

## Monogenean Microfauna of the Nile Catfish, *Clarias gariepinus* as Biomonitor of Environmental Degradation in Aquatic Ecosystems at the Nile Delta, Egypt

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**Abstract:** One tool to obtain data on the health of the aquatic ecosystem is the use of bioindicators or biomonitor. The present study aimed to test the validity of the oviparous and viviparous monogeneans of the Nile catfish, *Clarias gariepinus* as a tool for monitoring some aquatic ecosystems in the Nile Delta, Egypt. Three differing-water quality ecosystems were studied during the period from October 2015 to September 2016: Damietta Branch of the River Nile, Ammar Drain (Drain No. 2) and Telbanah Drain. Obtained data revealed that the studied monogenean species varied greatly in their numbers on the catfish host, with the oxygen-poor, pungent smell, highly turbid, dirty, shallow and small-sized Telbanah Drain was the most hospitable habitat for these parasites (4907 out of 7098 worms). This deteriorated waterway stored the highest amounts of  $Ca^{+2}$ ,  $Mg^{+2}$  and  $N_2$ . On the other hand, the relatively deeper, larger and agriculturally polluted water of Ammar Drain showed the highest levels of pH, EC, TDS,  $HCO_3^-$ ,  $SO_4^{+2}$ ,  $Cl^-$ ,  $Na^+$ ,  $K^+$  and P, and accommodated 1704 monogenean worms. All the monogeneans responded similarly to environmental parameters, however *Macrogyrodactylus clarii* was highly sensitive to water temperature whereas *Gyrodactylus* sp. was highly sensitive to TDS. The highly transparent, oxygen-rich, much deeper, and larger River Nile showed the lowest infestation levels (487 worms). The highest number of monogenean worms was attained by the most dominant oviparous *Quadriacanthus aegypticus* (3058 worms), followed by *Quadriacanthus clariadis* (1786 worms) and *Quadriacanthus kearnii* (1375 worms). The dominant *Q. aegypticus* attained the highest infection variables (prevalence, mean intensity and abundance) at Telbanah Drain and Ammar Drain. *Macrogyrodactylus congolensis* recorded the greatest number (549 worms) among viviparous monogeneans. A total of 146 worms was scored for *Macrogyrodactylus clarii*, however *Gyrodactylus rysavyi* recorded 96 worms on the skin and 71 worms on the gills. The satellite monogeneans, *Gyrodactylus* sp. and *Paraquadriacanthus nasalis* recorded 14 and 3 worms, respectively. The present findings revealed that only *Quadriacanthus* species are good biomonitor and sensitive pointers of environmental fluctuations in the aquatic ecosystems; these organisms apparently proliferate in polluted water and stressed fish.

**Keywords:** Monogenean Microfauna, *Clarias gariepinus*, Biomonitor, Water Pollution, Nile Delta, Egypt.

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### I. Introduction

Over the past decades, the freshwater ecosystems and their biota in the Nile Delta have been subjected to a composite of anthropogenic wastes. Aquatic ecosystems receiving huge amounts of industrial, agricultural, suburban and urban, and sewage effluents have become a rising anxiety in Egypt; great amounts of waste are discharging into these vital resources. Exposure to sublethal levels of pollutants for a long period of time can produce morphological, anatomical, physiological, functional and/or behavioural alterations [1, 2, 3, 4, 5, 6, 7]. These impacts are manifesting at the community, population, individual, organ and cellular levels [8, 9, 10, 11, 12]. [13, 14] suggested that the aquatic ecosystems can be monitored either directly by monitoring abiotic and biotic parameters at regular intervals or indirectly by employing biomonitor that actively respond to environmental degradation.

Environmental pollution of the aquatic ecosystem may either increase or decrease the worm (parasite) burden in aquatic organisms such as fish host [9, 15, 16, 17]. According to [9], the skin and gill dwellers of the fish hosts tend to proliferate with the severity of the pollution circumstances, however internal helminth fauna of these hosts tend to show comparatively lower population size. [18] reported that the monogenean, *Paradiplozoon ichthyoxantho* was absent from the smallmouth yellowfish, *Labeobarbus aeneus* inhabiting streams which are rich in the electrical conductivity (EC) and trace metals. [16] hypothesized that the type of pollutant plays an important role in shaping the response of aquatic organisms to the pollution gradient. Marked sensitivity in fish ectoparasites toward environmental alterations may be attributed to their microhabitats on the outer surface of the host which are exposed to the full force of the surrounding

environment [16]. [19] observed that the level of monogenean infestation declined with elevated concentrations of the pulp and paper mill discharges, however it increased in response to oil exposure in the marine environment. [20] illustrated that the specialist monogenean species were more sensitive toward water pollution than generalists. These authors concluded that such ecological shifts are good pointers of environmental pollution [20].

Monogeneans are regarded among the fundamental groups and the most dominant brands of fish helminth fauna [21, 22, 23]. Fish monogenean communities are good bioindicators of the ecosystem health and environmental pollution [24, 25, 13, 26, 27, 28, 29, 30, 31]. This parasitic group has a simple and direct life cycle, and are in direct contact with both the fish host and environment, fully or merely exposed to the hydrodynamic forces acting on their microhabitats, highly sensitive to the changes of environmental factors, available in considerable numbers, easily identified, discriminated, readily involved in statistical approaches, experience variable modes of reproduction and have brief life time, and can proliferate under optimal conditions. To all these advantages, ectoparasitic monogeneans are regarded as successful bioindicators or biomonitor of the human interference and disturbance. Moreover, living in/on living environment (a monogenean on the host fish) always provokes a dramatic change in the quality of the niche [32, 33]. According to [34], the fish host providing a monogenean with a peaceful microhabitat and a rich source of energy soon becomes deadly ground containing phagocytic cells, cytotoxic cells, antibodies, proteolytic enzymes, epidermal regeneration, etc.

The catfish *Clarias gariepinus* is a successful survival of the aquatic ecosystems polluted with agricultural, urban and/or industrial discharges. It is a target host for a group of highly specialized monogenean species belonging to the genera *Gyrodactylus*, *Macrogryrodactylus* (viviparous monogeneans), and *Paraquadriacanthus* and *Quadriacanthus* (oviparous monogeneans). Each of these monogeneans occupies an ecological niche and appears to interact variably with its environment. Understanding the ecological aspects of these monogeneans under natural conditions could provide baseline data for the welfare of the catfish stock in extensive aquaculture systems during the coming years. A biomonitor means a living organism that provides quantitative information on the properties of the ambient environment [35]. In this respect, a powerful biomonitor will point to the occurrence of the pollutant or contaminant and also may provide further evidence about the level of introductions and human intervention. Individual organisms as well as their populations or communities seem likely to reflect environmental modifications by fluctuating in physiological, chemical and/or behavioural aspects. According to [36], the population and community structure, behavioural responses, biochemical manifestations and morphological as well as anatomical features are the most relevant tools in biomonitoring environmental pollution. The present investigation aimed at analyzing the structure of the monogenean communities on the gills and skin of the Nile catfish, *Clarias gariepinus* inhabiting three differing-water quality ecosystems. One of the primary objectives of the present study was to test the biomonitor ability of two brands of helminth parasites, namely oviparous and viviparous monogeneans harbouring a highly tolerant, keystone teleost, namely *C. gariepinus* and to demonstrate the relationship between physicochemical/biological profiles of the aquatic ecosystem and fish health, in particular their susceptibility to monogenean invasion.

## II. Materials and Methods

### 2.1. Study area:

The study area is located in the Nile Delta, an amazing and highly populated geographical region in the north part of Egypt. Three differing-water quality ecosystems were selected, namely Ammar Drain (Figures 2A, 2B and 2C), Damietta Branch of the River Nile (Figures 3A and 3B), and Telbanah Drain (Figures 4A and 4B). The River Nile locality was selected at Al-Tawilah suburban area, Talkha City, Dakahlia Province, north Egypt. Ammar Drain is located nearby Belqas district, 50 km north Mansoura City. Ammar Drain is known as Drain No. 2; it is one of the main agricultural drains, flows close to the International Coastal Road (Google Map: 31°22'46.4556" N 31°29'13.2432" E), and receives a variety of discharges originating from suburban and agricultural areas along its sides. The downstream point of Ammar Drain is a complex network of agricultural/industrial drains, discharging directly into the Northern Coast of Egypt in the Mediterranean Sea at Gamasa. The drain is employed as an irrigation canal to water thousands of acres of vegetables and some economic crops. Mansoura Drain (common name: Telbanah Drain) is located at Telbanah suburban area, Mansoura; it is considered as industrially and agriculturally polluted locality. This annoying drain receives considerable amounts of industrial pollution from Dakahlia Spin Wear Company and Oil and Soap Company which play an important role in the economy of this highly populated geographical area.

### 2.2. Host sampling and Dissection:

Specimens of the African Sharptooth Catfish, *Clarias gariepinus* Burchell, 1822 (Figure 1) were caught monthly, with special nets, between October 2015 and September 2016. Each monthly collected sample

comprised approximately 25 fish from each locality (total harvest = 989 fish). Freshly caught individuals of the catfish were immediately preserved in 20% formaldehyde and safely transported to the Laboratory of Environmental Sciences at Mansoura University. Thereafter, the total catfish length was measured in centimetres (cm) from the tip of snout to the posterior margin of the tail fin. Each catfish was weighed in grams (g) with METLAR MD-2000 Electronic balance. Host sex was differentiated into males and females through the examination of the external genitalia and dissection of the internal reproductive organs (ovary in males and testis in females).

### 2.3. Collection of the monogenean parasites:

The gills of the catfish host were removed carefully and transferred to a mixture of 1:4000 formaldehyde/water according to [37]. *Quadriacanthus aegypticus* was identified according to the original description made by [38], *Quadriacanthus clariadis* according to [39], *Quadriacanthus kearnii* according to [40], *Macrogyrodactylus clarii* according to [41], *Macrogyrodactylus congolensis* according to [42, 43], *Gyrodactylus rysavyi* according to [44] and *Paraquadriacanthus nasalis* according to [45]. Monogenean worms were dislodged from the gill filaments and gill lamellae, and discriminated depending on comparison of the body dimensions, architecture of the copulatory structures, measurements of the sclerotized hamuli, hooks, and connective bars of the haptor. To dislodge monogenean worms off their attachment loci on the gills, a sharp needle was introduced safely below the fixed haptor and then the parasite was pushed gently and driven to the bottom of the dish. Monogenean worms were then transferred individually on a glass slide, and flattened properly with the aid of a coverslip to illustrate the distinctive features of each monogenean species (haptor sclerites, copulatory complex and vaginal tube).

### 2.4. Community structure and species diversity of monogeneans:

Parasitological indices evaluated in this study included dominance, prevalence, mean intensity and abundance. The community structure indices included dominance, prevalence, mean intensity and abundance. The dominance of monogenean parasite species was calculated according to [46] as follows:

$$\text{Dominance index} = \frac{N}{N_t}$$

Where N = total number of individuals belonging to a particular species, and  $N_t$  = total number of individuals of different species in the community.

Community Similarity (similarity coefficients for binary data):

#### 2.4.1. Sorenson's Coefficient (coefficient of community):

Sorenson's coefficient according to [47], provides a value between 0 and 1. The closer the coefficient value is from zero, the complete community dissimilarity is. On the other hand, the closer the coefficient value is from 1, the more the community overlap is.

$$S_s = \frac{2c}{S_1 + S_2}$$

$S_s$  = Sorensen's similarity coefficient.

c = the number of species common to (or shared by) both communities.

$S_1$  = the number of species unique to the first community.

$S_2$  = the number of species unique to the second community.

#### 1. Jaccard Index:

The Jaccard index (Jaccard similarity coefficient) is a statistic employed for comparing the similarity and diversity of two sample groups (binary data)[48]. It is based mainly on the local presence or absence of the living organism. According to [49], the Jaccard similarity coefficient is simple to compute, reflects species shifts and trends between stations, is applicable at low population densities or sample size, can be adapted to qualitative data, and minimizes the effects of seasonal or annual variation on population trends. [50] reported that the Jaccard similarity coefficient works directly with density differences for individual species and may reflect fluctuations in species proportion. Jaccard's Equation is formulated as follows:

$$S_j = \frac{100c}{a + b - c}$$

$S_j$  = Jaccard similarity coefficient.

a = the number of species common to (or shared by) both communities.

b = the number of species unique to the first community.

c = the number of species unique to the second community.

#### 2.4.2. Shannon-Weiner index:

The Shannon index is an informative statistic index, which means it assumes all species are represented in a sample and that they are randomly sampled. Shannon index, p is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ln is the natural log,  $\Sigma$  is the sum of the calculations, and s is the number of species.

Shannon created a formula that measured information as a function of probabilities, with  $p_i$  being the probability of each message:

$$H = -\sum_{i=1}^n (p_i \times \ln p_i) [51]$$

4. Simpson's Diversity Indices:

Simpson's Index (D) measures the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species) [52]. There are two versions of the formula for calculating D, either is acceptable, but be consistent.  $D = \sum (n / N)^2$

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

n = the total number of organisms of a particular species.

N = the total number of organisms of all species.

The value of D ranges between 0 and 1. With this index, 0 represents infinite diversity and 1, no diversity. That is, the bigger the value of D, the lower the diversity. This is neither intuitive nor logical, so to get over this problem, D is often subtracted from 1 to give:

#### 2.4.3. Infection variables:

The prevalence (percentage of infection) is defined as the number of individuals of a host parasitized by a particular parasite divided by the number of examined hosts:

$$\text{Prevalence (P)} = \frac{\text{Total number of fish infected}}{\text{Total number of examined fish}} \times 100$$

Mean intensity is the number of individuals of a particular parasite divided by the number of infected host individuals (mean number of parasites per infected host).

$$\text{Mean Intensity (MI)} = \frac{\text{Total number of single parasite species}}{\text{Total number of infected fish}}$$

Abundance is the number of individuals of a particular parasite species divided by the number of host individuals examined (mean number of parasites per examined host).

$$\text{Abundance (A)} = \frac{\text{Total number of single parasite species}}{\text{Total number of examined fish}}$$

The terms, prevalence and mean intensity were used in accordance with the recommendations of [53].

#### 2.5. Heavy Metals analysis:

The microelements chromium (Cr), zinc (Zn), nickel (Ni), iron (Fe), cobalt (Co), copper (Cu), cadmium (Cd), manganese (Mn) and lead (Pb) were analyzed in the collected water samples with the aid of an atomic absorption spectrophotometer (WFXAA Spectrophotometer model-130B) according to [54].

#### 2.6. Statistical analysis and treatment of data:

All data were recorded as (Mean  $\pm$ SD). Differences in the prevalences, mean intensities and abundances of the monogenean species among the three environments were tested statistically using One-Way ANOVA test on SPSS package (version 20). Furthermore, a PostHoc test, namely Tukey's range test (Tukey's HSD) was employed to detect the differences between each pair of localities. The ordination (CCA: Canonical Correspondence Analysis) [55, 56] was determined using MVSP software program (version 3.2). This analysis aimed to clarify the relationship between the physicochemical environmental parameters and the infection variables of the studied monogeneans. Probability values  $\leq 0.05$  were designed significant, those  $\leq 0.01$  as highly significant,  $\leq 0.001$  as very highly significant, while  $> 0.05$  as non-significant.

### III. Results

#### 3.1. Water quality:

The water of Telbanah Drain is black in colour, more turbid than that of Ammar Drain, which was comparatively more turbid (cloudy) than that of the River Nile. The mean water depth of the River Nile, Ammar Drain and Telbanah Drain was approximately  $10.00 \pm 2.00$  m,  $3.00 \pm 1.00$  m and  $0.50 \pm 0.20$  m, respectively. The mean amount of total dissolved solids recorded its highest value in Ammar Drain ( $1324.85 \pm 376.62$  ppm) followed by Telbanah Drain ( $601.84 \pm 171.95$  ppm) and the River Nile ( $232.31 \pm 40.19$  ppm). Similar trend was recorded for water sulphates and chlorides, with mean values of  $323.78 \pm 162.25$  and  $300.43 \pm 171.83$  ppm,  $133.94 \pm 114.58$  and  $107.64 \pm 42.53$  ppm, and  $64.24 \pm 30.21$  and  $24.53 \pm 17.63$  ppm at Ammar Drain, Telbanah Drain and the River Nile, respectively.

The electrical conductivity of water (EC) showed its greatest level at Ammar Drain, with a mean value of  $2.26 \pm 0.48$  ds/m. On the other hand, the mean values of this physical parameter were  $1.05 \pm 0.31$  and  $0.40 \pm 0.04$  ds/m at Telbanah Drain and the River Nile, respectively. The amount of water dissolved oxygen varied from obviously higher levels at the River Nile locality ( $6.19 \pm 0.89$  mg/l) to comparatively lower values at Ammar Drain ( $3.96 \pm 1.39$  mg/l) and Telbanah Drain ( $3.65 \pm 1.73$  mg/l). Hydrogen ion concentration (pH)

differed slightly among the studied ecosystems; the recorded values lied within the weak alkaline scale and varied from  $7.23 \pm 0.18$  at Telbanah Drain to  $7.55 \pm 0.25$  at the River Nile and  $7.57 \pm 0.39$  at Ammar Drain.

Chemical analysis of water indicated that Ammar Drain was more alkaline ( $\text{HCO}_3^- = 324.55 \pm 143.79$  ppm) than Telbanah Drain ( $224.23 \pm 122.25$  ppm) and the River Nile ( $114.01 \pm 63.33$  ppm). Chemical analysis of water revealed also that Telbanah Drain received higher amounts of nitrogen ( $12.99 \pm 5.61$  ppm) than Ammar Drain ( $9.30 \pm 1.79$  ppm) and the River Nile ( $7.80 \pm 1.47$  ppm). The River Nile accumulated more phosphorous ( $0.62 \pm 1.28$  ppm) than Telbanah Drain ( $0.35 \pm 0.71$  ppm) and Ammar Drain ( $0.14 \pm 0.02$  ppm).

Water sodicity, a term assumed to the quantity of sodium held in water, was higher at Ammar Drain ( $392.66 \pm 156.81$  ppm) than Telbanah Drain ( $121.57 \pm 76.43$  ppm) and the River Nile ( $32.61 \pm 23.00$  ppm). Regarding calcium and magnesium content of water, Telbanah Drain stored higher levels of these two essential minerals ( $35.25 \pm 24.67$  and  $25.19 \pm 11.85$  ppm) than Ammar Drain ( $34.20 \pm 32.41$  and  $21.93 \pm 9.79$  ppm) and the River Nile ( $26.95 \pm 15.58$  and  $9.94 \pm 5.44$  ppm), respectively. The amount of potassium estimated in the water sampled from Ammar Drain ( $9.25 \pm 6.50$  ppm) and Telbanah Drain ( $8.76 \pm 7.41$  ppm) was nearly twice that collected from the River Nile ( $4.96 \pm 1.96$  ppm).

### 3.2. Heavy Metals:

The highest concentrations for iron ( $0.255 \pm 0.390$  ppm), copper ( $0.744 \pm 0.582$  ppm), zinc ( $0.054 \pm 0.041$  ppm) and cobalt ( $1.177 \pm 0.912$  ppm) were registered at Ammar Drain, while the highest concentrations of cadmium ( $0.812 \pm 0.775$  ppm), nickel ( $1.186 \pm 1.253$  ppm) and chromium ( $0.153 \pm 0.304$  ppm) were measured at Telbanah Drain. The heavy metals manganese and lead recorded their highest levels at the River Nile, with mean values of  $0.244 \pm 0.177$  and  $0.411 \pm 0.326$  ppm, respectively.

### 3.2. Monogenean worm burden:

A total of eight ectoparasitic monogenean species were encountered on *C. gariepinus* dwelling the River Nile, Telbanah Drain and Ammar Drain. Gill monogeneans comprised three oviparous monogeneans, namely *Quadriacanthus aegypticus*, *Q. clariadis* and *Q. kearni*, and two viviparous monogeneans, namely *Macrogyrodactylus clarii* and *Gyrodactylus rysavyi*. Skin monogeneans comprised the viviparous monogeneans *Macrogyrodactylus congolensis* and *Gyrodactylus rysavyi*. Two monogenean species, namely *Gyrodactylus* sp. and *Paraquadriacanthus nasalis* were recorded from the nasal cavities of *C. gariepinus*.

Out of 989 fish examined, a total of 7098 monogenean worms were encountered on the Nile catfish, *Clarias gariepinus* inhabiting the River Nile, Telbanah Drain and Ammar Drain at the Nile Delta, Egypt. According to their microhabitats, 6436 monogenean worms were recorded on the gills, 645 worms on the skin and only 17 monogenean worms in the nasal cavity of the catfish host. Regarding the sexual dimorphism of the monogeneans infestation on *C. gariepinus*, 31.73% of the monogenean worm burden was calculated on female hosts, whereas 68.27% was estimated on male hosts.

Regarding the gill monogeneans, 4606 worms were found on the catfish dwelling Telbanah Drain, 1383 worms at Ammar Drain and 447 worms at the River Nile. Concerning the skin monogeneans, the highest number (318 worms) was counted on *C. gariepinus* dwelling Ammar Drain. On the other hand, the number of monogenean worms found on the host's skin from Telbanah Drain and the River Nile was 287 and 40 worms, respectively.

*Gyrodactylus* worms were recorded in the nasal cavity of *C. gariepinus* inhabiting Ammar Drain (3 worms) and Telbanah Drain (11 worms). However, no *Gyrodactylus* worms were encountered in the nasal cavity of *C. gariepinus* at the River Nile locality. Irrespective of an intensive search on 989 specimens of *C. gariepinus*, only 3 worms of the oviparous monogenean *Paraquadriacanthus nasalis* were recorded from the nasal cavities of the catfish host dwelling Telbanah Drain. Berger-Parker Dominance index was modified to calculate the sharing ratio for each species in the monogenean assemblage of *Clarias gariepinus*. The sharing ratios were obtained in the following order: 43.08% for *Q. aegypticus*, 25.16% for *Q. clariadis*, 19.37% for *Q. kearni*, 7.74% for *M. congolensis*, 2.06% for *M. clarii*, 2.35% for *G. rysavyi*, 0.20% for *Gyrodactylus* sp. and 0.04% for *P. nasalis* (Figure 5).

### 3.3. Infection variables:

As shown in Table 1, the oviparous *Quadriacanthus aegypticus* scored the highest infection variables (prevalence, mean intensity and abundance), followed by the congeneric members, *Q. clariadis* and *Q. kearni* at Telbanah Drain and Ammar Drain. At the River Nile, a slight difference could be noticed between the mean prevalences of the two cohabitants, *Q. aegypticus* and *Q. clariadis*, however the highest mean intensity and mean abundance levels were recorded for *Q. clariadis* (Table 1). The viviparous *Gyrodactylus* sp. was completely absent from the catfish host dwelling the River Nile, however this monogenean showed light infestation levels at Telbanah Drain and Ammar Drain (Table 1). Similarly, the isolationist *Paraquadriacanthus*

*nasalis* was completely absent from the catfish host inhabiting the River Nile and Ammar Drain. Moreover, this oviparous exhibited irregular occurrence, with dramatic decline of the infection variables (Table 1).

One-Way ANOVA test (SPSS: version 20) indicated very highly significant differences in the prevalence ( $F=52.665$ ,  $P < 0.001$ ), mean intensity ( $F=10.084$ ,  $P < 0.001$ ) and abundance ( $F=10.564$ ,  $P < 0.001$ ) of the oviparous *Quadriacanthus aegypticus* among the three aquatic ecosystems. Further statistical analysis (Tukey's range test) detected significant differences in the prevalence and mean intensity between Telbanah Drain and the River Nile as well as Ammar Drain. Similar output was estimated between Ammar Drain and the River Nile. Tukey's HSD showed also significant differences in the abundance of *Q. aegypticus* between Telbanah Drain and the River Nile as well as Ammar Drain.

One-Way ANOVA test (SPSS: version 20) indicated a very highly significant difference in the prevalence ( $F=22.276$ ,  $P < 0.001$ ), a highly significant difference in the abundance ( $F=7.784$ ,  $P < 0.01$ ) and a significant difference in the mean intensity ( $F=10.564$ ,  $P < 0.001$ ) of the oviparous *Quadriacanthus clariadis* among the three aquatic ecosystems. Further statistical analysis (Tukey's range test) detected significant differences in the prevalence and abundance between Telbanah Drain and the River Nile as well as Ammar Drain. Moreover, a significant difference in the mean intensity of this monogenean was estimated between Telbanah Drain and Ammar Drain.

There was a very highly significant difference in the prevalence (One-Way ANOVA test:  $F=21.383$ ,  $P < 0.001$ ) and highly significant differences in the mean intensity ( $F=6.278$ ,  $P < 0.01$ ) and abundance ( $F=6.740$ ,  $P < 0.01$ ) of the oviparous *Quadriacanthus kearnii* among the three aquatic ecosystems. Further statistical analysis (Tukey's range test) detected significant differences in the prevalence, mean intensity and abundance between Telbanah Drain and the River Nile as well as Ammar Drain. Moreover, a significant difference was estimated for the prevalence of *Q. kearnii* between Ammar Drain and the River Nile.

One-Way ANOVA test revealed that the differences in the prevalence, mean intensity and abundance of the skin and gill monogenean *Gyrodactylus rysavyi* ( $P > 0.05$ ) among the three aquatic ecosystems was non-significant statistically. Similarly, One-Way ANOVA test revealed that the differences in the abundance of the skin monogenean *Macrogyrodactylus congolensis* ( $P > 0.05$ ) among the three aquatic ecosystems was non-significant statistically.

Highly significant variations were recorded by One-Way ANOVA test for the prevalence ( $F=6.245$ ,  $P < 0.01$ ) and abundance ( $F=6.101$ ,  $P < 0.01$ ) of the gill monogenean *Macrogyrodactylus clarii* among the three aquatic ecosystems. Further statistical analysis (Tukey's range test) detected significant differences in the prevalence and abundance between Telbanah Drain and the River Nile as well as between Ammar Drain and the River Nile.

As shown in Figure 6, the vast majority of the studied monogenean species are concentric, i.e. located in the vicinity of center of the CCA biplot, and therefore responded similarly to the fluctuations of the measured physicochemical environmental parameters. It can be also seen that the prevalences of these parasites, particularly *Macrogyrodactylus clarii* and *Gyrodactylus rysavyi*, are strongly affected by dissolved oxygen, manganese, magnesium, chlorides and electrical conductivity.

As revealed by the ordination CCA in Figure 7, all the studied monogenean species, except for *Gyrodactylus* sp. (from the nasal cavity), are concentric and thus reacted equally to the variations of the selected physicochemical environmental parameters, with no definite parameter controlling or regulating their occurrence (mean intensity). It could be also noticed from Figure 7 that the electrical conductivity, total dissolved solids (sulphates, chlorides and bicarbonates), and the essential minerals: sodium, potassium, calcium and magnesium, in addition to nitrogen positively influenced the mean intensity values of the studied monogeneans (data pooled from the three ecosystems). It is also clear that the viviparous monogenean *Gyrodactylus* sp. from the nasal cavity of the catfish host is the most sensitive species to these environmental changes (Figure 7). In contrast, some physicochemical factors such as water temperature, hydrogen ion concentration and dissolved oxygen, in addition to some heavy metals such as iron, zinc and lead appear to have negative impacts of the number of monogenean worms, particularly *Gyrodactylus* sp., aggregating on an individual fish.

From Figure 8, it is obvious that the viviparous *Macrogyrodactylus clarii* exhibits significant positive correlation with water temperature and moderate negative correlation with nitrogen and the heavy metals iron, cadmium and nickel. On the other hand, the viviparous monogeneans *Gyrodactylus* sp., *G. rysavyi* and *Macrogyrodactylus congolensis* exhibited strong negative correlations with EC, TDS (sulphates, chlorides, bicarbonates) and the essential minerals: sodium, potassium, calcium and magnesium, in addition to the heavy metal chromium (Figure 8). However, these monogeneans are positively correlated with water dissolved oxygen, biological oxygen demand, cobalt, phosphorous and zinc (Figure 8).

### 3.4. Community structure and species diversity:

From the pooled data of the three communities (River Nile, Telbanah Drain and Ammar Drain), it can be noticed that *Quadriacanthus aegypticus* was the dominant species. This monogenean attained the highest

dominance index (0.431) (Table 2) and the highest Shannon-Weiner index (162.06). However, at the River Nile, the congeneric *Quadriacanthus clariadis* recorded the highest dominance index (0.462) (Table 2), Shannon-Weiner index (177.090) and Simpson index (0.016). At Telbanah Drain, *Q. aegypticus* was the most dominant species and recorded the highest dominance index (0.4603), Shannon-Weiner (176.24) and Simpson index (0.212) (Table 2). Also, at Ammar Drain, *Q. aegypticus* was the most dominant species and recorded the highest dominance index (0.389), Shannon-Weiner (142.45) and Simpson index (0.151) (Table 2).

The similarity index (Jaccard Similarity Coefficient and Sørensen's Coefficient) between Ammar and Telbanah Drain was 100%. The similarity index (Sørensen's Coefficient) between Ammar Drain and the River Nile was as well as between Telbanah Drain and the River Nile was 93.33%. Also, the similarity index (Jaccard Coefficient) between Ammar Drain and the River Nile was as well as between Telbanah Drain and the River Nile was 87.50%.

#### IV. Discussion

One strategy to understand and prevent the outcomes of the accelerating environmental degradation is the use of biological indicators, for example free-living or parasitic species that respond to habitat alterations with changes in their numbers, physiology or chemical composition. In recent years, the utilization of fish parasites as biomonitoring in the aquatic ecosystem is a common trend over the globe [29, 30, 57]. To date, in Egypt, only few studies have been made on this topic [30, 58, 59, 60]. As fish parasites, in particular the monogeneans, are highly sensitive bioindicators of environmental pollution, a good deal of attention should be paid to test and verify their validity as environmental biomonitoring. According to [9] accumulation bioindicators deliver more accurate evidence on the quality of the habitat than investigations measuring numerical fluctuations in parasite population or community. Only one study, namely [57] dealt with the monogeneans as tags to assess heavy metal pollution in the Red Sea. Both digenean and monogenean parasites were found by [16] to be successful bioindicators of heavy metal pollutants, and recommended that digenean parasites are proper bioindicators of eutrophication.

In the present study, the monogeneans belonging to the genus *Quadriacanthus* showed relatively high infestation levels, followed by members of the genus *Macrogyrodactylus*. However, *Gyrodactylus* sp. and *Paraquadriacanthus nasalis* recorded dramatically low infestation levels. The catfish host dwelling Telbanah Drain harboured greater numbers of monogenean worms than Ammar Drain and the River Nile. This small-sized, shallow drain exhibits repellent odours and is characterized by low dissolved oxygen and high cloudiness. Moreover, it received the highest levels of  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$  and  $\text{N}_2$ . Ammar Drain is a comparatively deeper and larger waterway, and stored great amounts of agricultural water. This drain attained the highest values of EC, TDS,  $\text{HCO}_3^-$ ,  $\text{SO}_4^{+2}$ , pH,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and P. Unlike Ammar Drain and Telbanah Drain, the River Nile is deeper and larger, and exhibited marked transparency. *Quadriacanthus aegypticus* was the most dominant, followed by *Quadriacanthus clariadis* and *Quadriacanthus kearni*. On the other hand, the viviparous monogeneans *Macrogyrodactylus* and *Gyrodactylus* species recorded comparatively lower infestation levels. Owing to its scarceness, the oviparous monogenean *Paraquadriacanthus nasalis* may be considered as a satellite or endangered species.

The present investigation has demonstrated that *Quadriacanthus aegypticus* attained the highest infestation order, followed by *Q. clariadis* and *Q. kearni* at Telbanah Drain and Ammar Drain. On the other hand, regarding the River Nile locality, a minor variance was found between the mean prevalences of *Q. aegypticus* and *Q. clariadis*. The monogenean *Q. clariadis* recorded the highest mean intensity and mean abundance levels. The *Gyrodactylus* sp. disappeared from the catfish host at the River Nile and exhibited a light infestation at Telbanah Drain and Ammar Drain. Similar disappearance was noticed from *Paraquadriacanthus nasalis* from the catfish host at the River Nile and Ammar Drain, however this parasite showed irregular existence, with marked drop of the infection parameters.

The recorded data combined with field observations indicated significant variations in the transparency/turbidity, stream velocity, water depth, electrical conductivity, total dissolved solids, dissolved oxygen, bicarbonate alkalinity, sulphates, chlorides, nitrogen, phosphorous and the amount of water flowing across the three streams. Telbanah Drain accommodated the highest values of calcium, magnesium and nitrogen, Ammar Drain stored the greatest amounts of the pH, electrical conductivity, total dissolved solids, bicarbonate alkalinity, sulphates, chlorides, sodium, potassium and phosphorous, whereas the River Nile was more transparent, larger in size, slightly warmer and received the highest amounts of the dissolved oxygen. Except for zinc that recorded values under the permissible limits in all studied water bodies, undesirable limits were obtained for all the heavy metals exceeded the permissible limits set by the Egyptian Organization for Standardization and Quality [61], European Commission [62], World Health Organization [63] and Environmental Protection Agency [64].

The degree of water turbidity in the studied ecosystems was in the following order: Telbanah Drain > Ammar Drain > River Nile. Turbidity reduces the clarity of water, which becomes visually horrible, with low photosynthetic activity and high thermal gradient. The cloudy, highly turbid water of the small-sized and

shallow drain of Telbanah can influence the aquatic life in many ways. The cloudiness of water indicates that more particles, both organic and inorganic, penetrate into the water column. In contrast, the clarity of water indicates that it is free or almost free of particles. It can be suggested that the successful survival of the oviparous and viviparous monogeneans in the highly turbid, small-sized and shallow water of Telbanah Drain may indicate an adaptation to environmental degradation.

According to [31], the diversity of parasitic species seems to decrease as watershed size becomes smaller and habitat quality decreases. However, overall abundance of parasites, mainly determined by fluctuations in the prevalence and intensity appears to increase under these circumstances. [65] hypothesized that reducing habitat accessibility may increase host population density, increasing the opportunities of transmission of parasite, showing direct life cycle, from one host individual (species) to another. The author added that decreasing habitat availability may increase the density of the host population, leading to the exposure of a greater number of fish individuals to secondary or intermediate host(s) as habitat necessities overlap. Many authors reported that large-sized streams show higher parasite diversity [66, 67, 31]. In this respect, increased growth and diversity of parasite populations may be correlated with the complexity of the habitat and connectivity of greater ecosystems [31].

Hydrogen ion concentration (pH) appears to have no effect on the infection variables of the monogeneans under investigation. It showed no marked variation among the three water courses, which showed weak alkaline values (from 7.57 in Ammar Drain, 7.55 in the River Nile to 7.23 in Telbanah Drain). Blood imaging in fish indicates that the hydrogen ion concentration attains an average level of 7.4 [12]. [68] suggested that the favourable pH values for fish farming lie between 6.7 and 9.5, whereas the ideal values for optimum growth ranges between 7.5 and 8.5. [69] assumed that hydrogen ion concentrations below 6.5 and above 9 likely represent a stressor to fish. Owing to these findings, it seems likely that the monogenean invasions on the catfish host in all investigated ecosystems could not be related to the hydrogen ion concentration of water.

Water dissolved oxygen is regarded as a vital element to the survival of aquatic fauna and flora. Oxygen is a key element in cellular respiration and a good indicator of the environmental health. Oxygen penetrates into water through diffusion from the atmosphere to water surface, aeration as water flows over rocks, waves and winds, and photosynthetic activity conducted by aquatic vegetation. At the River Nile, which showed the lowest levels of monogenean infestations, oxygen-rich water seems likely to support the general health conditions and immune mechanisms of the catfish host, leading to a reduction in the propagation of monogenean infection. [12] suggested that fish inhabiting degraded ecosystems become more vulnerable to monogenean invasion. These authors demonstrated that water temperature and dissolved oxygen are the key factors determining the growth of monogenean populations. They added that monogeneans residing in degraded ecosystems proliferate in response to high thermal regimes and low oxygen levels following organic loading and oxygen sag, a phenomenon related to the release of organic contaminants or nutrient components. Oxygen sag likely stimulates the proliferation of oxygen-dependent microorganisms.

Oxygen drop is a crucial pointer of water quality and the impacts of pollution. Declines of dissolved oxygen influence the aquatic life and specify the manifestation of eutrophication, a phenomenon in which there is an excessive growth of particular organisms, leading to low oxygen content. In the present study, Telbanah Drain showed the lowest amount of dissolved oxygen; however the River Nile recorded the highest value. Therefore, poor oxygenation of water may be regarded as a key factor determining the proliferation of monogenean populations. [11] used monogenean parasites of some fishes in the Lake of 16 Tishreen Dam to monitor the environmental degradation and found that the highest monogenic infestation rate was obtained in summer at high temperature, low dissolved oxygen, and higher biological oxygen demand. These authors also found that *Cichlidogyrus sclerosus*, a gill monogenean of *Tilapia zillii*, was the most successful biomonitor of the environmental degradation in the lake.

Telbanah Drain accommodated moderate amount of the total dissolved solids, Ammar Drain showed the highest level, while the River Nile recorded the lowest value. Similar distribution pattern was recorded for the electrical conductivity, sulphates and chlorides among the studied environments. Water sodium attained its maximum value at Ammar Drain, followed by Telbanah Drain and the River Nile. Telbanah Drain recorded greater amounts of both minerals, followed by Ammar Drain and the River Nile. The levels of potassium in Ammar Drain 9.25 ppm and Telbanah Drain (8.76 ppm) were nearly twice the level estimated for this essential mineral in the River Nile (4.96 ppm).

Poor quality of water in the aquatic ecosystems such as Telbanah Drain and Ammar Drain likely creates a stressful circumstance that weakens the immune system and alters the defense tactics of the aquatic organisms, leading to poor fish health. Therefore, stressed fish become susceptible to a variety of health problems such as helminth invasions. Owing to their direct life succession and better ability to adapt to the ambient environment, monogeneans are regarded as good biomonitor candidate in comparison to other microorganisms that show multiple and indirect life cycles. Fish in healthy environments, donated with



optimum resistance that helps facilitate the control of monogenean microfauna, areskilfulinstrugglingagainst parasites as well as other contaminants.

[70, 71] hypothesized that the interplay between the host parasite association and the ambient environment is complicated and hard to interpret because these relationships are shaped by a complementary set of abiotic and biotic determinants. [72] found that settings for persistence and proliferation may be promoting at one site, and become inhibiting at another site. Pollution may increase the infection level through its adverse influence on the host defense mechanisms, so increasing host susceptibility for infection [73, 74]. [75] studied the influences of different types of pollutants on the richness and pattern of parasite distribution in addition to the combined influence of pollutants and parasites on the host's health. [12] reported that as monogeneans anchor and reproduce on/in their favourable host(s), they may obtain more food items from heavily secreted mucus and loosened epithelial cells. They added that organic overload rich in nutrients and microorganisms enriched may act as a promoting factor for the growth of the parasite.

[12] stressed that gyrodactylid monogeneans are usually overlooked as bioindicators for conveying the impacts of pollution and the degradation of aquatic ecosystems. These authors highlighted the importance of this brand of monogeneans as an important tool for monitoring the status of aquatic environment and to telescope the performance of the biota under acute or chronic circumstances. In the present investigation, the viviparous monogeneans (*Macrogyrodactylus* and *Gyrodactylus* species) and the oviparous *Paraquadriacanthus nasalis* attained markedly lower numbers and percentages of infections than the oviparous monogeneans of the genus *Quadriacanthus*. Accordingly, *Quadriacanthus* species can be used as informative tools for monitoring the changes of the aquatic ecosystems in the Nile Delta.

[76] studied the effects of stream flow, hydrogen ion concentration and temperature on the infection variables of the monogenean *Pseudodactylogyryrus anguillae* and the crustacean copepod *Ergasilus celestis* from the gills of the wild eel, *Anguilla rostrata* cultured in 8 watersheds at Novascotia. They found that the localities characterized by lowest stream flow and highest pH (i.e. on the alkaline scale) showed the highest infestation levels of *P. anguillae* and *E. celestis* on the *A. rostrata*. However, watershed with low pH levels (i.e. on the acidic scale) and high stream flow showed comparatively lower monogenean infestation levels. [76] suggested that the velocity of the stream pose a negative influence on the transmission tactics planned by parent monogenean worms to spread their offspring on a large proportion of the host population.

In the present study, the lowest infestation levels were recorded on the catfish *C. gariepinus* inhabiting the considerably lotic fresh water stream (i.e. Damietta Branch of the River Nile). In contrast, comparatively higher infestation levels were found on the catfish host inhabiting more lentic Telbanah and Ammar Drains. In addition to the velocity of the water, the water depth and water volume may affect the host-parasite relationship. As the water volume and water depth increase, the parasites undergo a greater challenge in searching, localizing and finding the host. In contrast, shallow and decreased amount of water help facilitate the host-parasite contact and rapid arrival of the free swimming oncomiracidia to their microsettlement sites on the catfish host. [77] suggested that dactylogyrid monogeneans are rheophilic. [78] estimated that individuals of the cyprinid host inhabiting sluggish (lotic) and hypoxic (oxygen-poor) habitats were more infested than their conspecifics in the fast-flowing (lotic) and well oxygenated (oxygen-rich) aquatic habitats. According to [78], the fast-flowing water may limit invasion of the cyprinid fish host by *Afrodiplozoon polycotyleus*.

[79] showed that *Neodiplozoon polycotyleus* was highly abundant throughout the dry season, which is characterized by low current speed and little amount of dissolved oxygen in water. [79] recorded high prevalence level (47.2%) for *N. polycotyleus* from the gills of *Barbus neumayeri* inhabiting an irregular (periodic) stream with low oxygen content (2.5 mg/liter), indicating high tolerance to hypoxic conditions in this diplozoid gill monogenean. Similar tolerance to poor water oxygenation was recorded in the gill monogenean *Pseudodactylogyryrus anguillae* from the eel host dwelling lentic stream of the lake mouths than in faster flowing stream [76]. In the present investigation, high abundances obtained for the vast majority of the studied monogeneans on *C. gariepinus* at Telbanah Drain probably reflect a similar tolerance to the hypoxic conditions of this waterway. [80] studied the monogenean *Diplozoon paradoxum* on the roach, *Rutilus rutilus* and the bream, *Abramis brama* in two differing-water quality streams, one with fast-moving water and the other more lentic. The author estimated a prevalence level of 22.2 % in the lotic stream and 9.3 % in the more lentic stream and thereby suggested that diplozoids of the roach are rheophilic; a rheophile is an organisms which prefers to live in rapid water currents [81]. [82] found that the monogenean *Dactylogyryrus gracile* was more prevalent on hosts collected from narrow and shallow streams of the Renault Basin in France, which are characterized by benthic numerous refuge areas and high host densities. Contraction of the aquatic habitat, lower oxygen availability and higher host density in Telbanah Drain are probably the key factors determining the abundance level and transmission rate of the monogeneans within host population.

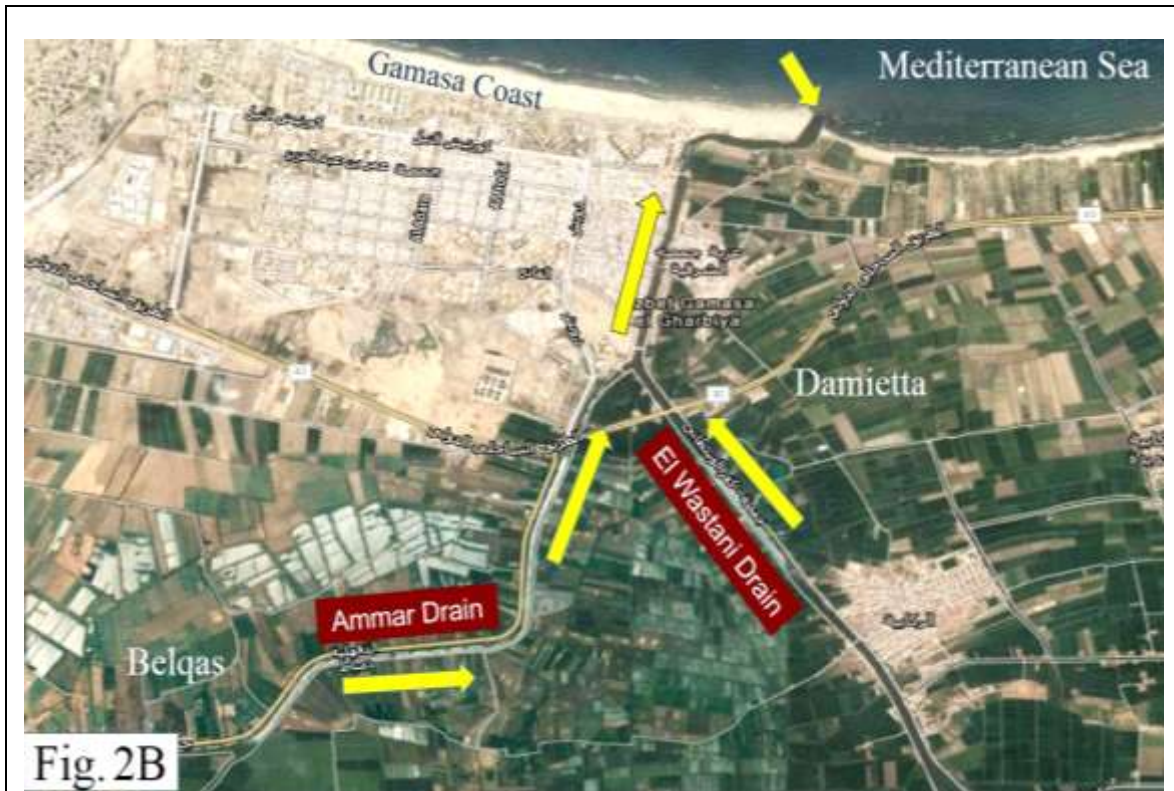
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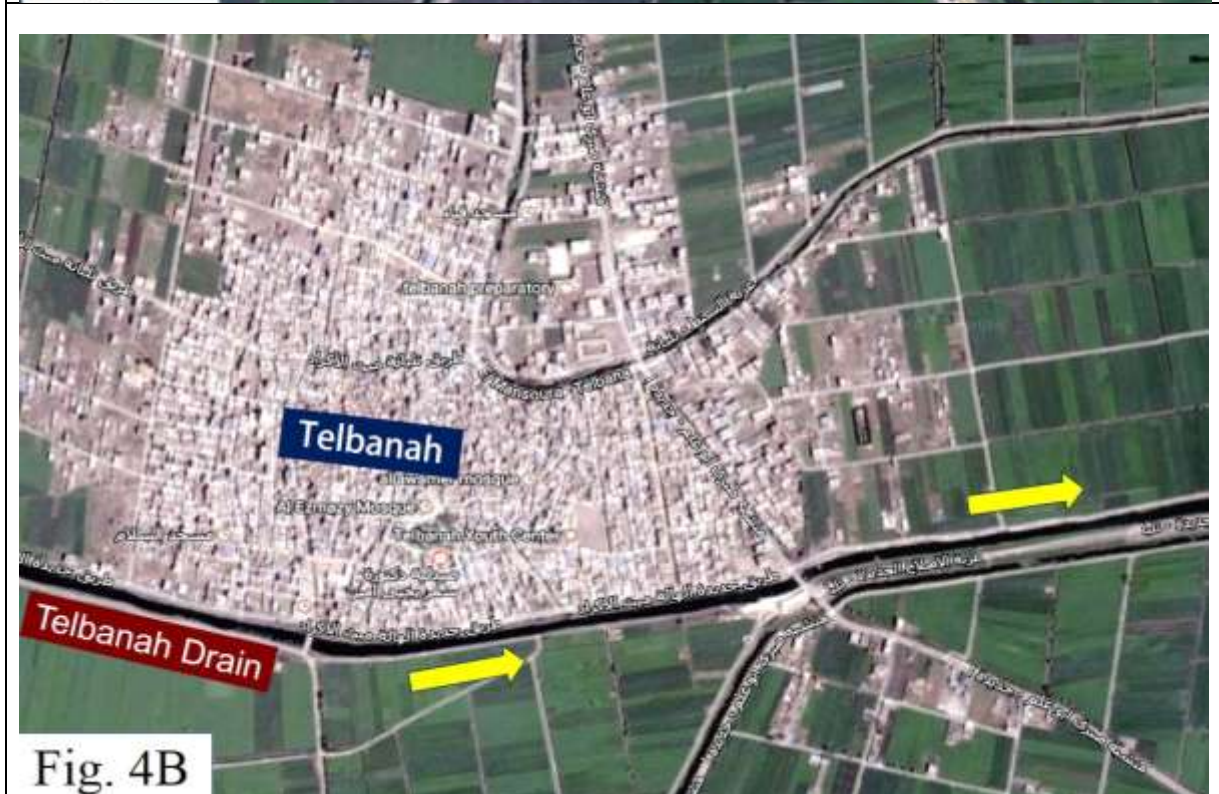
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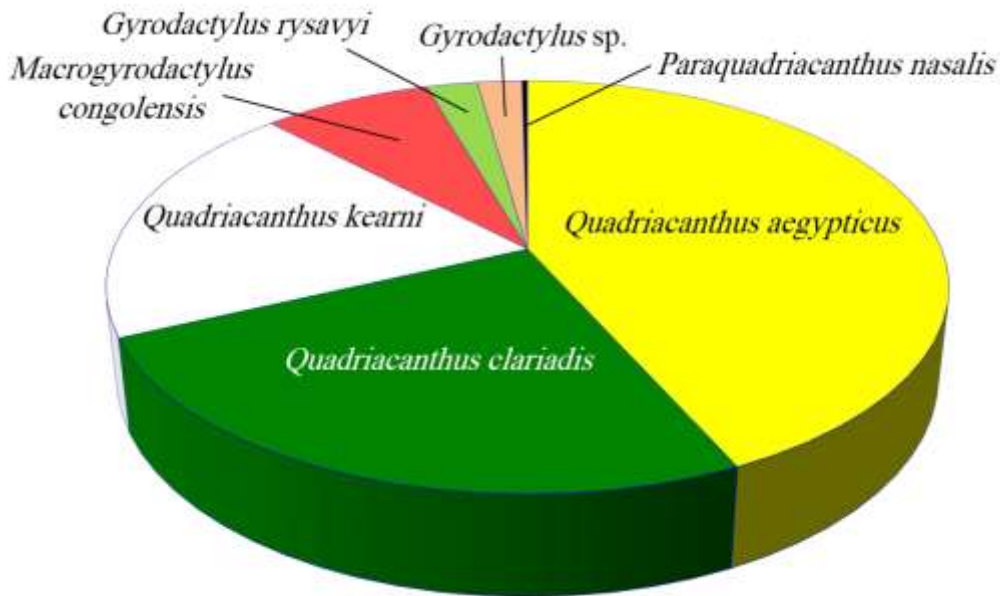
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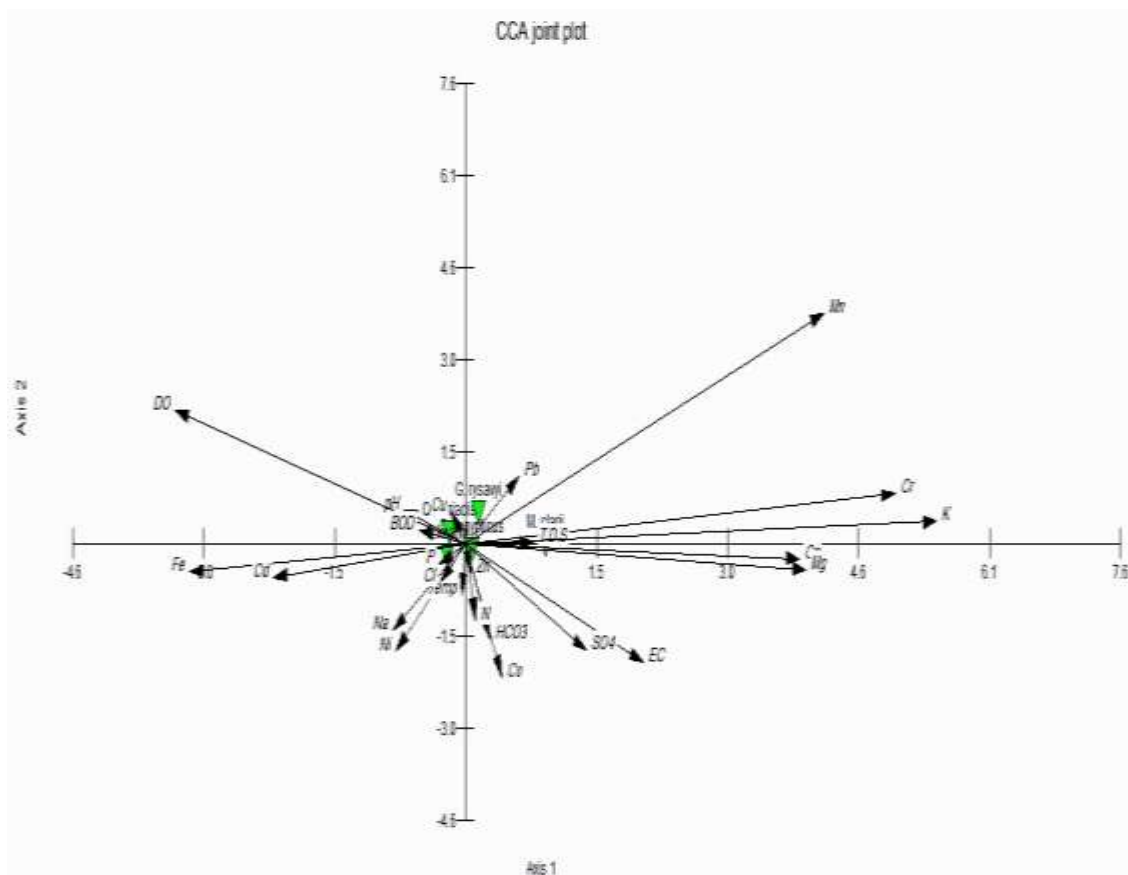






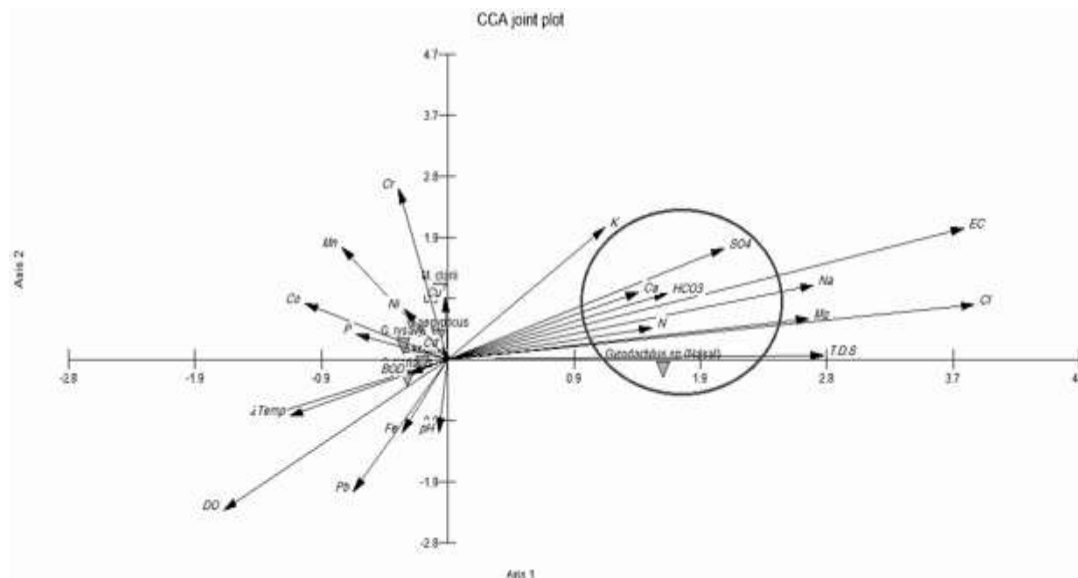


**Fig. 5:** Pie chart showing the sharing ratio of members of *Quadriacanthus*, *Macrogyrodactylus*, *Gyrodactylus* and *Paraquadriacanthus* in the monogenean assemblage of *Clarias gariepinus* (data pooled from the three ecosystems).

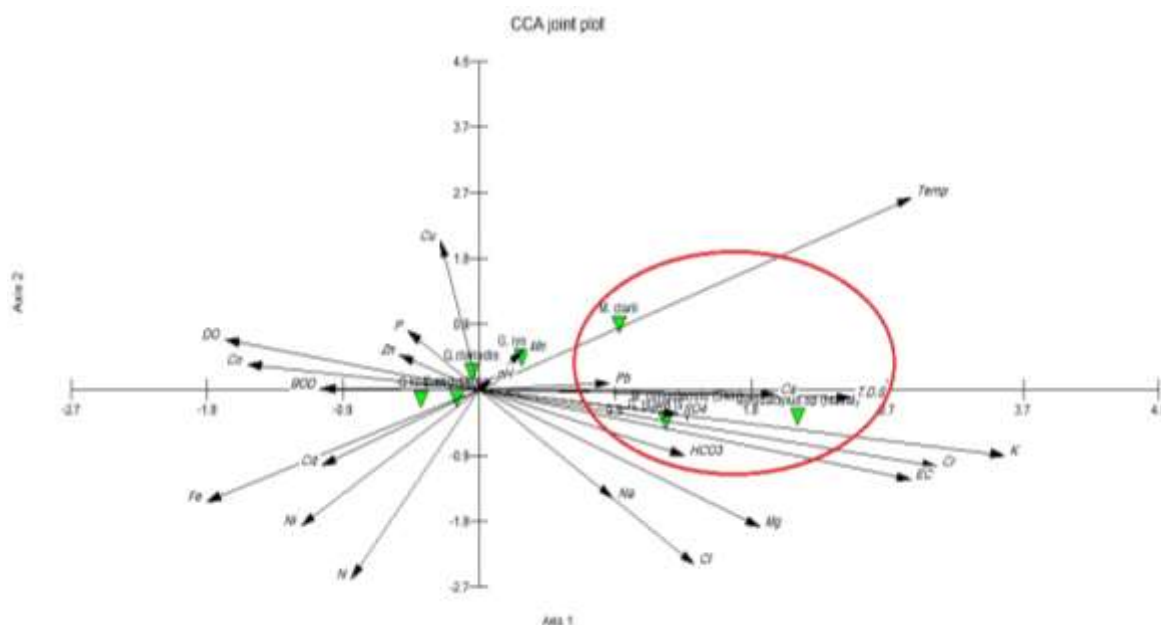


**Fig. 6:** Canonical Corresponding Analysis (CCA) ordination diagram of the prevalence values of the different parasites infested catfish according to the gradient of environmental variables (arrows) during the 12 months in the study sites.





**Fig. 7:** Canonical Corresponding Analysis (CCA) ordination diagram of the mean intensity values of the different parasites infested catfish according to the gradient of environmental variables (arrows) during the 12 months in the study sites.



**Fig. 8:** Canonical Corresponding Analysis (CCA) ordination diagram of the abundance values of the different parasites infested catfish according to the gradient of environmental variables (arrows) during the months in the study sites.

**Table 1:** The infection variables (prevalence, mean intensity and abundance) of the oviparous and viviparous monogeneans of *Clarias gariepinus* from the River Nile, Telbanah Drain and Ammar Drain. Values are given as mean  $\pm$  standard deviation.

Month	River Nile			Telbanah Drain			Ammar Drain		
	P%	MI	A	P%	MI	A	P%	MI	A
<i>Quadriacanthus aegypticus</i>	19.33 $\pm 8.27$	1.30 $\pm 0.63$	0.48 $\pm 0.26$	79.35 $\pm 12.41$	7.52 $\pm 5.70$	6.88 $\pm 5.68$	55.43 $\pm 18.70$	2.68 $\pm 1.40$	2.02 $\pm 1.60$
<i>Quadriacanthus clariadis</i>	19.26 $\pm 10.56$	1.93 $\pm 2.68$	0.79 $\pm 1.16$	67.70 $\pm 19.71$	4.06 $\pm 2.98$	3.69 $\pm 2.93$	35.36 $\pm 20.04$	1.43 $\pm 0.95$	1.10 $\pm 0.94$
<i>Quadriacanthus kearni</i>	10.32 $\pm 6.89$	0.53 $\pm 0.46$	0.17 $\pm 0.13$	58.51 $\pm 23.03$	3.52 $\pm 3.50$	3.21 $\pm 3.42$	30.74 $\pm 18.04$	1.13 $\pm 0.84$	0.89 $\pm 0.83$
<i>Macrogyrodactylus clarii</i>	3.69 $\pm 5.32$	0.12 $\pm 0.16$	0.04 $\pm 0.05$	16.44 $\pm 11.73$	0.27 $\pm 0.28$	0.23 $\pm 0.20$	12.46 $\pm 7.68$	0.29 $\pm 0.15$	0.17 $\pm 0.10$

<i>Gyrodactylus rysavyi</i> (gills)	3.72 ±2.71	0.19 ±0.17	0.06 ±0.05	7.49 ±4.95	0.11 ±0.08	0.10 ±0.07	6.66 ±4.75	0.10 ±0.06	0.07 ±0.05
<i>Gyrodactylus</i> sp.	—	—	—	0.77 ±1.81	0.03 ±0.07	0.02 ±0.05	1.00 ±2.38	0.01 ±0.03	0.01 ±0.02
<i>Macrogyrodactylus congolensis</i>	—	—	0.15 ±0.22	—	—	0.64 ±0.95	—	—	0.86 ±1.07
<i>Paraquadracanthus nasalis</i>	—	—	—	0.59 ±0.22	1.50 ±0.08	±0.03	—	—	—

**Table 2:** Sharing Ratio, Dominance Index and Simpson Diversity of the oviparous and viviparous monogeneans of *Clarias gariepinus* from the River Nile, Telbanah Drain and Ammar Drain.

Monogenean species	Sharing Ratio	Dominance Index			Simpson Diversity		
		River Nile	Telbanah Drain	Ammar Drain	River Nile	Telbanah Drain	Ammar Drain
<i>Quadracanthus aegypticus</i>	43.08 %	0.281	0.4603	0.3891	0.002	0.212	0.151
<i>Quadracanthus clariadis</i>	25.16 %	0.462	0.2458	0.2083	0.016	0.060	0.043
<i>Quadracanthus kearni</i>	19.37 %	0.107	0.2099	0.1731	0.001	0.044	0.030
<i>Macrogyrodactylus clarii</i>	2.06 %	0.025	0.0171	0.0293	0.007	0.000	0.001
<i>Gyrodactylus rysavyi</i> (gills)	2.35 %	0.039	0.009	0.0147	0.082	0.000	0.000
<i>Gyrodactylus</i> sp.	0.20 %	0.000	0.0022	0.0018	0.000	0.000	0.000
<i>Macrogyrodactylus congolensis</i>	7.74 %	0.076	0.0471	0.1649	0.005	0.002	0.027
<i>Paraquadracanthus nasalis</i>	0.04 %	0.000	0.0006	0.000	0.000	0.000	0.000

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