive behaviour in *Tilapia zilli*:

3.1.1. Nest localization, construction and guarding:

Earlier in April, males and females of the nest-breeding tilapia, *Tilapia zilli* intending to spawn arrived to the inshore, shallow water zone atMansouria Canal. The mating partners were observed to select a muddy spawning ground (Figure 2A), exposed to the direct sunlight. In some occasions, spawning couples were observed,on the video-camera system, to select their nests on relatively flattened surface of stones (Figure2B) or other solid objects settlingat the bottom. Furthermore, some large-sized mates were observed to occupy vertical grooves in the shoreline and dig a few pits on the base of these grooves (Figure 3A). Intensive field observations revealed that both males and females of the breeding tilapia began to colour up on the commencement of the spawning season(Figure 3B). Nesting forms appeared slender and were confined to the ground throughout the spawning time. Both sexes attained shiny, attractive yellowish coloration, with a prevailing red throat (red belly).

Morphometric measurements revealed that males were usually larger and likely older than females. Spawning couples constructed a variable number of bowl-shaped excavations in the muddy substratum. Deposited eggs were hardly seen on the grayish or yellowish surface of the stones or other solid materials. On the muddy substrate, more than 10 couples were seldom observed to construct closely-spaced spawning nests on wide arenas. These spawning grounds showed reciprocal stimulation of adjacent nest mates and aggressiveness of the mating partners towards intruders.

Frequently, large-sized males and females were recognized in the vicinity of the spawning grounds, however they exhibited less ornamentation and were not involved in the spawning course. Non-nesting forms were fat, whitish-grey in colourand displayed random and vigorous movements nearby the nesting mates that fight and frightened the intruders either by aggressive, mouth-to-mouth, biting or rapid physical displacements.Close inspection with the aid of the video-camera system revealed that the spawning nest was guarded by both male and female fish. On the one hand, nesting males were more movable than females, and defended the periphery of the spawning nest. On the other hand, nesting females were relatively quiescent, occupied the median area of the spawning nest, and defended their eggs/offspring against intruders and contaminants driven by the water current.

3.1.2. Egg rearing and care of offspring:

The egg clusters (Figure4A) were usually observed on relatively flattened, solid objects in the spawning area. The emerging fries were never seen in the vicinity of the spawning nest during the first few days after egg hatching. A few days later, many mating partners as well as their developing fries left the spawning ground and swam freely in water close to the shoreline. With the aid of video-camera system, the fries were seen moving as a unified shoal according to the instructions of the parent female that was located in the center of the shoal (Figure 4B). On the other hand, the parent male was observed to occupy the marginal area of the territorial swimming shoal.

3.1.3. Biological associations (intraspecific and interspecific):

Many biological associations (interactions) and behavioural patterns were documented throughout the spawning period of the nest-breeding tilapia atMansouria Canal. These biological associations include competition, predation, egg cannibalism and altruism. The behavioural aspects comprised nest building, nest guarding, parental care, social life (cooperative defense and colonial breeding), communication and agnostic behaviour. Simple styles of learned behaviour were recorded during the period of water decline by the end of the breeding season.

Egg cannibalism was common either by pirates of the non-nesting tilapia, swimming actively close to the margin of the spawning ground, or other intruders such as the Dayglowfulu*Haplochromisbloyeti*, the two spotted jewelfish *Hemichromisguttatus*, the white tilapia *Oreochromisniloticusniloticus*, the mango tilapia *Sarotherodongalilaeusgalilaeus*, the crayfish *Procambarus clarkia* or the freshwater crab *Liocarcinusvernalis*. The latter was observed to prefer more sheltered places beneath stones and solidobjects. The crayfish was highly aggressive and frequently attacked the spawning nests, preyed upon nesting couples and occupied their vacant niches. The crayfish was observed to make menacing gestures with the aid of their massive claws. They were observed to burrow deep, inshore holes into the mud and frequently threatened the life of the nesting tilapia. These omnivorous and scavengers were observed to frighten the spawning couples at regular intervals.The aggressivebehaviour was practiced by the nest mates, however females were more aggressive than males. The nest mates showed strong offense displays to secure their territory against the crayfish, crab, conspecifics and other sympatric species.

From September 2016 onwards, Mansouria Canal showed an irregular filling and draining cycles (Figure 1C). At this time, nest mates were found to experience simple learning tactics; each mating partner prepared two alternative nesting grounds and allocated the building and guarding efforts between the two grounds. However, the two nesting ground were constructed at the same depth, close the shoreline.

3.2. Monthly occurrence of ectoparasiticmonogeneans on the gills of *Tilapia zilli* during the spawning period:

Out of 155 examined fish, a total of 337 monogenean worms were isolated and identified. The breeding forms of the green tilapia were infested with 270 worms, whereas the non-breeding forms were parasitized by 67 worms. The monogenean*Cichlidogyusarthracanthus* recorded the highest number (163 worms), followed by*C. aegypticus* (53 worms) and *C. tilapiae* (47 worms). However, the monogenean*C. hallitypicus* attained the lowest number (16 worms). A total of 178 monogenean worms were collected from the gills of 51 breeding males, whereas a total of 92 monogenean worms were collected from the gills of 25 the breeding females of the green tilapia. On the other hands, a total of 50 monogenean worms were found on the gills of 52 non-breeding males, whereas a total of 17 monogenean worms were encountered on the gills of 27 non-breeding males of tilapia.

As shown in Table 1 and Figures 5 and 6, the monthly prevalence values of all the studied monogenean speciesare higher in the breeding than non-breeding forms of *Tilapia zilli*. The monogenean*C. arthracanthus* is the core (most dominant) species in the breeding and non-breeding forms, with mean prevalence values of 64.19 and 21.11% in the two forms respectively. The cohabitant monogenean*C. aegypticus* comes second, with mean prevalence values of 26.67 and 4.22% in the breeding and non-breeding forms respectively. The monogenean*C. hallitypicus* recorded the lowest mean prevalence values in the two forms of tilapia (Table 1).

The monthly mean intensities of the monogeneans*C. arthracanthus*, *C. aegypticus*, *C. hallitypicus* and *C. tilapiae* on the gills of the breeding and non-breeding forms of *T.zilli* are recorded in Table 2 and graphically represented in Figures 7 and 8.Regarding the breeding forms of tilapia, the highest mean intensity value was recorded for *C. arthracanthus*(3.21), while the lowest level was found for *C. tilapiae* and *C.hallitypicus* (1.50). On the other hand, on the non-breeding forms of tilapia, the maximum mean intensity value was recorded for *C. arthracanthus*(1.07), while the lowest value was found for *C. hallitypicus* (0.50).

It can be noticed from Tables1, 2 and 3 that all the studied monogeneans were completely absent from the gills of the non-breeding forms of tilapia during May and June. Except for the most dominant *C.*

arthracanthus, all monogeneans were not encountered on the gills of the non-breeding tilapia during August. A similar disappearance in August was recorded for the monogeneans*C. tilapiae* and *C. hallitypicus* on the gills of the breeding tilapia. The rare monogenean*C. hallitypicus* was also absent from the gills of the breeding and non-breeding tilapia during July.

From Table 3 and Figures9 and 10,the abundance values of the monogenean parasites on the breeding were higher than those on non-breeding forms of *T. zilli*.The highest mean abundance value was detected for *C. arthracanthus*(2.28) in the breeding forms, however the lowest mean abundance value was obtained for *C. aegypticus* and *C. hallitypicus* (0.06). Also, a markedly low mean abundance level (0.08) was found for *C. tilapiae* on the breeding tilapia (Table 3).

3.3. Hematological and Biochemical parameters of *Tilapia zilli* during the spawning period:

3.3.1. Hematological parameters

Tables 4A and 4B show the monthly variations of the hematological parameters in the blood of *T.zilli* caught from Mansouria Canal during the period from April to August 2016. The hematological parameterscomprisedhemoglobin (HGB), red blood cells (RBCs), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), white blood cells (WBCs), Lymphocytes (LYM), platelets (PLT), red cell distribution width (RDWc), mean platelet volume (MPV), and Platelet distribution width (PDWc). Except for a considerable difference in the count of thrombocytes, there are slight variations in the levels of hematological parameters between the two forms of tilapia (Tables4A and 4B).

From Table 4A, the hemoglobin level varied between 6.90 and 13.1×10^3/µl. The mean hemoglobin value in the breeding forms was 11.2 (±1.10) × 10^3/µl and in non-breeding forms 10.84 (±1. 90) ×10^3/µl.The mean RBCs count in the breeding forms was 1.71 (±0.16) × 10^6/µl and in the non-breeding forms 1.81 (±0.21) ×10^6/µl. As shown in Table 4A,the mean values of HCT in the breeding forms of *T. zilli*was 24.98 (±3.11) % and in the non-breeding 25.13 (±3.40) %. The mean value of MCV in the breeding forms was 137.70 (±9.38) fl and in the non-breeding forms 133.24 (±12.22) fl (Table 4A). Records documented in Table 4Ashow that the values of MCH in the breeding and non-breeding forms of *T. zilli* were 62.3 (±3.59) pg and 58.50 (±8.91) pgrespectively.

From Table 4B, the mean WBCs value in the breeding forms was 91.90 (±11.38) ×10^3/µl and in the non-breeding forms 103.10 (±11.02) ×10^3/µl.The mean lymphocytes count in the breeding forms was 70.64 (±11.18) × 10^3/µl and in the non-breeding forms 68.56 (±16.25) ×10^3/µl. As shown in Table 4B, the mean PLT value in the breeding and non-breeding forms are 140.70 (±19.92) ×10^3/µl and 115.70 (±31.95) ×10^3/µl respectively. The mean RDWc value in the non-breeding forms was 17.00 (±3.63) % and in the breeding forms 13.50 (±2.37) %. Table 4B shows that the mean MPV value in the breeding forms is 8.92 (±0.83) fl and in non-breeding forms 8.43 (±1.06) fl. Data documented in Table 4B show that the mean values of PDWc in the breeding and non-breeding forms of the green tilapia are 36.52 (±8.81) and 35.57 (±10.08) % respectively.

3.4. Biochemical parameters

3.4.1. Hormones

Tables 5A and 5Brepresent the output of the analysis of sex hormones Estrogen and Testosterone respectively.From Table 5A, the level of estrogen hormone attained considerablyhigher values in the breeding females than non-breeding females. The mean values of the hormone in the breeding and non-breeding females were 500.66 (±249.07) pg/ml and 396.82 (±202.01) pg/ml respectively. Concerning the breeding forms, the highest level was estimated during July, while the lowest level was recorded during April at the commencement of the spawning period (Table 5A). Regarding the non-breeding forms, the highest level was estimated during April, while the lowest level was recorded during August at the termination of the spawning period (Table 5A).
It is obvious form Table 5B that the level of testosterone hormone attained considerably higher values in the breeding females than non-breeding females. The mean values of the hormone in the breeding and non-breeding females were 4.82 (±3.34) ng/mland 3.59 (±3.07) ng/ml respectively. Concerning the breeding forms, the highest level was estimated during April, while the lowest level was recorded during August (Table 5B). Regarding the non-breeding forms, the highest level was estimated during August, while the lowest level was recorded during May (Table 5B).

3.4.2. Antioxidant activity

Obtained values of the nitric oxide (NO), catalase (CAT), malondialdenhde (MDA) andglutathione (GST, GSH) are recorded in Table 6. The breeding forms attained higher levels of NO and GSH than non-breeding forms. In contrast, the non-breeding forms showed higher levels of CAT, MDA and GST than breeding forms of the green tilapia
Student's t−Test on SPSS program (version 20) revealed significant differences in the number of PLT ($t=2.100$, $p \leq 0.05$), MDA ($t= -2.102$, $p \leq 0.05$) and CAT ($t= -2.425$, $p \leq 0.05$) between the breeding and non-breeding

forms of *T. zilli*. However, the two forms of the green tilapia showed no significant variations in the other hematological and biochemical parameters. ($p > 0.05$ in all cases).

3.4.3.Bactericidal activity

The bactericidal activity for*Vibrio cholera, Pseudomonas* and *Salmonella*was analyzed in the blood serum of monthly collected samples. There was no activity against any different bacterial species (*Vibrio cholera, Pseudomonas*, and *Salmonella*) in the blood serum (Figures 11A, 11B and 11C).

IV. Discussion

The blood parameters are regarded as a successful bioindicator or biomarker of the environmental stress as they reflect the physiological and immunological conditions of the aquatic organisms. [27]suggested that data obtained from the hematological analysis of fish might provide valuable information about the characteristic features of the waterbody in stressed ecosystems. [28, 29] highlighted the effects of fish gender on the hematological parameters.[30] highlighted the importance of hematological approaches as an understanding tool of the relationship between elements of the blood and the habitat, and adaptive responses of the living organism to the environment. The authors recommended the establishment of normal blood profiles and standard hematological criteria for different fish species. The present study providesa baseline data for the blood profile of the nest-breeding tilapia, *Tilapia zilli* during the breeding season and associated physiological and behavioral patterns. These information may help facilitate the maintenance and improvement of the fish populations atMansouria Canal. [31] stressed that monitoring fish hematology requires reference data, close to the normal levels of different blood parameters as reliable descriptors of the fit body of fish under ordinary circumstances.[5] employed the blood parameters as a mirror reflecting the health condition of an array of fish to estimate the physiological alterations as a result of exposure to pollution and hypoxic conditions, in addition to other waterborne stressors. [8] reported that fish as well as other aquatic organisms are directly influenced by the fluctuations of the aquatic environment and stressed that the blood profile readily reflects the internal environment of the body of fish. This can represent an early sign prior to the development of disease.

Antioxidant enzymes are important for ideal fish development. Moreover,they support the body immune system. The antioxidant arrangements in the living organism may be categorized into two forms: one is signified by enzymes, e.g. superoxide dismutase, catalase and peroxidases thateradicate reactive oxygen species (ROS). Other antioxidantspick up free radicals. According to [32], the manner of action of antioxidants is classified as follows: removing oxygen or decreasing local O_2 concentrations, removing catalytic metal ions, removing reactive oxygen species, scavenging initiating radicals, breaking the chain of an initiated sequence, quenching or scavenging singlet oxygen.[33] suggested that ROS- and free radicals induced antioxidant peroxidation may be greatlyharmful, leading to damage in cellular membranes.

According to [30], fish blood constitutes up to 7% of its body weight. Fish blood is one of the most vital components and is concerned with gas exchange between the body of fish and oxygen-holding water in the aquatic environment. [34] highlighted the importance of the blood parameters as an early alarming sign of the stress response in fish to abiotic and biotic factors. As a measure of the quality and quantity of RBCs, WBCs and PLT, the complete blood count (CBC) is regarded as an important and informative diagnostic tool in ecotoxicological reports and fish health assessment assays [35, 36]. The hematocrit (HCT)designates how many red blood cells are existing. Lower HCT levels might indicate the onset of anemia, while higher HCT mightpoint to dehydration. The hemoglobin (Hgb) can help determine how well the red blood cells are carrying oxygen to differenttissues. Higher numbers of the white blood cells (WBC) may be a response to an infection or inflammation [36]. In contrast, lower numbers of the white blood cells may indicate a serious infection or bone marrow dysfunction.The platelets count (PLT) measures the quantity of platelets, which are involved in the blood clotting process [37].

The hematological analysis indicated significantly higher levels of the blood platelets in the breeding than non-breeding forms of the green tilapia. The mean values of PLT were 140.70 ($\pm$ 19.92) x 10^3 /µl and 115.70 ($\pm$ 31.95) x 10^3/µl in the breeding and non-breeding forms, respectively. However, the red blood cells (RBCs) and white blood cells (WBCs) recorded lower, but non-significant, count in the blood of the breeding than non-breeding forms of *T.zilli*. The mean values of RBCs in the blood of the breeding and non-breeding forms were 1.71($\pm$ 0.16) x 10^6/µl and 1.81 ($\pm$0.21) x 10^6/ µl respectively. The mean values of WBCs in the blood of the breeding and non-breeding forms were 91.90 ($\pm$ 11.38) x 10^3/ µl and 103.10 ($\pm$11.02) x 10^3/ µl respectively.

The white blood cells (WBCs) act as the primary force of the immune system in aquatic animals. [38] suggested that this blood cell type has a significant role in the immune responses and defense tactics. Optimization of the white blood cells count seems likely to indicate integration of the cellular immune responses and increased ability to interplay with pathogens and multisource stressors. The relatively lower count of the white blood cells on the breeding forms of *T. zilli* may indicate a stress condition in the breeding

males and females. To verify a successful reproductive output, breeding forms of tilapia must direct their energetics to the production of high quality gametes, selection of the fertile partner, nest-building and guarding, optimal oviposition and intensive care of the fertilized eggs. These activities indeed overconsume the stored energy and assimilatory elements across the organ systems of the body. [3] studied the hematology of *O. niloticus* after acclimation to captivity and found that the stress condition in the fish created changes in the white blood cells and differential counts neutrophils, monocytes and lymphocytes. Owing to data obtained in the present work, neither the count of the red blood cells nor the level of hemoglobin showed marked variation between the breeding and non-breeding forms of *T. zilli*.

In the present study, hemoglobin levels varied insignificantly between the blood profile of the breeding and non-breeding forms of the green tilapia. The mean values obtained for HGB were 11.2 (±1.10) x 10^3/µl and 10.8 (± 1.90) x 10^3/µl in the breeding and non-breeding forms, respectively. Hemoglobin is a highly important biological molecule. It is a respiratory pigment capable of transporting oxygen molecules to the blood. The normal hemoglobin level in *T.zilli* has not been changed significantly between the two fish categories, indicating an adaptation of the breeding forms.

According to [39, 40, 41] the blood platelets (thrombocytes) attain the ability to phagocytose pathogens and play a role in the defense mechanisms. [42] suggested that the thrombocytes act as a link between the innate and adaptive immune systems of the fish. [11] showed that the blood platelets express surface and intracellular molecules, which are involved in immune function. Many studies indicated that fish thrombocytes are blood phagocytes, which form an important tool of the protective barriers in fish (e.g. [43, 44]) Significantly higher levels (counts) of the blood thrombocytes of the breeding forms of the green tilapia seem likely to represent an immune response and a healing process following frequent injuries induced by invaders of the nesting arenas such as antagonistic, non-breeding males of the cichlid fish and the freshwater carb, *Liocarcinusvernalis* and the crayfish,*Procambarusclarkii*.

The secondary blood indices, MCHC, MCH, MCV, MPV, RDWc, WDWc and PDWc showed non-significant difference between the mating partners and non-mating dwellers of the green tilapia. [45] reported that the mean cell hemoglobin level is an index used to assess the number of red cell swelling (low MCHC) or shrinkage (high MCHC) found in the blood sample.

Fish immune system possesses mechanisms responsible for defense against bacteria, through pathways mediated by macromolecules originating in the outside of the plasma membrane (humoral immunity), for example antibodies andantimicrobial proteins, stemming from a number of differentcauses or influences which aim at preventing bacterial colonization. On the other hand, tools of the innate mechanisms (cell-mediated immunity) comprise the production of antibacterial compounds, phagocytosis and inflammation [46, 47], macrophages, neutrophils and phagocytes showgreatamounts of lysosomal enzymes and reactive oxygen species todevastateattacking bacteria. In the present study, no bactericidal activity was recognized in the blood of the green tilapia, indicating an effective immune response against invading pathogens by destroying colony forming units and stimulating the reduction in its numbers in the plates. This immunoassay proved the raise in the level of serum protective proteins which occurs after a natural infection.

Table (1). Monthly Prevalence Values Of The Gill Monogeneans Of The Breeding And Non-Breeding Forms Of *Tilapia Zilli* During The Period From April To August (2016).

Month (2016)	Breeding forms				Non-breeding forms			
	C. arthracanthus	*C. aegypticus*	*C. tilapiae*	*C. hallitypicus*	*C. arthracanthus*	*C. aegypticus*	*C. tilapiae*	*C. hallitypicus*
April	83.33	45.83	33.33	12.5	38.89	11.11	11.11	11.11
May	50.00	16.67	16.67	16.67	absent	absent	absent	absent
June	46.15	23.08	23.08	15.38	absent	absent	absent	absent
July	56.25	12.5	0.23	absent	36.67	10	10.00	absent
August	88.24	35.29	absent	absent	30.00	absent	absent	absent
Mean ±SD	64.19 ±19.57	26.67 ±13.74	14.66 ±14.55	8.91 ±8.27	21.11 ±19.55	4.22 ±5.79	4.22 ±5.79	2.22 ±4.97

Table (2). Monthly Mean Intensity Values Of The Gill Monogeneans Of The Breeding And Non-Breeding Forms Of *Tilapia Zilli* During The Period From April To August (2016).

Month (2016)	Breeding forms				Non-breeding forms			
	C. arthracanthus	*C. aegypticus*	*C. tilapiae*	*C. hallitypicus*	*C. arthracanthus*	*C. aegypticus*	*C. tilapiae*	*C. hallitypicus*
April	3.70	2.27	2.88	1.00	1.57	1.50	1.50	2.50
May	4.33	4.00	2.00	5.00	absent	absent	absent	absent
June	2.00	1.67	1.00	1.50	absent	absent	absent	absent
July	2.22	2.00	1.60	absent	2.45	1.33	2.33	absent
August	3.80	1.33	absent	absent	1.33	absent	absent	absent
Mean ±SD	3.21 ±1.04	2.25 ±1.04	1.50 ±1.08	1.50 ±2.04	1.07 ±1.06	0.57 ±0.78	0.77 ±1.09	0.50 ±1.12

Table (3). Monthly Abundance Values Of The Gill Monogeneans Of The Breeding And Non-Breeding Forms Of *Tilapia Zilli* During The Period From April To August (2016).

Month (2016)	Breeding forms				Non-breeding forms			
	C. arthracanthus	*C. aegypticus*	*C. tilapiae*	*C. hallitypicus*	*C. arthracanthus*	*C. aegypticus*	*C. tilapiae*	*C. hallitypicus*
April	3.70	1.04	0.96	0.13	0.61	0.17	0.17	0.28
May	2.17	0.67	0.33	0.83	absent	absent	absent	absent
June	0.92	0.38	0.23	0.23	absent	absent	absent	absent
July	1.25	0.25	0.50	absent	0.90	0.13	0.23	
August	3.35	0.47	absent	absent	1.33	absent	absent	absent
Mean ±SD	2.28 ±1.23	0.56 0.31±	0.40 0.36±	0.24 ±0.35	0.57 ±0.58	0.06 0.08±	0.08 ±0.11	0.06 ±0.58

Table (4A). Complete Blood Picture (CBC) Of *Tilapia Zilli* During The Period From April To August 2016.

Fish Group	Month (2016)	Fish Sex	Complete Blood Picture				
			HGB 10^3/µl	RBCs 10^6/µl	HCT %	MCV Fl	MCH Pg
Breeding Forms	April	Male	13.10	1.30	31.50	153	64
		Female	11.00	1.70	23.35	144	66
	May	Male	11.40	1.71	26.30	152	67
		Female	9.60	1.70	19.39	127	67
	June	Male	10.20	1.81	25.10	138	59
		Female	10.60	1.80	25.17	140	58
	July	Male	10.00	1.67	22.70	136	60
		Female	11.70	1.90	24.96	131	61
	August	Male	11.50	1.83	24.50	134	63
		Female	12.40	1.70	26.89	125	58
Mean ± SD			11.15 ±1.10	1.71 ±0.16	24.99 ±3.11	138 ±9.55	62.3 ±3.59
Non-Breeding Forms	April	Male	9.30	1.35	17.4	129	69
		Female	9.69	1.70	23.15	133	55
	May	Male	12.50	1.87	26.60	102	67
		Female	13.10	1.90	24.40	132	67
	June	Male	6.90	1.80	25.50	130	38
		Female	11.70	1.80	26.90	147	58
	July	Male	12.90	2.11	30.50	133	61
		Female	10.80	1.70	26.50	134	58
	August	Male	11.10	2.04	27.50	134	54
		Female	10.40	1.80	28.50	136	58
Mean ± SD			10.84 ±1.90	1.81 ±0.21	25.70 ±3.56	131.04 ±11.21	58.50 ±8.91

Table (4B). Complete Blood Picture (CBC) Of *Tilapia Zilli* During The Period From April To August 2016.

Fish Group	Month (2016)	Fish sex	Complete Blood Picture					
			WBCs 10^3/µl	LYM 10^3/µl	PLT 10^3/µl	RDWc %	MPV fl	PDWc %
Breeding Forms	April	Male	115	62.90	93	11	8.00	38.00
		Female	97	45.20	173	12	8.00	11.70
	May	Male	82	75.40	140	11	9.60	39.00
		Female	74	64.20	133	15	8.00	38.20
	June	Male	99	72.40	150	12	8.10	38.00
		Female	86	72.20	141	12	9.20	38.20
	July	Male	99	75.20	148	18	9.60	40.00
		Female	90	80.90	141	15	10.00	41.50
	August	Male	92	72.60	148	13	9.80	41.00
		Female	85	85.40	140	16	8.90	39.60
Mean ±SD.			91.9 ±11.38	70.64 ±11.18	140.70 ±19.92	13.50 ±2.37	8.92 ±0.83	36.52 ±8.81
Non-breeding Forms	April	Male	120	40.40	122	12	8.80	39.00
		Female	99	69.50	81	21	8.50	39.00
	May	Male	104	40.40	120	12	8.80	39.00
		Female	100	71.90	140	18	8.90	39.80
	June	Male	118	69.20	171	23	5.70	7.00
		Female	98	80.50	137	15	9.70	40.00
	July	Male	115	68.30	73	20	7.90	37.00
		Female	95	83.20	137	16	8.50	38.50
	August	Male	95	75.10	86	16	8.50	38.50
		Female	87	88	115	18	9.00	37.90
Mean ±SD			103.10 ±11.02	68.56 ±16.25	115.70 ±31.95	17.10 ±3.63	8.43 ±1.06	35.57 ±10.08

WBCs: White blood cells PLT: Platelets MPV: Mean platelet volume LYM: Lymphocytes
RDWc: Red cell distribution widthPDWc: Platelet distribution width

Table (5A). Levels Of Estrogen In The Blood Of The Breeding And Non-Breeding Females Of *Tilapia Zilli*.

Fish Group	Month (2016)	Gender	Estrogen (pg/ml)
Breeding Forms	April	Female	164.80
	May		395.70
	June		453.2
	July		782.60
	August		707.00
Mean ±SD			500.66 ±249.07
Non-breeding Forms	April	Female	620.00
	May		150.10
	June		301.70
	July		578.50
	August		133.80
Mean ±SD			396.82 ±202.01

Table (5B). Levels Of Testosterone In The Blood Of The Breeding And Non-Breeding Males Of *Tilapia Zilli*.

Fish Group	Month (2016)	Gender	Testosterone (ng/ml)
Breeding Forms	April	Male	9.15
	May		7.53
	June		3.24
	July		2.84
	August		1.35
Mean ±SD			4.82 ±3.34
Non-breeding Forms	April	Male	6.62
	May		0.70
	June		2.70
	July		0.89
	August		7.06
Mean ±SD			3.59 ±3.07

Table (6). Antioxidant Activity Parameters In Fish Blood Of The Breeding And Non-Breeding Forms Of *Tilapia Zilli*.

Fish Group	Month (2016)	Fish Sex	Antioxidant Activity				
			NO µmol/l	Catalase u/tissue	MDA nmol/g. tissue	GST u/g. tissue	GSH Mg/g. tissue
Breeding Forms	April	Male	14.30	0.15	80.5	0.20	0.96
		Female	4.14	0.19	46.20	0.18	26.79
	May	Male	7.32	0.77	37.8	0.15	7.52
		Female	15.90	0.84	53.18	0.55	7.85
	June	Male	14.5	1.48	54.0	0.10	1.50
		Female	3.00	1.70	10.36	0.20	11.22
	July	Male	7.00	1.27	5.7	0.60	1.20
		Female	8.50	1.36	13.10	0.30	4.12
	August	Male	4.50	1.25	25.1	0.45	6.24
		Female	14.50	1.56	50.55	2.04	22.28
Mean ±SD			8.80 ±4.96	0.88 ±0.58	36.22 ±23.83	0.30 ±0.58	7.49 ±8.91
Non-Breeding forms	April	Male	11.15	0.49	132.8	0.30	1.49
		Female	17.50	2.82	70.75	1.37	7.49
	May	Male	3.00	1.77	48.10	0.20	2.45
		Female	5.5	1.73	69.20	0.11	3.06
	June	Male	2.50	1.49	70.50	0.44	3.91
		Female	2.00	1.90	45.50	0.30	1.20
	July	Male	3.00	2.04	124.80	3.35	9.37
		Female	5.00	1.94	39.17	0.39	9.58
	August	Male	7.50	1.59	19.00	0.65	2.49
		Female	13.00	1.51	72.54	1.53	5.20
Mean ±SD			6.35 ±5.24	1.75 ±0.58	68.87 ±35.87	0.79 ±1.00	4.56 ±3.15

NO: Nitric oxide GST, GSH: Glutathione MDA: Malondialdehyde in plasma

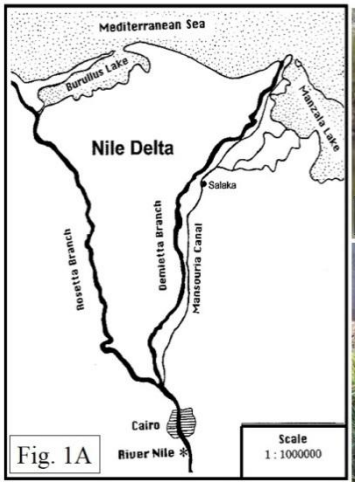


Fig. 1A


Fig. 1B


Fig. 1C

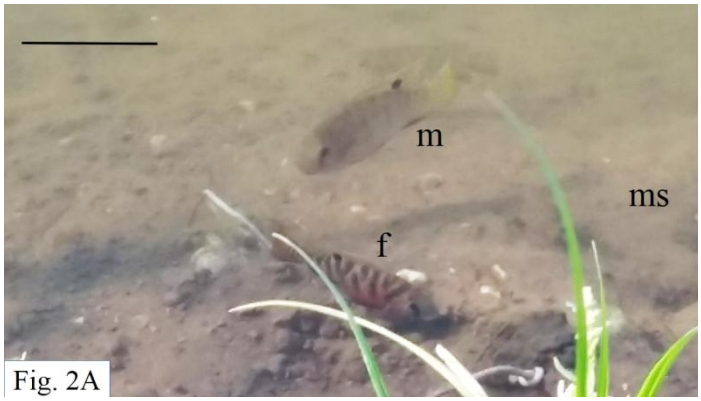


Fig. 2A

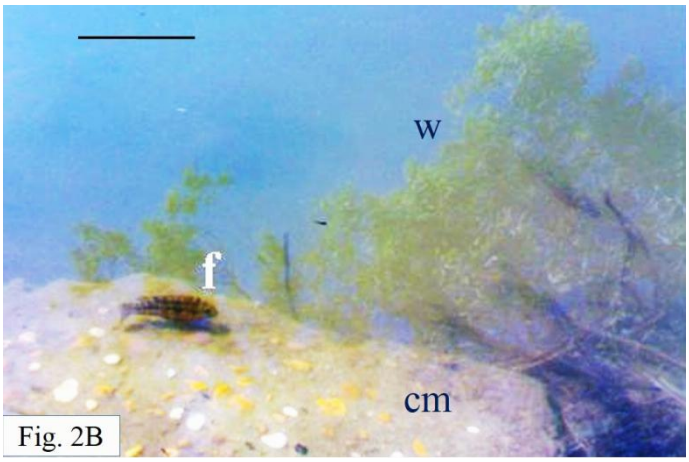


Fig. 2B

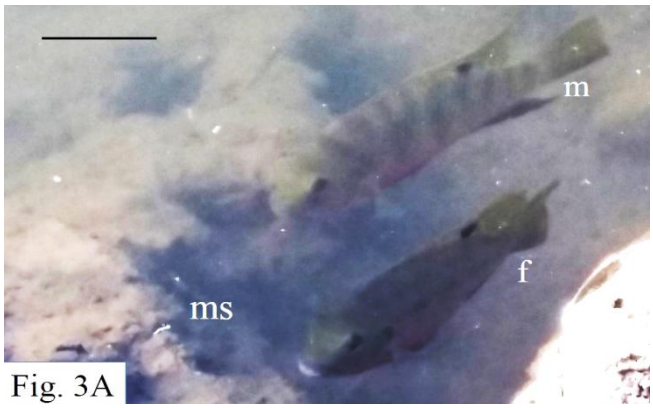


Fig. 3A

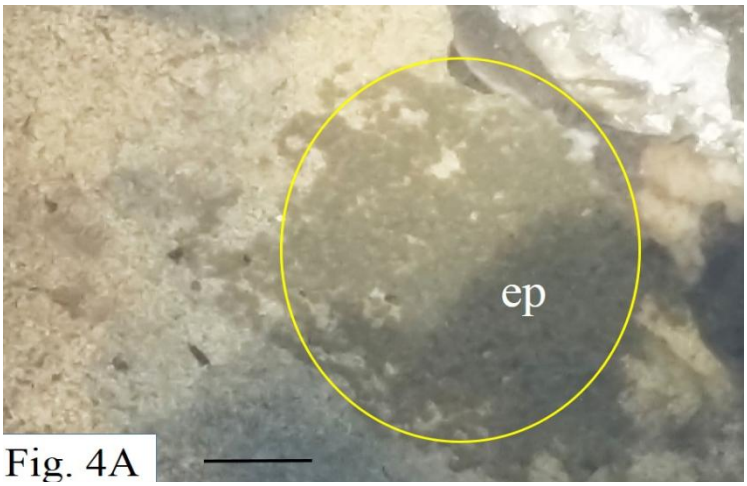
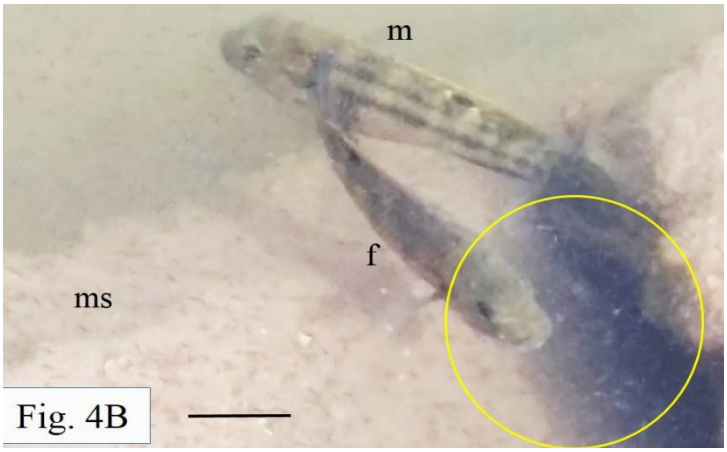


Fig. 4A



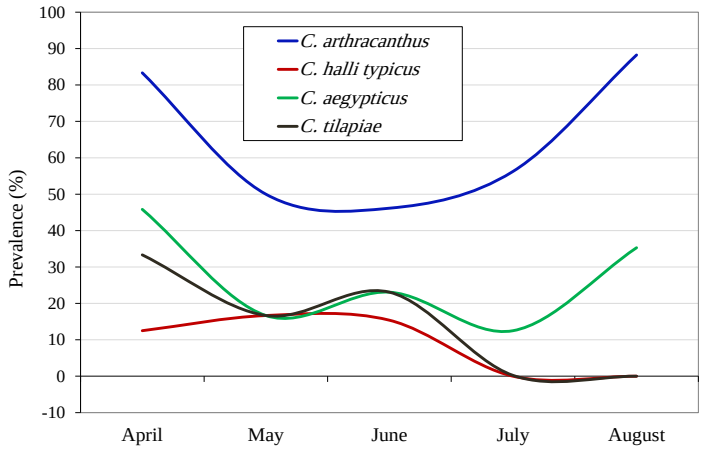


Fig. (5): Monthly Fluctuations Of The Prevalences Of The Gill Monogeneans On The Breeding Forms Of *tilapia Zilli*.

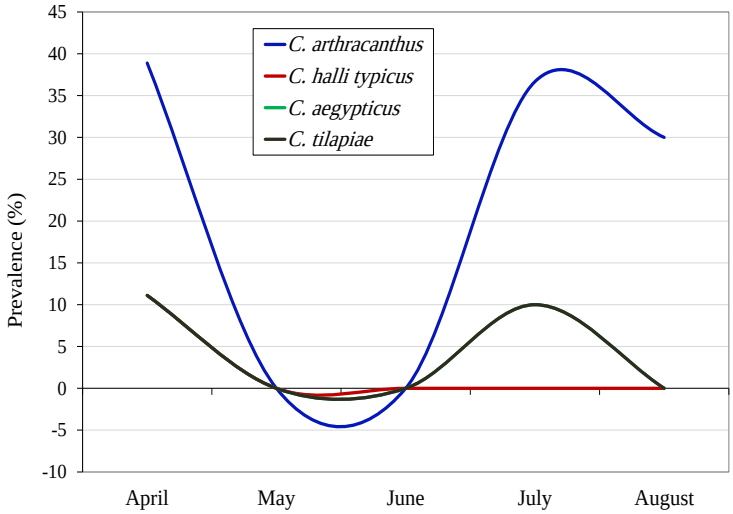


Fig. (6): Monthly Fluctuations Of The Prevalences Of The Gill Monogeneans On The Non-Breeding Forms Of *Tilapia Zilli*.

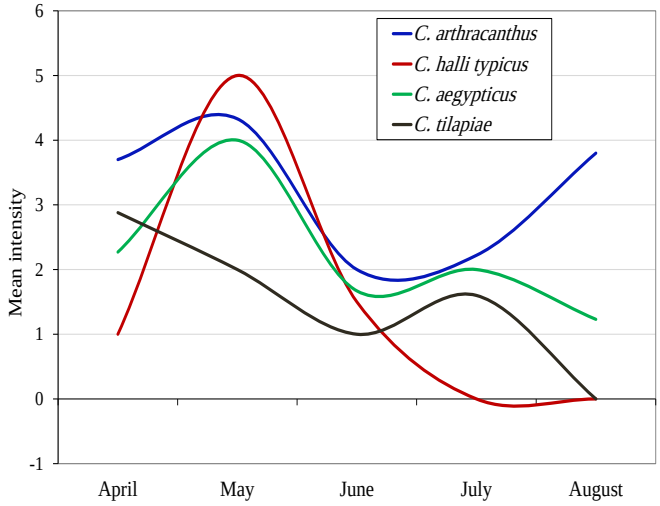


Fig. (7): Monthly Fluctuations Of The Mean Intensity Values Of The Monogeneanson The Breeding Forms Of *Tilapia Zilli*.

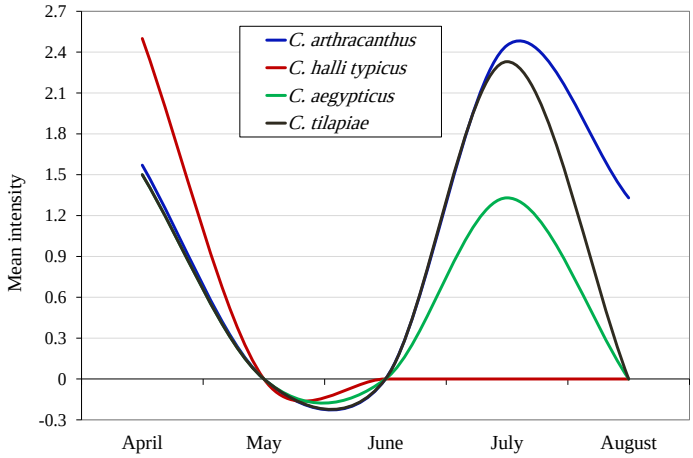


Fig. (8): Monthly Fluctuations Of The Mean Intensity Values Of The Gill Monogeneanson The Non-Breeding Forms Of *tilapia Zilli*.

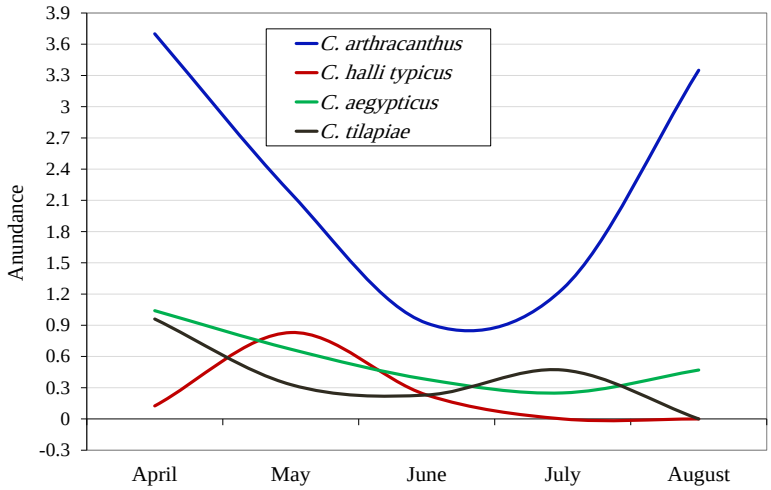


Fig. (9): Monthly Fluctuations Of The Abundances Of The Gill Monogeneanson The Breeding Forms Of *Tilapia Zilli*.

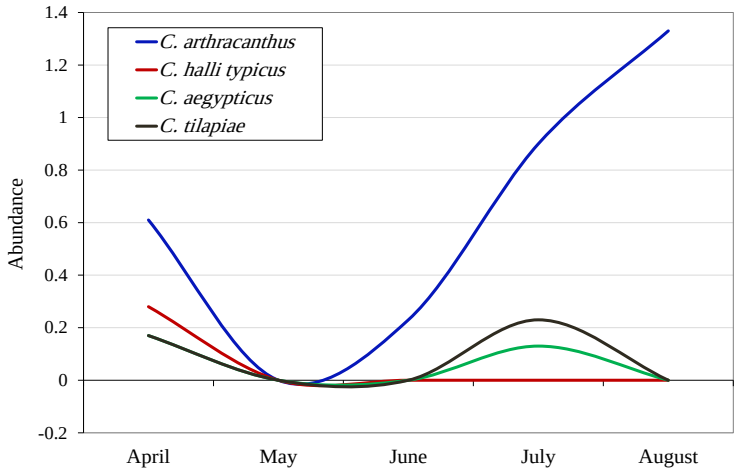


Fig. (10): Monthly Fluctuations Of The Abundances Of The Gill Monogeneanson The Non-Breeding Forms Of *Tilapia Zilli*.

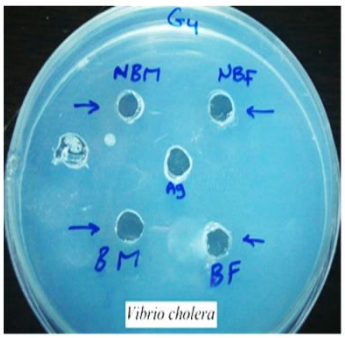


Fig. 11A

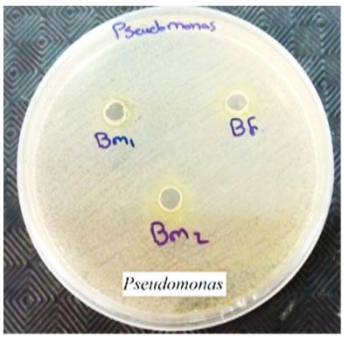


Fig. 11B

Fig. 11C

V. Explanation Of Figures

Fig. 1.Description of the investigation area. A) Map showing the location of Salaka Village atMansouria Canal in the vicinityof Mansoura City, Nile Delta, Egypt. B) Photograph showing one of the sources of contamination (dead animals) of the waterbodyatMansouria Canal. C) Photograph showing the marked drop in the level of water inMansouria Canal during September, 2016. D) Google Earth Satellite image showing a sector of Mansouria Canal nearby Mansoura City.

Fig. 2. Photomicrograph showing the nesting forms of *Tilapia zilli*. A) Breeding male andfemale begin to prepare the nest on a muddy substrate. Note the markedcolouration of the body of mating partners. Scale bar = 80 mm. B) Breeding female waiting a mating partner on a concrete mass. Scale bar = 90 mm. cm, concrete mass; f, female; m, male; w, aquatic weeds.

Fig. 3. Photomicrograph showing thebreeding forms of *Tilapia zilli*. A) Large-sized mates occupying a vertical groove close to the shoreline of Mansouria Canal. Scale bar = 40 mm. B) Breeding male attaining attractive colouration during the spawning period. Scale bar = 45 mm. Note the distinctive, dark vertical strands across the body of the breeding male,red throat and abdomen, and shiny yellowish colouration of the lateral sides of the body. f, female; m, male; ms, muddy substrate.

Fig. 4. Photomicrograph showing egg patch and parental carein*Tilapia zilli*. A) Hundreds of deposited eggs on contaminated, muddy substrate. Scale bar = 30 mm. B) Breeding male and female providing intensive care to the newly hatched larvae (yellow circle). Note that the male secures the margins of the nest, whereas the female devotes her effort to safeguard and guide the offspring. Scale bar = 40 mm. ep, egg patch; f, female; m, male; ms, muddy substrate.

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