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# Influence of Water Quality on the Seasonal Infestation of the Nile Tilapia with Gill Parasites in Different Localities Along Rosetta Branch, the River Nile

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## ABSTRACT

The present study was designed to reveal the relative impact of water quality changes induced by the climate's influence and some waste discharge on the existence and prevalence of some gill parasite species in the Nile tilapia inhabiting Rosetta Branch, the River Nile. Both fish (1600 Nile tilapia fish) and water samples were randomly collected seasonally from four sites (El-Kanater El-Khayria, El-Qatta, Tamalay, and Kafr El-Zayat) along the Rosetta Branch of the Nile, which receives some types of wastewater. Parasitological examination of fish revealed the presence of *Trichodina* sp., *Ambiphyra* sp., *Cichlidogyrus tilapiae*, the encysted metacercaria of *Clinostomum phalacrocoracis*, and the larval stage of the freshwater mussels, *Glochidia*. The highest parasite prevalence was recorded at Kafr El-Zayat station, followed by El-Qatta and Tamalay. On the other hand, the autumn season recorded the highest parasitic prevalence, followed by winter, summer, and spring. The histological examination of the gills of the infected fish revealed severe histological alterations and the presence of different histozoic parasites. The most significant water parameters influencing the parasitic prevalence were temperature, pH, DO, BOD, and ammonia. The outcomes reveal that water parameter, temperature, pH, DO, BOD, and TAN are important factors affecting the different parasite prevalence values of the infected tilapia fish.

## INTRODUCTION

Climate change can influence seasonal trends. Climatic variations represent a critical threat since they affect ecological interactions, including parasitism and climate changes such as the thermal performance curve, which may kill the parasite by rising temperature or, in some cases, increasing the intensity and pathologies of the parasite. In addition to the previously mentioned threats, climate-induced changes to the dissolved oxygen, $CO_2$, acidity, host, and parasite distributions **(Byers, 2021)** are of concern.

The Rosetta Branch is suffering from some waste types that have directly been poured into its mainstream, including industrial wastes at Kafr El-Zayat station, agricultural wastes at El-Kanater El-Khayria, and sewage wastes at El-Qatta and Tamalay stations **(Abd El-Rahman, 2013)**. Climate and wastewater have a significant effect on both the occurrence and distribution of parasites **(Valon *et al*., 2013)**.

Many studies have concerned the relation between parasitism, water quality, and fish susceptibility to diseases **(Tayel *et al.,* 2020)**. The tilapias, especially the Nile tilapia and *Oreochromis niloticus,* are Egypt's most common commercial fish species. Parasitic infestation is usually associated with a reduction in fish productivity and marketability. Moreover, in some cases, parasites may be zoonotic **(El-Shahawy *et al.,* 2017)**.

The parasites' two main categories are ectoparasites and endoparasites. Ectoparasites are parasites that infect the gills, skin, mouth, and fins. Ectoparasites may be opportunistic, ubiquitous, and obligate parasites **(Ojwala *et al*., 2018)**. Ectoparasites are usually identified by scratches, ulcerations, hemorrhagic spots on the skin, and injured fins and usually lead to high mortality in fish **(El-Seify *et al*., 2011)**. The prevalence of ectoparasites can be very high in the wild and cultured Nile tilapia populations in the future due to concerns about the high temperatures resulting from climate change **(Gehman *et al*., 2018)**.

External protozoa, monogenetic, and digenetic trematodes (encysted metacercariae, EMC) of freshwater fish could be considered the most prevalent disease-causing agents affecting fish skin and gills. It usually results in inflammation and distortion of the normal gill anatomy, subsequently impairing its main functions. In addition to their respiratory function, gills are a primary site of nitrogenous waste excretion and play an important role in ionic balance **(Noga, 2010; Marzouk *et al*., 2013)**.

Trichodinids are common parasitic infections in fish. Trichodinid parasites in fish are ecto-commensals, attach for a little while, and actively swim on the surface of the host's body to obtain food on particles in water. Generally, trichodinids have low intensity in healthy fish with no effect **(Anshary *et al*., 2022)**. *Ambiphrya ameiuri* is a solitary sessile ciliate with a large vase-shaped body. The body is divided externally by a permanent equatorial ciliary girdle into an oral region and a motionless, ribbon-like macronucleus. The micronucleus is rounded and situated adjacent to one end of the macronucleus. Moreover, food vacuoles are distributed in the oral cavity **(Tayel *et al.,* 2020)**.

Monogenetic trematodes could be considered one of the most prevalent parasitic agents affecting skin and gills, causing irritation and destruction of gills, leading to impairment of breathing as well as tremendous losses. *Dactylogyridae* is the most common gill parasite in freshwater fish, especially young fish. Young stages of the tilapia were more susceptible to monogeniasis than adult stages **(Saleh & El-Nobi, 2003)**.

The yellow grub disease is caused by the metacercariae of *C. phalacrocoracis* in both the cultured and wild Egyptian tilapias **(Hamouda & Younis, 2021)**. Heavy freshwater mussel larval stage (*glochidia*) loads can lead to fish mortality and cause clear harm to the fish host **(Taeubert & Geist, 2013)**.

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants **(Ahmed *et al.,* 2019)**. Therefore, the present study aimed at recording the parasitic prevalence in the gills of the tilapia inhabiting different localities along the Rosetta Branch and investigating the histopathological alterations induced in the gills of these fish in relation to climate change and water contamination.

## MATERIAL AND METHODS

### Fish and water sampling

Water samples and a total number of 1600 Nile tilapia (*O. niloticus*) fish were randomly collected from four locations; El-Kanater El-Khayria (I), El-Qatta (II), Tamalay (III) and Kafr El-Zayat (IV) (Table 1), along Rosetta Branch (Fig. 1), during the four seasons starting summer 2020 to spring 2021. Both fish and water samples were immediately transported to the Fish Diseases Laboratory and Water Analysis Laboratory, respectively, at the National Institute of Oceanography and Fisheries (NIOF), El-Kanater El-Khayria, Cairo, Egypt.

**Table 1.** Description of Rosetta Branch sampling sites

| Site | Latitude | Longitude | Distance from El-Kanater El-Khyria |
|---|---|---|---|
| **El-Kanater El-Khayria (before bifurcation)** | 30010/22// N | 31008/22// E | 0.0 Km |
| **El-Qatta (Rosetta Branch)** | 30013/26// N | 30058/24// E | 20.0 Km |
| **Tamalay (Rosetta Branch)** | 30030/30// N | 30050/02// E | 72.2 Km |
| **Kafr El-Zayat (Rosetta Branch)** | 30049/29// N | 30048/31// E | 126.0 Km |


**Fig. 1.** Description of Rosetta branch sampling sites

## Water analysis

Analysis of water samples was immediately conducted in the Water Analysis Laboratory at NIOF. The physicochemical properties of water were measured and detected according to the American Public Health Association standard methods for water and wastewater analysis **(APHA, 2012; Tayel *et al*., 2020)**. Water temperature was measured during the time of sampling using an ordinary centigrade thermometer (0 $^{o}$C). The hydrogen ion concentration (pH value) was determined by means of the Orion Research Ion Analyzer 399A pH meter. The modified Winkler's method was used to measure the dissolved oxygen (DO). Nunsen's bottle was used to fill special oxygen bottles to the brim with water for each depth. Fixation was done immediately on the spot by adding 1ml of $MnSO_4$ and then 1ml of KI solution while thoroughly shaking the bottles. Two milliliters of concentrated $H_2SO_4$ were added, stirred, and allowed to stand for five minutes in the lab. After titrating the generated yellow color against a sodium thiosulfate solution (0.0245 N) until a faint, pale yellow hue was obtained, 0.5ml of starch was added, and the titration process was continued until the blue color completely vanished. The following formula was used to determine the dissolved oxygen concentration: **DO mg /l = N x V x 8 x 1000 / ml of sample**

N: normality of sodium thiosulphate

V: volume of sodium thiosulphate

The biochemical oxygen demand (BOD) was determined using a five-day incubation method. Specifically designed bottles were filled with water from the chosen DO stations and incubated at 20°C for five days. Then, using the previously mentioned dissolved oxygen approach, fix and treat, the difference between the initial and final dissolved oxygen concentrations was used to compute BOD. Aerated samples with low dissolved oxygen content (very contaminated) were diluted with water (distilled water plus 1 milliliter of each phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solution for every liter of distilled water) to raise the original DO concentration **(APHA, 2012)**

Using the Phenate method, total ammonia nitrogen (TAN) was measured: 50ml of sample water plus 2ml of phenol solution, followed by 2ml of sodium nitroprosside solution plus 5ml of oxidizing solution (mixed with alkaline citrate solution and 25ml of sodium hypochlorite). Ammonia was determined by adding 1ml, and after 60 minutes, measure the color produced at 640nm. The calibration curve was prepared using a standard solution of ammonium chloride concentrations (0– 2mg), and the unknown sample was identified (µg/l) **(APHA, 2012)**.

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## Parasitological examination

In the Fish Diseases Laboratory of NIOF, clinical examination of the collected fish was conducted according to **Noga (2010)**. Then, an immediate parasitological examination was carried out. According to **Ali (1992)**, wet mount smears were made by scraping the gills on a glass slide, allowing them to air dry before fixing them with 100%

methanol and staining them with geimsa to investigate protozoal infection. Additionally, small portions of gill filaments were cut with a drop of physiological saline to make a wet mount preparation. The gills were then covered with a cover slide and inspected using a dissecting binuclear microscope to observe monogenetic trematodes **(Osman, 2005)**. After being gently compressed between two glass slides to excite the worms, the encysted metacercaria discovered in the branchial chamber were stained with carmine stain **(Pritchard & Kruse, 1982)**.

### Histopathological examination

Immediately after fish dissection, specimens from gills were carefully collected in 10% neutral buffered formalin. These formalin-fixed samples were then dehydrated in ascending grades of ethyl alcohol and cleared in xylene. The fixed, cleared tissues were embedded in paraffin wax and sectioned at 4- 6μm. Sections were stained with hematoxylin and eosin stain according to **Bernet *et al.* (1999)** and **Ahmed *et al.* (2022)**. The stained sections were microscopically examined for any histological alteration.

### Statistical analysis

Microsoft Excel software was used to code and save all the counts gathered from the fields along the Rosetta Branch. Using the correlation Pearson test, ANOVA test, and Tukey (HSD), data obtained were examined using XL State software.

## RESULTS

### Clinical and parasitological findings

The fish infested with gill parasites showed dark skin coloration, and in some cases, congestion of gills was recorded (Fig. 2). Parasitic examination of fish gills revealed the presence of protozoa (*Trichodina* sp. and *Ambiphyra* sp.) and metazoans, particularly the monogenetic trematode *Cichlidogyrus tilapiae*, the encysted metacercaria (EMC) of *Clinostomum phalacrocoracis*, and the larval stage of freshwater mussels, *Glochidia*. Wet-mount examination of fish gills revealed a heavy infestation with *Trichodina* sp., which is characterized by a saucer-like adhesive disc shape. The ray of its denticles was long, thin, slightly curved, and had a sharp pointed tip (Fig. 3). Moreover, a stained smear from the gills showed *Ambiphyra* sp. which is characterized by its vase shape with a ribbon-shaped macronucleus. Most of the time, there was a mixed infestation with *Trichodina* sp. As shown in Fig. (4), the monogenetic trematode *Cichlidogyrus tilapiae* was also detected in the wet mount smear as a flat, elongated worm (Fig. 5). The EMC of *Clinostomum phalacrocoracis* was detected in the branchial cavity of fish as yellow nodules embedded in the branchial chamber. The metacercaria was elongated and whitish yellow in color, as shown in Fig. (6). *Glochidia*, the larval stages of freshwater mussels, were seen encysted and attached to the fish gill filaments Fig. (7).

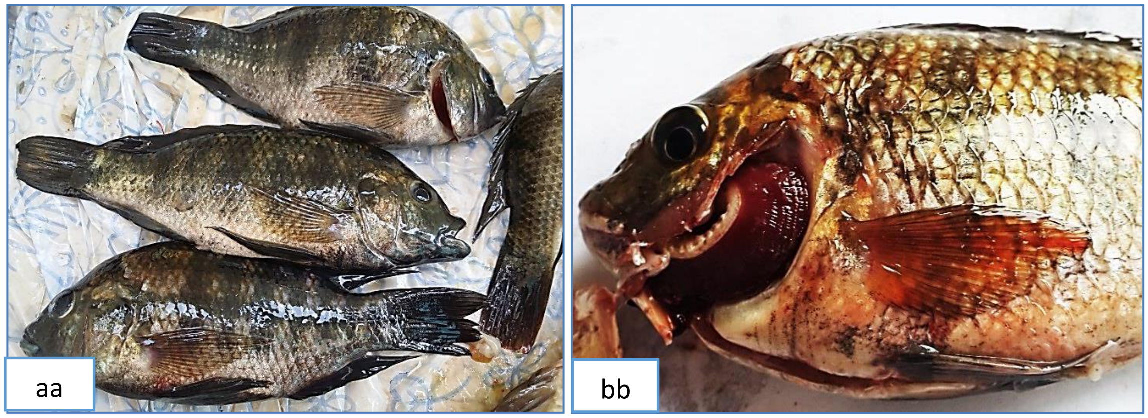


**Fig. 2.** *Oreochromis niloticus* infested with gill parasites showing: (a) Dark skin coloration, and (b) Congestion of gills

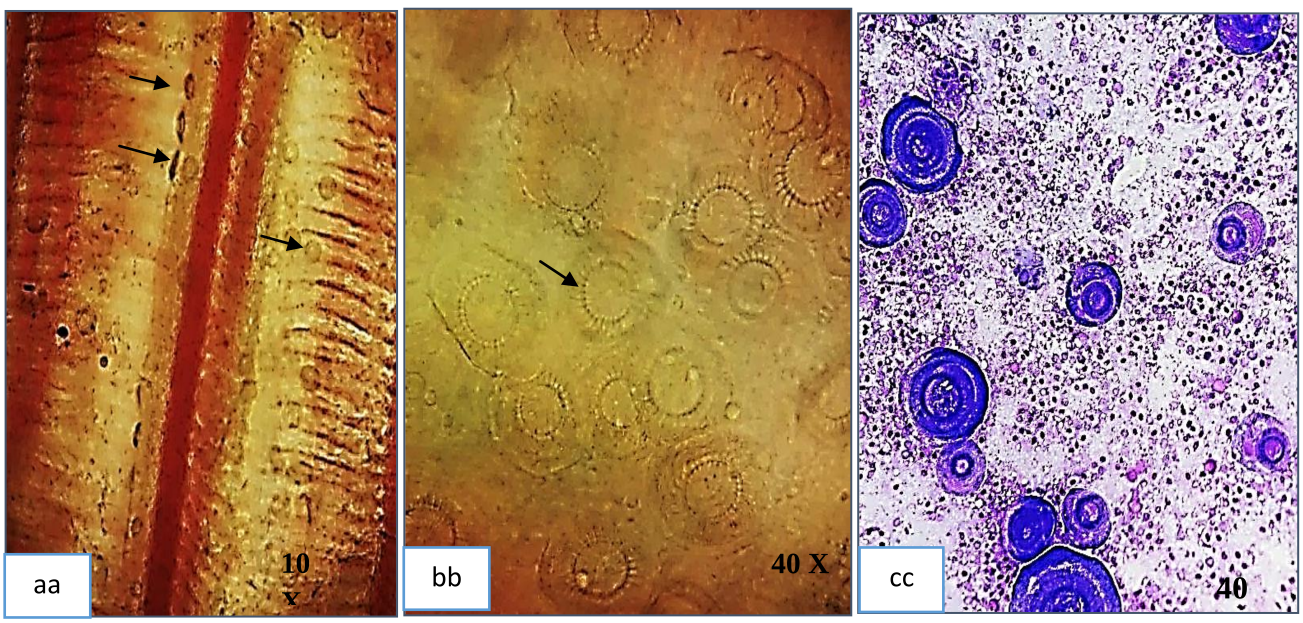


**Fig. 3.** Wet mount of gills displaying: (a & b) *Trichodina* sp. infestation. (c) *Trichodina* sp. from fish gills stained with Giemsa stain

**Regional and seasonal prevalence of gill parasites infestation among examined tilapia fish**

As presented in Table (2), the prevalence of gill parasitic infestation among *Oreochromis niloticus* inhabited the investigated areas showed the following order: site IV (73.5%) > site II (54% > site III (52.5%) > site I (27.5%). While, the seasonal prevalence was as follows: autumn (73.3%) > winter (56.7%) > summer (46.7%) > spring (30.7%) (Table 3).

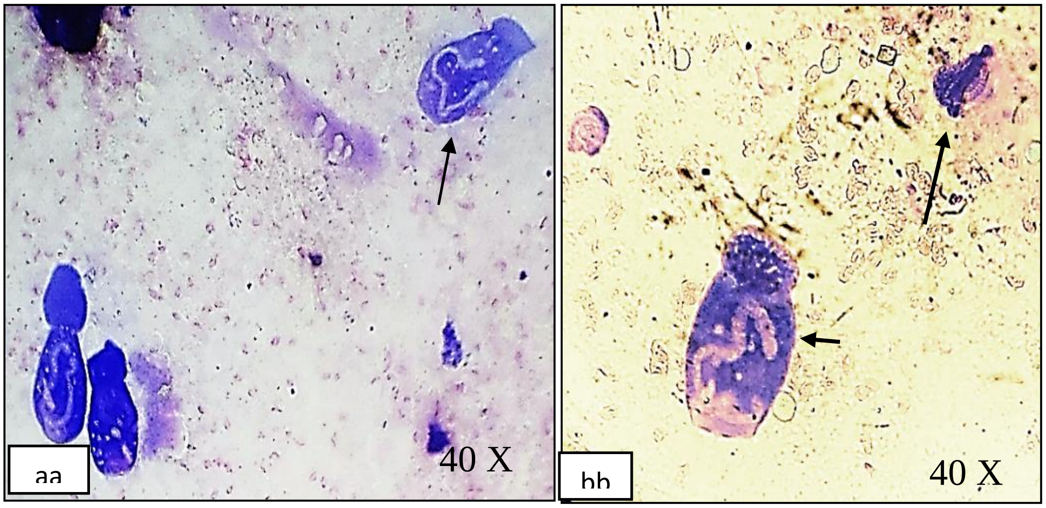


**Fig. 4.** *Ambiphyra* sp. from fish gills stained with Giemsa stain. *Ambiphyra* sp. *Trichodina* sp. in a mixed infestation and stained with Giemsa stain

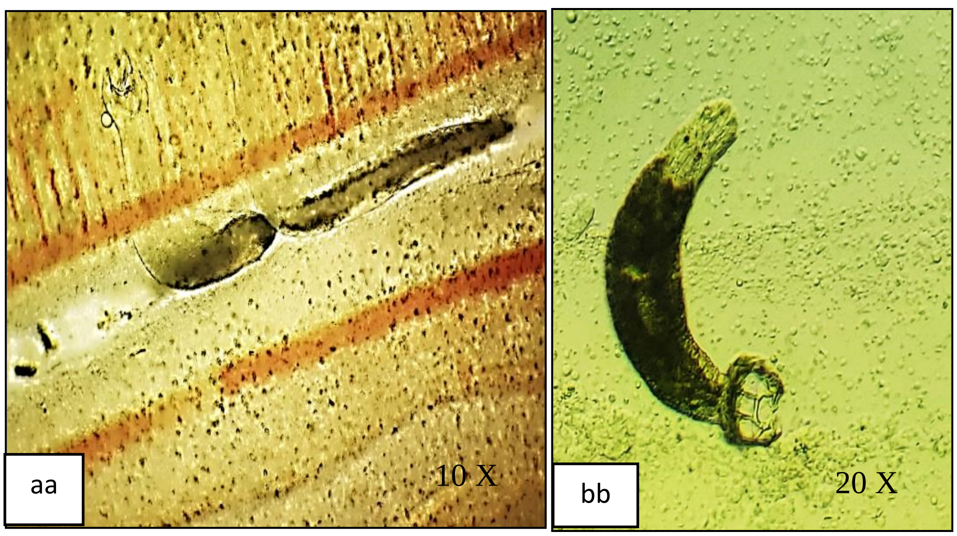


**Fig. 5.** Wet mount of gills showing: (a) *Cichlidogyrus tilapiae*; (b) *Cichlidogyrus tilapiae* from fish gills exhibiting two large hocks in its posterior end and four eye spots in the anterior part

**Table 2.** The regional prevalence of gill parasitic infestation among *Oreochromis niloticus* inhabiting the investigated areas

| Investigated area | **I** | **II** | **III** | **IV** | **Total** |
|---|---|---|---|---|---|
| No. of examined fish | 400 | 400 | 400 | 400 | 1600 |
| No. of infested fish gills | 110 | 216 | 210 | 294 | 830 |
| Percentage | (27.5%) | (54%) | (52.5%) | (73.5%) | (51.87%) |

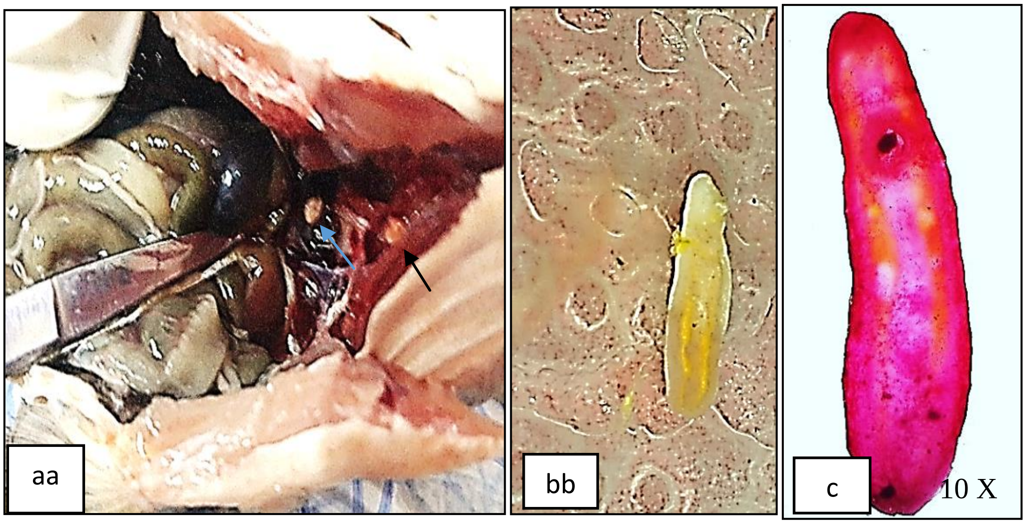


**Fig. 6.** (a) *Clinostomum phalacrocoracis* EMC (whitish yellow nodules) in the branchial cavity of *Oreochromis niloticus*. (b) *Clinostomum phalacrocoracis* metacercaria fresh mount and (c) stained with acetic acid carmine stain


**Fig. 7.** Wet mount of gills showed Glochidia, the larval stages of freshwater mussel

**Table 3.** Seasonal prevalence of gill parasitic infestation among *Oreochromis niloticus* inhabiting the investigated areas

| Season | Summer | Autumn | Winter | Spring |
|---|---|---|---|---|
| No. of examined fish | 400 | 400 | 400 | 400 |
| No. of infested fish | 187 | 293 | 227 | 223 |
| Percentage | (46.7%) | (73.3%) | (56.7%) | (30.7%) |

**The prevalence of detected gill parasites in different localities and seasons**

As shown in Table (4), the gill parasites that were mainly detected from site IV were *Trichodina* sp. (72% in autumn), the monogenetic trematode *Cichlidogyrus tilapiae* (46% in summer), and *Ambiphyra* sp. (27%in winter). Whereas, *Glochidia* (33%) was found at site II in summer and *Clinostomum phalacrocoracis* (33%) at site III in winter. On the other hand, *Trichodina* sp. was mostly detected in the cold months (autumn and winter). In addition, *Ambiphyra* sp. was noticed mainly during the cold season at site IV. While *Cichlidogyrus tilapiae* was highly recorded during the warm months (summer and spring), *Glochidia* was not detected during the winter and spring seasons or at site IV. However, the presence of *Clinostomum phalacrocoracis* was noticed at all sites and during all seasons, except for site IV, where it was observed during spring and summer.

**Table 4.** Prevalence of different gill parasites among *Oreochromis niloticus* inhabiting the investigated areas during different seasons

| Season | Station | *Trichodina* sp. | *Cichlidogyrus tilapiae* | *Ambiphyra* sp. | *Clinostomum phalacrocoracis* | Glochidia |
|---|---|---|---|---|---|---|
| Summer | I | 0 | 12 | 0 | 9 | 0 |
| | II | 0 | 25 | 0 | 23 | 33 |
| | III | 0 | 0 | 0 | 19 | 20 |
| | IV | 0 | 46 | 0 | 0 | 0 |
| Autumn | I | 0 | 22 | 0 | 11 | 20 |
| | II | 21 | 0 | 0 | 32 | 3 |
| | III | 38 | 0 | 0 | 32 | 0 |
| | IV | 72 | 0 | 14 | 28 | 0 |
| Winter | I | 0 | 0 | 0 | 23 | 0 |
| | II | 30 | 0 | 0 | 21 | 0 |
| | III | 35 | 0 | 0 | 33 | 0 |
| | IV | 48 | 0 | 27 | 10 | 0 |
| Spring | I | 0 | 10 | 0 | 3 | 0 |
| | II | 13 | 5 | 0 | 10 | 0 |
| | III | 13 | 0 | 0 | 20 | 0 |
| | IV | 26 | 23 | 0 | 0 | 0 |
| **Total %** | | 74% | 35.75% | 10.25% | 68.5% | 19% |

## Water quality

The seasonal variation of physicochemical parameters of water samples collected from the investigated areas is tabulated in Table (5). The maximal value (31.3°C) of water temperature was recorded at site IV during the summer season, while the minimal value (17.50°C) was recorded during winter at site I. The pH value declined from 8.30 during the summer at site I to 6.95 during the autumn at site II. The reduction of DO concentration (2.1mg/ l) was noticed at site II during summer, while an elevated level (8.2mg/ l) occurred at site I during winter. The maximal value (38.8mg/ l) of BOD was recorded at site II during winter, while the minimal value (3.50mg/ l) was recorded at site I during spring. The total ammonia concentration (TAN) ranged from 0.450mg/ l at site I during spring and summer to 12.50mg/ l at site II during winter.

**Table 5.** Seasonal variations of physicochemical parameters of water samples collected from the investigated areas

| Season | site | Temp. ($^0$C) | pH | DO mg/l | BOD mg/l | TAN mg/l |
|---|---|---|---|---|---|---|
| summer | I | 31.0 | 8.30 | 7.2 | 3.70 | 0.450 |
| | II | 31.1 | 7.30 | 2.1 | 35.9 | 10.90 |
| | III | 31.0 | 7.85 | 2.7 | 30.5 | 4.300 |
| | IV | 31.3 | 8.20 | 5.4 | 13.5 | 3.600 |
| Autumn | I | 21.6 | 7.80 | 7.8 | 3.80 | 0.580 |
| | II | 22.9 | 6.95 | 2.2 | 37.6 | 11.00 |
| | III | 22.9 | 7.50 | 3.2 | 33.8 | 5.900 |
| | IV | 22.6 | 7.60 | 5.5 | 21.0 | 4.300 |
| Winter | I | 17.5 | 8.25 | 8.2 | 3.70 | 0.600 |
| | II | 19.9 | 7.42 | 2.4 | 38.8 | 12.50 |
| | III | 20.0 | 7.47 | 3.5 | 35.9 | 6.200 |
| | IV | 19.5 | 7.90 | 5.6 | 22.1 | 5.100 |
| Spring | I | 24.5 | 8.10 | 7.9 | 3.50 | 0.450 |
| | II | 24.6 | 7.30 | 2.8 | 32.0 | 9.900 |
| | III | 25.0 | 7.70 | 3.5 | 30.0 | 5.700 |
| | IV | 26.0 | 7.80 | 5.8 | 14.1 | 3.900 |

## Histopathological findings

The histological examination of gills infested with *Trichodina* sp. showed curling (Cr) and degeneration (D) of secondary lamellae. Hyperplasia (Hp) in the epithelial cells of primary and secondary lamellae leads to fusion (Fu) and the presence of parasitic Trichodina. Hemorrhage, congestion (C) in the blood vessels of primary lamellae, and separation (S) in the epithelial cells of secondary lamellae are depicted in Fig. (8). The histological examination of gills infested with monogeneans (*Cichlidogyrus tilapiae*) revealed hyperplasia (Hp) in epithelial cells of primary and secondary lamellae, leading to fusion (Fu) with parasitic monogeneans (M) and congestion (C) in primary lamellae (Fig. 9). The histological examination of gills infested with metacercaria (*Clinostomum phalacrocoracis*) showed the presence of metacercaria in the cartilage of primary lamellae and severe degeneration in secondary lamellae (Fig. 10a). The histological examination of gills infested with *Glochidia* revealed the presence of parasitic bivalve stages in the cartilage of primary lamellae, degeneration (D) in the epithelial cells of secondary lamellae, and necrosis (N) (Fig. 10b).

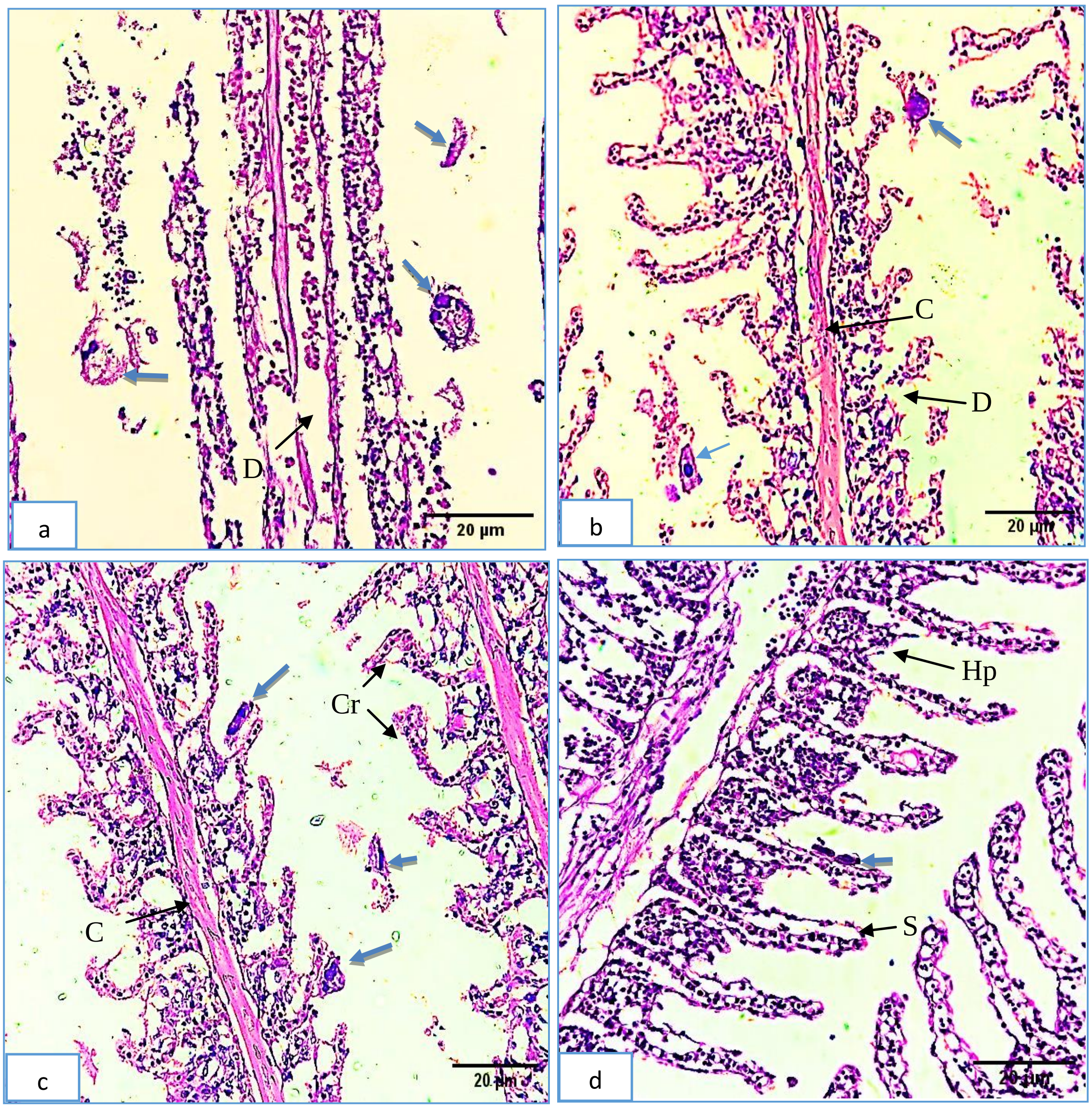


**Fig. 8.** Histopathological section of *O. niloticus* gills infested with *Trichodina* sp. (blue arrow) showed degeneration (D), congestion of blood vessel of the primary lamellae (C), curling of gill filament (Cr), hyperplasia in-between the secondary lamellae (Hp) and separation of epithelial lining of secondary lamellae (S)

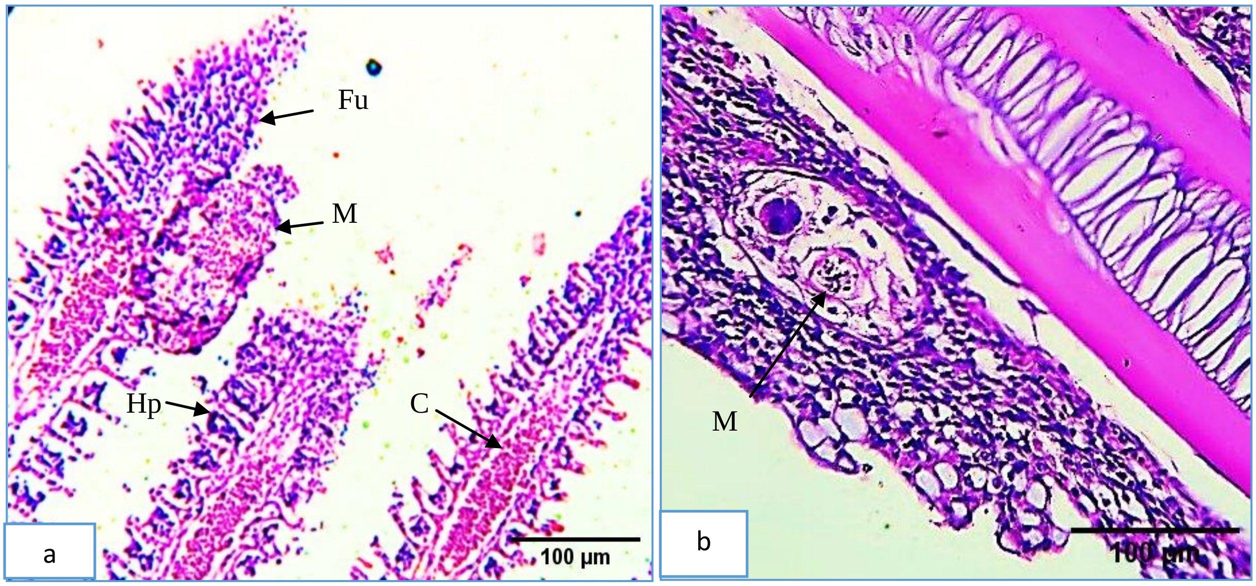


**Fig. 9.** Histopathological section of *O. niloticus* gills infested with Monogenetic trematodes (M), show Hyperplasia between secondary lamellae epithelium (Hp) and congestion in primary lamellae blood vessels (C).


**Fig. 10.** (a) Histopathological section of *O. niloticus* gills showing encysted metacercaria embedded in primary lamellae surrounded by fibrous tissue (arrow). (b) *Glochidia*, larval stage of freshwater mussels embedded in primary lamellae (arrow), the gill tissue revealed presence of degeneration (D) and necrosis (N) of secondary lamellae epithelium

**Statistical analysis**

A) ANOVA analysis of water parameter indicates that temperature, pH, DO, BOD and TAN were significantly different ($P< 0.05$) among the four seasons and the four locations of field study (Tables 6, 7).

B) *Trichodina* and *Clinostomum* metacercaria were significantly different ($P< 0.05$) among the four season and the four locations of field study, while Monogenean, *Ambphyra* and *Glochidia* (mussel) were not significantly different ($P> 0.05$) among seasons and locations of study (Tables 8, 9).

C) Tukey test indicates that *Trichodina* was significantly different in autumn from summer, while was significantly different ($P< 0.05$) in Kafer El- Zaiat from El- Kanater. *Clinostomum* metacercaria was significantly different ($P< 0.05$) in autumn from spring, while it was significantly different in Tamalay from Kafr El- Zaiat and El- Kanater. The other biological results are non significant ($P> 0.05$).

**Table 6.** Correlation of water parameter during different seasons

| | Temp.($^0$C) | pH | DO mg/l | BOD mg/l | TAN mg/l |
|---|---|---|---|---|---|
| Winter | 19.225 d | 7.760 a | 4.925 ab | 25.125 a | 6.100 a |
| Spring | 25.025 b | 7.725 a | 5.000 a | 19.900 c | 4.988 b |
| summer | 31.100 a | 7.913 a | 4.350 c | 20.900 bc | 4.813 b |
| Autumn | 22.500 c | 7.463 b | 4.675 b | 24.050 ab | 5.445 ab |
| Pr > F(Model) | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Significant | Yes | Yes | Yes | Yes | Yes |

**Table 7.** Correlation of water parameter between different sites

| | Temp.($^0$C) | pH | DO mg/l | BOD mg/l | TAN mg/l |
|---|---|---|---|---|---|
| IV | 24.850 a | 7.875 b | 5.575 b | 17.675 c | 4.225 c |
| III | 24.725 a | 7.630 c | 3.225 c | 32.550 b | 5.525 b |
| II | 24.625 a | 7.243 d | 2.375 d | 36.075 a | 11.075 a |
| I | 23.650 b | 8.113 a | 7.775 a | 3.675 d | 0.520 d |
| Pr > F(Model) | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Significant | Yes | Yes | Yes | Yes | Yes |

**Table 8.** Correlation of parasite during different seasons

| | Trichodina | Monogenea | Ambphyra | Clinostomum | Gloshidia |
|---|---|---|---|---|---|
| Autumn | 29.833 a | 4.125 a | 3.111 a | 26.611 a | 4.736 a |
| Summer | 0.000 b | 20.750 a | 0.000 a | 12.750 b | 13.250 a |
| Winter | 31.611 a | 0.458 a | 8.481 a | 18.981 ab | -0.227 a |
| Spring | 13.000 ab | 9.500 a | 0.000 a | 8.250 b | 0.000 a |
| Pr > F(Model) | 0.018 | 0.199 | 0.167 | 0.004 | 0.484 |
| Significant | Yes | No | No | Yes | No |

**Table 9.** Correlation of parasite between different sites

| | Trichodina | Monogenea | Ambphyra | Clinostomum | Gloshidia |
|---|---|---|---|---|---|
| IV | 36.500 a | 17.250 a | 10.250 a | 9.500 b | 0.000 a |
| II | 16.000 ab | 7.500 a | 0.000 a | 21.500 a | 9.000 a |
| III | 21.500 ab | 0.000 a | 0.000 a | 26.000 a | 5.000 a |
| I | 0.444 b | 10.083 a | 1.343 a | 9.593 b | 3.759 a |
| Pr > F(Model) | 0.018 | 0.199 | 0.167 | 0.004 | 0.484 |
| Significant | Yes | No | No | Yes | No |

## DISCUSSION

Fish parasitic infestations are fascinating and gaining an increasing interest as bioindicators of environmental pollution such as sewage and industrial pollution **(Ali *et al*., 2015)**. The present study deals with the most prevalent parasitic diseases infecting fish gills among the naturally infested Nile tilapia in relation to the seasonal prevalence and water parameters from different stations at the Rosetta Branch of the Nile River, which receive different types of pollution, such as industrial pollution at Kafer El- Zaiat and sewage pollution at El- Qata and Tamalay, while El- Kanater receives agricultural waste, resulting in a nearly similar picture to the clinical and postmortem picture reported in the studies of **Eissa *et al.* (2011)** and **Tayel *et al.* (2020)**. The parasitic examination in the current work revealed the existence of various gill parasites, including *Trichodina* sp., *Ambiphyra* sp., *Cichlidogyrus tilapiae*, the encysted metacercaria of *Clinostomum phalacrocoracis*, and the larval stage of freshwater mussels, *Glochidia*. The presence of some of these parasites is well correlated with water quality. The highest prevalence of the detected parasites from different sites was as follows: site IV, which was subjected to industrial waste, revealed the presence of *Trichodina* sp. (72%), *Cichlidogyrus tilapiae* (46%) and *Ambiphyra* sp. (27%). These results are in line with those of **Reda (2011)** and **Tayel *et al.* (2020)**. In addition, *Ambiphyra* sp. was only present mixed with *Trichodina* sp. at site IV. According to **Sures *et al.* (2017)**, monogeneans are one of the most prevalent parasites in fish that reflect environmental pollution. *Clinostomum phalacrocorasis* isolated from gills was 33% at site III in winter. This dataset is nearly identical to that of **Eissa *et al.* (2011)**, **Tayel *et al.* (2020)** and **Hamouda and Younis (2021)**. According to previous reports, Clinostomum phalacrocorasis was found in the pharyngeal area and branchial chamber **(Shehata *et al*., 2018; Tayel *et al*., 2020)**.

*Glochidia*, the larval stage of freshwater mussels, was recorded in the Nile tilapia collected from the Rosetta Branch in Egypt for the first time, according to our knowledge. It was only recorded at sites II and III during the summer (33, 20%, respectively), and also at site I during the autumn (22%). *Glochidia* was affected by the attachment position and the abiotic conditions, especially water temperature, as reported by **Haag (2012)** and **Taeubert *et al.* (2014)**.

The total prevalence of gill parasite infestation was at its highest at site IV (73.5%), followed by site II (54%), then site III (52.5%), and the lowest was recorded at site I (27.5%). The high load of parasites may be attributed to industrial and sewage pollution, which may be accompanied by an increase in the prevalence of parasitic infestation. These results agree with those of **Eissa *et al.* (2011)**, who reported that the increase in parasitic infestation was accompanied by sewage and industrial pollution.

In the present study, the winter and autumn seasons showed a high peak of parasitic infestation, whereas the minimum values were mostly attained during spring. This may be traced back to the high winter water temperatures in Egypt that are relatively enough to support parasite reproduction. In addition, such temperatures are below the level at which antibodies are produced by the fish, as reported by **Abu El-Enien (2007)**. The disappearance of some gill parasites during some seasons in El-Qatta (site II), Tamalay (site III), and Kafr El-Zayat (site IV) sites may be due to the amounts of waste drainage, sewage water, and/or industrial effluents that may affect the environmental water conditions and become unfavorable for the life and reproduction of parasites. Furthermore, the water might be converted to a hypo-osmotic environment that could inhibit the tissue functions of the parasites, as reported by **Abdel-Aziz *et al.* (2015)**.

Histopathological alterations have been widely used as biomarkers in the evaluation of fish health exposed to biological infectious agents and non-biological factors such as parasite infestation and changes in water physicochemical parameters **(Soliman & Sayed, 2020**; **Vankara *et al.*, 2022)**. In the present study, several histopathological alterations, including parasitic stages, hyperplasia, fusion, curling, congestion, degeneration, necrosis, and hemorrhage, were all observed in the gills. These alterations occur mainly in fish collected from sites II and IV during the cold seasons (autumn and winter). This observation is parallel with the prevalence of parasitic infestation at these sites and during these seasons, the elevation of TAN and BOD concentrations, the depletion of dissolved oxygen concentrations, and the presence of *Trichodina* sp., *Ambiphyra* sp., Monogenea, *Clinostomum phalacrocorasis*, and *Glochidia*. This observation was also noticed in previous studies **(Forouhar *et al*., 2018;**

**Tayel *et al*., 2020; Suliman *et al*., 2021; Ahmed *et al*., 2022)**. Ectoparasites e.g. *Trichodina*, Monogenea, and *Ambiphyra* are attached to fish body parts that are directly exposed to the external environment, viz. gills and skin, by their specialized morphological structures. This attachment could result in the degeneration of the gill epithelium cells, leading to a loss in the respiratory surface area. Additionally, congestion, hemorrhage from the ruptured blood vessels and a reduction in the number of chloride cells, which play an essential role in the ionic equilibrium, could occur. Moreover, hyperplasia of the epithelial cells and fusion of secondary lamellae are patterns of defense mechanisms of the gills, reducing the surface area of effective respiration. This explanation agree with those of **Sufardin *et al*. (2021)** and **Workeale and Melkamu (2021)**.

The results of the water physicochemical parameters in the present study revealed that the investigated areas suffered from the influences of climate change and the various waste types discharged at these sites. Temperature is an important factor in the aquatic environment since it affects directly or indirectly not only the survival and distribution of aquatic organisms at any life stage but also their growth rate, development, activity, activation of reproduction processes and susceptibility to diseases (**Abdo, 2013)**. The changes in water temperature may depend on the climate and meteorological conditions, variations in air temperature, latent heat of evaporation, and the different times, sites, and seasons of sampling **(Gopko *et al*., 2020)**. The elevation of the water temperature recorded at site IV in the summer may be due to industrial waste discharged at this site. This observation corroborates with that obtained by **Tayel *et al*. (2020)** in the same investigated area. The reduction of pH value at site II during autumn may be related to the lower activities of phytoplankton; however, the increase of pH value at site I during summer may be due to the increase of phytoplankton activities, as recorded by **Saad *et al.* (2011)**. **Abdel-Aziz *et al*. (2015)** reported a positive relationship between the infestation with external parasites and the pH value. The depletion of dissolved oxygen at site II during the summer may be due to the aerobic decomposition of organic matter and domestic wastes that were discharged in addition to the action of high temperatures. While, the increase in dissolved oxygen at site I during winter may be due to the clarity and transparency of the water that might be positively reflected on the photosynthesis activity of phytoplankton. This finding agrees with that obtained by **Abd El-Rahman (2013)**. The increase in BOD values at Site II during winter indicated the presence of more organic matter, which might be discharged into this site. While, the decrease in BOD values at site I during spring may be due to a lower organic matter load, photosynthetic activity, and abundance of phytoplankton, as reported by **Abdel-Aziz *et al*. (2015)**. Increasing the total ammonia at Site II during the winter may be due to the presence of organic wastes and their decomposition, which elevated the total ammonia level. On the other hand, the decrease in the total ammonia concentrations at sites may be related to the good nitrification process occurring in the water column, as reported by

**Moustafa *et al.* (2010)**. The high concentration of total ammonia with a low oxygen level may adversely affect the host gill tissue, making the fish more susceptible to parasitic infestation, which coincides with the outcome of **Abdel-Aziz *et al.* (2015)**. ANOVA analysis of water parameters and parasite prevalence values indicates that temperature, pH, DO, BOD, and TAN are important water parameters affecting the different parasite prevalence values of the infected tilapia fish at the different study sites throughout all seasons. This result nearly matches those of **Eissa *et al.* (2011)** and **Abdel-Aziz *et al.* (2015)**.

## CONCLUSION

Changes in the water quality parameters induced by the influences of climate change and water pollution could affect the susceptibility of the Nile tilapia to gill parasites. Water parameter and parasite prevalence values indicate that temperature, pH, DO, BOD, and TAN are important water parameters affecting the different parasite prevalence values of the infected tilapia fish. Furthermore, the various histological alterations noticed in the infested gills might negatively impact the vital functions of fish gills.

**Declarations of competing interests**

The authors declare no competing interests or personal relationships that could influence the work presented in this paper.

**Conflict of interest**

I, as the corresponding author, declare, on behalf of all authors of the paper, that no financial conflict of interest exists concerning the work described.

**Ethical approval**

The specimen is not under the listed category of experimental animals which needs ethical approval.

**Data availability statement**

Derived data supporting the findings of this study are available from the corresponding author on request.

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