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## Abundance and annual distribution of freshwater snails and some trematode cercariae at Damietta Governorate, Egypt

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### Summary

In addition to being a good food source, freshwater snails are considered one of the positively and negatively influencing invertebrates on the environment. Several biotic and abiotic factors are thought to affect the distribution and the dynamics of these snails. Freshwater snails are the most dominant and conspicuous individuals of the freshwater biota in Damietta Governorate. In this study, freshwater snails were collected monthly from 5 geographically and environmentally different sites at Damietta Governorate at the north of Egypt during June 2017-May 2018. Seven snail species; *Lanistes carinatus*, *Lanistes varicus*, *Pila wernei*, *Segmentorbis angustus*, *Melanoides tuberculata*, *Biomphalaria alexandrina*, and *Gabbiella senoriansis*, were identified. *Lanistes carinatus* was the most abundant species (56.4%) in the collection sites. Besides, Al-Inaniyyah village had the highest snail species abundance and variety. The abundance of the most recorded species varied seasonally; the highest abundance was recorded during summer while the lowest was during winter. The abundance of some snail species correlated positively or negatively with some physico-chemical parameters; water temperature, dissolved oxygen, conductivity, total dissolved salts, and electrical conductivity. Two types of trematode cercariae; gymnocephalus cercariae and xiphidiocercariae, were harvested from *Lanistes carinatus*. The highest prevalence of both cercarial types was detected during summer. The correlation between the prevalence of both cercarial types and the physico-chemical parameters were determined. Xiphidiocercariae were the most predominant cercarial type in Damietta Governorate. Our findings suggest that the invasion of some freshwater snails is likely to be the cause of the low prevalence or complete absence of some medically important intermediate snail hosts. We recommend further characterization of the distribution of freshwater snails and the interrelationships among them in Egypt, and suggest a controlled use of selected snail species to combat their medically important analogues.

**Keywords:** Snails; cercariae; abundance; seasonal; physico-chemical; *Lanistes*

### Introduction

Freshwater snails have vital roles in life where they represent a food source, contribute in recycling nutrients, and serve as bioindicators of metals in the ecosystem (Elder & Collin, 1991).

Moreover, snails are considered one of the influencing invertebrates and they have economic importance (Sharma *et al.*, 2013). They help in maintaining good water quality for other benthic aquatic invertebrates by consuming harmful algal blooms, detritus, and organic matter (Ukam *et al.*, 2018). However, some snail species

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can cause economic losses, for example the invasive golden apple snails (*Pomacea canaliculata*) have caused a significant ruin to the newly planted rice fields (Joshi *et al.*, 2001; Greene, 2008). Besides, several species of the apple snails (*Pomacea canaliculata* and *Pila polita*) may also serve as intermediate hosts for larval nematodes, e.g. *Angiostrongylus cantonensis* that causes eosinophilic meningitis in human (Tesana *et al.*, 2008 & Chen *et al.*, 2011).

Most species of snails act as intermediate hosts, in which several developing larval stages such as sporocysts, rediae, and cercariae are formed (Elsheikha & Elshazly, 2008). These snails are infected by the penetrating miracidia or by ingesting the eggs (Bdir & Adwan, 2011). Larvae of trematodes (cercariae) emerge from the snail tissue and might find the suitable secondary intermediate host or definitive host (Bdir & Adwan, 2011; Chantima *et al.*, 2018). The genera of *Biomphalaria*, *Bulinus*, *Lymnaea*, *Melanoides*, *Pomacea*, and *Asolene* are important intermediate hosts for the trematode parasites *Schistosoma*, *Stomylotrema*, *Echinochasmus*, *Phaneropsolus*, and *Fasciola* (Besprozvannykh *et al.*, 2013; Dida *et al.*, 2014; Pinto *et al.*, 2015; Dellagnola *et al.*, 2019). Therefore, freshwater snails perform significant roles in the human and veterinary health (Okafor & Ngang, 2004).

Snail control methods are, thus, a requisite in order to reduce the parasites and the diseases transmitted by them (Madsen & Hung, 2014). Several biological control programs used the snail

*Melanoides tuberculatus* as a competitor of the snail intermediate hosts of *Schistosoma* (Pointier & Jourdane, 2000). Furthermore, the freshwater apple snail *Lanistes varicus* has been considered for many years as effective biological control against snail intermediate hosts of schistosomiasis (Pointier & Jourdane, 2000; Anto & Bimi, 2017).

Environmental factors are known to affect the distribution patterns, life cycles, and population dynamics of snails (Rollinson *et al.*, 2001). There are several biotic and abiotic factors that affect the distribution and the ecology of snails. The abiotic factors include physical factors such as water current, temperature, turbidity, transparency, and distribution of suspended solids and chemical factors such as ion concentration and dissolved gases in water. The biotic factors include availability of food, competition, and predator-prey interactions (Ofoezie, 1999). Moreover, the aquatic macrophytes (vegetation) have been shown to play vital roles in the distribution of snails in different parts of Africa (Ofoezie, 1999). One of the two River Nile branches in Egypt, Damietta Nile Branch, runs for about 242 km from Delta Barrage to the Mediterranean Sea. This branch serves as the major source of water for municipal, industrial, and agricultural uses and enriches many governorates such as El-Qalubia, El-Gharbyia, El-Dakahlyia, and Damietta (El-Amier *et al.*, 2015). Unfortunately, freshwater ecosystem of Damietta governorate receives polluted waters from different sources including industrial, agricultural and urban sewage (Badr



Fig. 1. Location map showing study area in Damietta Governorate. Five sites were selected for this study; Al-Inaniyyah village; Izbat Al-Basartah village; EL-Shenawy village; EL-Serw and Kafr EL-Manazilla village.

et al., 2013; El-Amier et al., 2015). Freshwater snails were the most dominant and evident individuals of the freshwater biota at different regions in Damietta Governorate; Bostan, Adlyia, Damietta, El-kashf, and El-Serw drainage stations (Mahmoud & Sayed 2018).

Surveying snails, especially the species involved as intermediate hosts of digenetic trematodes, are needed for disease control (Rozendaal, 1997). It is crucial to know the habitat, ecological factors, and distribution of the freshwater snails especially the medically and economically important species. Unfortunately, few studies addressed the ecology and population dynamics of the freshwater snails in Egypt (Hussein et al., 2011; Marie et al., 2015; El-deeb et al., 2017; Mahmoud & Sayed 2018). Because of this and the unique freshwater ecosystem of Damietta governorate, the present study aimed to (1) survey the freshwater snails in selected sites at Damietta governorate, (2) determine the abundance of these snails and the seasonal changes in their abundance, (3) investigate whether there is a correlation between the abundance of these snails and some physicochemical parameters, and (4) address the seasonal changes in the snails' cercarial contents and investigate whether there is a correlation between their prevalence and some physicochemical parameters.

## Materials and Methods

### Study area

The study area covered 5 sites at Damietta Governorate, Egypt (Fig. 1). These sites were Al-Inaniyyah village, Izbat Al-Basartah

village, EL-Shenawy village, EL-Serw, and Kafr EL-Manazlla village. Selection of these sites depended on the differences in water source and the human and domestic animal activities. The water sources were drainage canal at Al-Inaniyyah village, irrigation canal at Izbat Al-Basartah, EL-Shenawy village, and EL-Serw, and the Nile's Damietta Branch at Kafr EL-Manazlla village. Al-Inaniyyah village is located at the northern-east of Damietta Governorate; at Manzala Lake borders between 31.39°N and 31.81°E. Izbat Al-Basartah is located at the northern-east of Damietta Governorate between Al-Inaniyyah and Izbat Al-Nahdah villages; between 31.38°N and 31.79°E. EL-Shenawy village is located at the southern-east of Damietta Governorate at the east of Nile's Damietta Branch; between 31.44°N and 31.29°E. EL-Serw is located at the south of Damietta Governorate at the east of the Nile's Damietta Branch; between 31.20°N and 31.63°E. Kafr EL-Manazlla village is located at the west of Damietta Governorate between 31.35°N and 31.68°E.

### Snail sampling and cercariae harvesting

Snails were collected monthly from June 2017 to May 2018 by a fishing net or by hand picking from rocks and plants. The collected snails (Fig. 2) were brought to the laboratory in labeled plastic bottles for identification and nomenclature. Identification of snails depended on the shape and size of shell, aperture shape, and number of whorls (Brown, 1994; Lotfy & Lotfy, 2015). After separation of specimens into species, they were then counted, and their abundance was determined.

Cercariae were harvested by exposing the snails to a strong

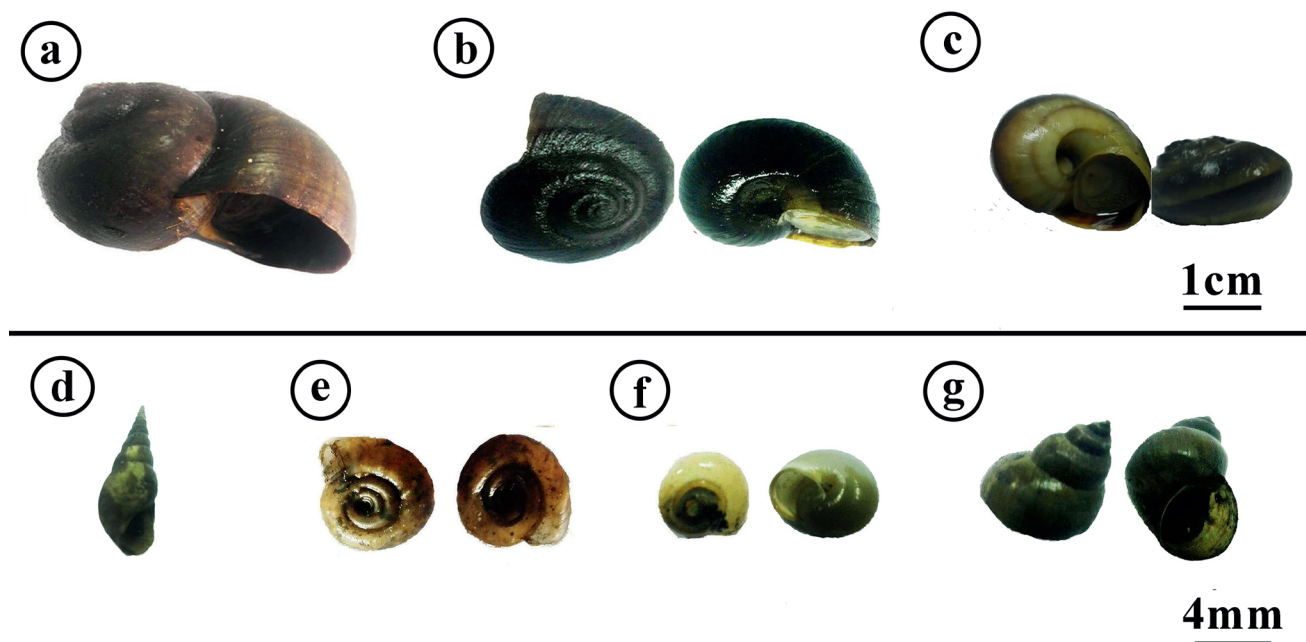


Fig. 2. Snail species collected from different sites in Damietta Governorate. a: *Pila wernei*; b: *Lanistes carinatus*; c: *Lanistes varicus*; d: *Gabbiella senoriansis*; e: *Biomphalaria alexandrina*; f: *Segmentorbis angustus*, and g: *Melanoides tuberculata*. (Scale bar: 1cm and 4mm)

artificial illumination for a period of 4 – 6 hours. The collected cercariae were transferred to Petri dishes for identification and their prevalence was determined. According to Luhe (1909), morphological identification of cercariae is based on the number and position of body suckers, the shape and relative dimensions of the cercarial tail, and the presence or absence of various specialized tegumental structures like stylet, spiny collar, and sensory papillae types. The harvested cercariae were picked up and transferred to 1.5 ml tube containing 70 % ethanol for fixation for 2 hours. The fixed cercariae were stained using Mayer's Paracarmine.

#### Collection of water samples and physicochemical analysis

Samples of freshwater were collected monthly from the five sites under study using tightly-stoppered bottles. Water temperature (T) was recorded with a Celsius thermometer, and the total dissolved salts (TDS) and water conductivity (EC) were measured monthly using YSI Model 33 S-C-T Meter (Yellow springs). pH of water samples was measured monthly electrometrically in the same sampling day using an Orion electrode standardized with pH buffer kit. Dissolved oxygen (DO) was measured monthly according to Winkler's method.

#### Statistical analysis

Snails diversity was assessed by calculating some general diversity indices, like Shannon diversity index (H), evenness index (E), and richness index (d) following Hossain *et al.* (2017). The significance of seasonal variations and the fluctuations of snail abundance were determined by ANOVA test using SPSS package (version 25). The significance difference was set at  $p \leq 0.05$ . Pearson's correlation coefficient was used to assess the correlation between both of the snail abundance and cercariae prevalence and the physicochemical parameters. Canonical Correspondence Analysis (CCA) was performed by CANOCO (version 4.5) to assess the association of snail species with the environmental factors. Monte Carlo test was used to evaluate the significance of the environmental variables through 499 permutations.

## Results

#### Abundance of snails and the harvested cercariae

A total of 1524 snails (Table 1) were collected from the study area and they were classified according to Brown (1994) into seven species (Fig. 2). These species are *Lanistes carinatus* (Olivier, 1804), *Lanistes varicus* (Müller, 1774), *Pila wernei* (Philippi, 1851), *Segmentorbis angustus* (Jickeli, 1874), *Melanoides tuberculata* (Müller, 1774), *Biomphalaria alexandrina* (Ehrenberg, 1831), and *Gabbiella senoriansis* (Küster, 1852). The freshwater snails *Lanistes carinatus* and *L. varicus* existed in all the 5 sites of study. But, the snail species *Segmentorbis angustus* existed in 4 sites and was absent from Kafr EL-Manazlla village. The snails *Pila wernei*, *Melanoides tuberculata*, and *Gabbiella senoriansis* were

collected only from Al-Inaniyyah village and Izbat Al-Basartah village. The snail *Biomphalaria alexandrina* was collected from Al-Inaniyyah village only. *Lanistes carinatus* was the highest snail in occurrence (56.4 %). The Shannon diversity index for snails was high at Al-Inaniyyah village and low at Kafr EL-Manazlla village, while the evenness index was high at EL-Serw and low at Izbat Al-Basartah village (Table 2). One-way ANOVA revealed no significant differences in the values of Shannon and evenness indices between different sites ( $P=0.086$  and  $P = 0.64$ , respectively). The highest value of richness was detected at Al-Inaniyyah village, while the lowest value was recorded at Kafr EL-Manazlla village (Table 2). One-way ANOVA revealed a significant difference in the value of richness between different sites ( $F = 7.46$  and  $P = 0.002$ ). Because of the high abundance of *Lanistes carinatus*, this snail was selected for cercariae harvesting. Cercariae harvested from this snail were identified into two types; gymnocephalus cercariae and xiphidiocercariae based on light microscopy of stained cercariae (Fig. 3), and the identification was confirmed by other tools (not included here). The body of the harvested gymnocephalus cercaria is ovate in shape, 165  $\mu\text{m}$  long and 120  $\mu\text{m}$  wide. Tail is 170  $\mu\text{m}$  long, 30  $\mu\text{m}$  wide, without fin-folds and with some sensory papillae. The oral sucker is sub-terminal and 26  $\mu\text{m}$  in diameter. The ventral sucker is well developed and larger than the oral sucker. The digestive system and cystogenous gland were observed (Fig. 3). While, the body of harvested xiphidiocercaria is cylindrical in shape, 72  $\mu\text{m}$  long and 40  $\mu\text{m}$  wide. Tail is 75  $\mu\text{m}$  long, 13  $\mu\text{m}$  wide and without fin-folds. The oral sucker is sub-terminal and armed with stylet. The diameter of the ventral sucker is almost equal to that of the oral sucker. The prevalence of these two types of cercariae was determined (Table 1). The gymnocephalus cercariae were recorded only at Al-Inaniyyah and Kafr EL-Manazlla villages. While, the xiphidiocercariae existed in all the 5 study and their total prevalence (4.5 %) was higher than that of the gymnocephalus cercariae (2.3 %). The drainage canal of Al-Inaniyyah village had the highest prevalence of xiphidiocercariae (6 %), while the Nile's Damietta Branch at Kafr EL-Manazlla village had the highest prevalence of gymnocephalus cercariae (9.52 %).

#### Relationship between the seasonal changes and the abundance of snail species and cercarial types

The seasonal changes of snail abundance and cercaria prevalence are shown in Table 3. All the snails collected during the present investigation existed during spring. Most snails existed during summer except for the snail *Segmentorbis angustus*. Two snails, *Melanoides tuberculata* and *Biomphalaria alexandrina*, disappeared during winter and autumn. Statistical analysis revealed a significant difference between the different seasons in the abundance of the snails *Lanistes carinatus*, *Lanistes varicus*, *Pila wernei*, and *Segmentorbis angustus* ( $P<0.05$ ), while there was no significant variation between the seasons in the abundance of the snails *Biomphalaria alexandrina* and *Gabbiella senoriansis*.

Table 1. Abundance of the collected snail species and prevalence of the cercarial types recovered from five sites in Damietta Governorate.

Snail species	Al-Inaniyyah village		Izbat Al-Basartah village		EL-Shenawy village		EL-Serw		Kafr EL-Manazila village		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>Lanistes carinatus</i>	200	43.1	172	43.77	174	68.77	167	69.01	147	85.47	860	56.43
<i>Lanistes varicus</i>	117	25.22	79	20.10	44	17.4	41	16.94	25	14.53	306	20.08
<i>Pila werneri</i>	24	5.17	19	4.83	0	0	0	0	0	0	43	2.82
<i>Segmentorbis angustus</i>	50	10.78	82	20.87	35	13.83	34	14.05	0	0	201	13.2
<i>Melanoides tuberculata</i>	24	5.17	18	4.58	0	0	0	0	0	0	42	2.76
<i>Biomphalaria alexandrina</i>	24	5.17	0	0	0	0	0	0	0	0	24	1.57
<i>Gabbiella senoriansis</i>	25	5.39	23	5.85	0	0	0	0	0	0	48	3.14
<b>Total No. of snail species in each site</b>	<b>464</b>		<b>393</b>		<b>253</b>		<b>242</b>		<b>172</b>		<b>1524</b>	
<b>Cercariae type %</b>												
<b>Gymnocephalus cercariae</b>	<b>3</b>		<b>0</b>		<b>0</b>		<b>0</b>		<b>9.52</b>		<b>2.32</b>	
<b>Xiphidiocercariae</b>	<b>6</b>		<b>5.81</b>		<b>2.87</b>		<b>2.39</b>		<b>5.44</b>		<b>4.53</b>	

Values represent the number (No.) and abundance (%) in case of a snails and the prevalence (%) in case of cercariae in each sampling site. Prevalence refers to the number of infected of snail species divided by the total number of the collected snail species. Abundance refers to the number of a specific snail species divided by the total number of the collected snail

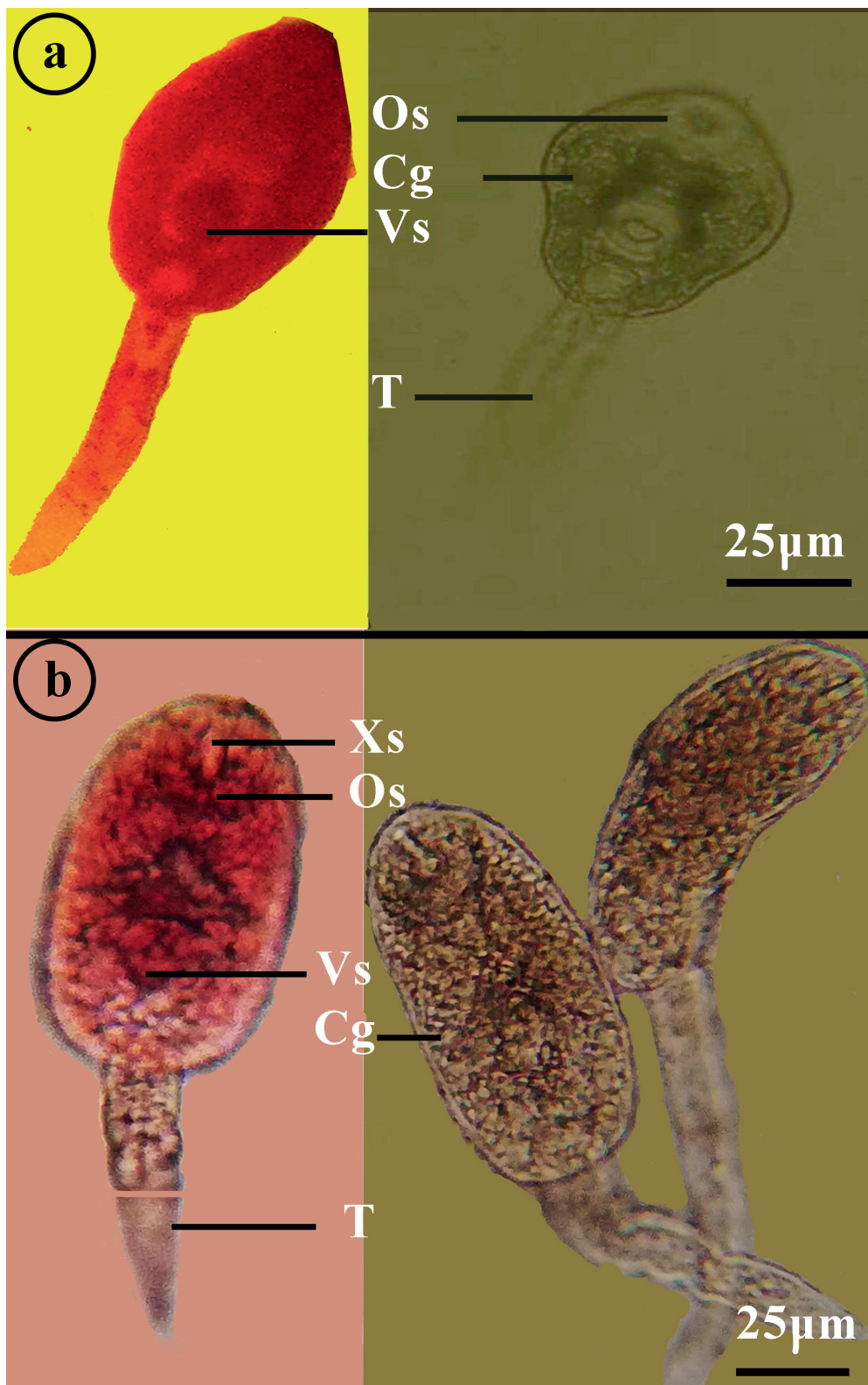


Fig. 3. Types of cercariae harvested from *Lanistes carinatus* collected from different sites in Damietta Governorate. a: Gymnocephalus cercariae; b: Xiphidiocercariae. Bo, Cercarial body; Cg, Cystogenous gland; Os, Oral sucker; T, Cercarial tail; Vs, Ventral sucker. (Scale bar: 25µm)

Table 2. Biodiversity indices of the freshwater snails collected from five sites in Damietta Governorate.

Snail species	Al-Inaniyyah village	Izbat Al-Basartah village	EL-Shenawy village	EL-Serw	Kafr EL-Manazlla village	P- value
Shannon – index (H)	1.44±0.49	1.21±0.27	0.77±0.16	1.04±0.71	0.62±0.13	0.086
Richness – index (d)	0.69±0.24	0.5±0.13	0.2±0.2	0.27±0.1	0.19	0.64
Evenness – index (E)	0.86±0.16	0.78±0.36	0.89±0.21	1.1±0.56	0.9±0.18	0.002

P values were considered significant if  $\leq 0.05$

The highest abundance of the snails *Lanistes carinatus*, *Lanistes varicus*, *Biomphalaria alexandrina*, and *Gabbiella senoriansis* was 7.28 %, 2.80 %, 0.25 %, and 0.60 %, respectively, during summer. While, the highest abundance of the snails *Pila wernei* and *Segmentorbis angustus* was 0.41 % and 1.94 %, respectively, during winter, and the highest abundance of the snail *Melanooides tuberculata* was 0.33 % during spring.

The gymnocephalus cercariae existed during spring, summer, and autumn. The highest prevalence of this cercarial type was recorded during spring (4.05 %), while the xiphidiocercariae existed during the four seasons. The highest prevalence of the xiphidiocercariae was recorded during summer (9.57 %). Statistical analysis revealed a significant difference between the different seasons in the prevalence of the xiphidiocercariae ( $p \leq 0.001$ ), while there was no significant variation between the different seasons in the prevalence of the gymnocephalus cercariae.

#### Fluctuation of water physicochemical parameters at the different snail collection sites

Table 4 summarizes the water physico-chemical parameters at the five sites under study. Generally, the water temperature and pH were almost similar among the five sites. Al-Inaniyyah village

had the highest water TDS and EC (823.58 ppm and 1380  $\mu\text{S/cm}$ , respectively). On the other hand, Kafr EL-Manazlla village had the lowest TDS and EC (385.5 ppm and 570  $\mu\text{S/cm}$ , respectively). Besides, this site had the highest DO (7.02 mg/L).

#### The relationship between water physicochemical parameters and snail abundance and cercariae prevalence

The relationship between the water physico-chemical parameters and the abundance of different snail species was analyzed by Pearson correlation test (table 5). The abundance of the snails *Lanistes carinatus*, *Lanistes varicus*, and *Gabbiella senoriansis* was positively correlated with water temperature with a high significance ( $p=0.00$ , 0.00 and 0.003, respectively). The abundance of the snails *Melanooides tuberculata* and *Biomphalaria alexandrina* was positively correlated with water temperature ( $p=0.346$ , and 0.227, respectively). However, the abundance of the snails *Pila wernei* and *Segmentorbis angustus* was negatively correlated with water temperature with a high significance ( $p \leq 0.001$ ). The prevalence of gymnocephalus cercariae and xiphidiocercariae was positively correlated with water temperature with a high significance for xiphidiocercariae ( $p \leq 0.001$ ).

The abundance of the snail *Segmentorbis angustus* was positively

Table 3. Seasonal changes in the abundance of snail species and the prevalence of cercaria types in Damietta Governorate.

Snail species	Seasons				P- value
	Winter	Spring	Summer	Autumn	
	%±SD	%±SD	%±SD	%±SD	
<i>Lanistes carinatus</i>	2.85±2.84	4.90±2.23	7.28±1.43	5.20±1.81	0.000
<i>Lanistes varicus</i>	0.26±0.33	1.56±0.71	2.80±0.65	1.55±0.93	0.000
<i>Pila wernei</i>	0.41±0.57	0.04±0.16	0.01±0.05	0.18±0.3	0.007
<i>Segmentorbis angustus</i>	1.54±1.93	1.02±2.02	0	0	0.006
<i>Melanooides tuberculata</i>	0	0.33±0.93	0.22±0.62	0	0.26
<i>Biomphalaria alexandrina</i>	0	0.11±0.44	0.25±0.62	0	0.3
<i>Gabbiella senoriansis</i>	0	0.21±0.46	0.60±0.78	0.04±0.16	0.003
Cercariae type	%±SD	%±SD	%±SD	%±SD	P-value
Gymnocephalus cercariae	0	4.05±7.13	3.76±9.24	0.55±2.15	0.14
Xiphidiocercariae	1.5±3.98	0.36±1.42	9.57±7.66	1.64±4.62	0.00

SD, standard error; P values were considered significant if  $\leq 0.05$

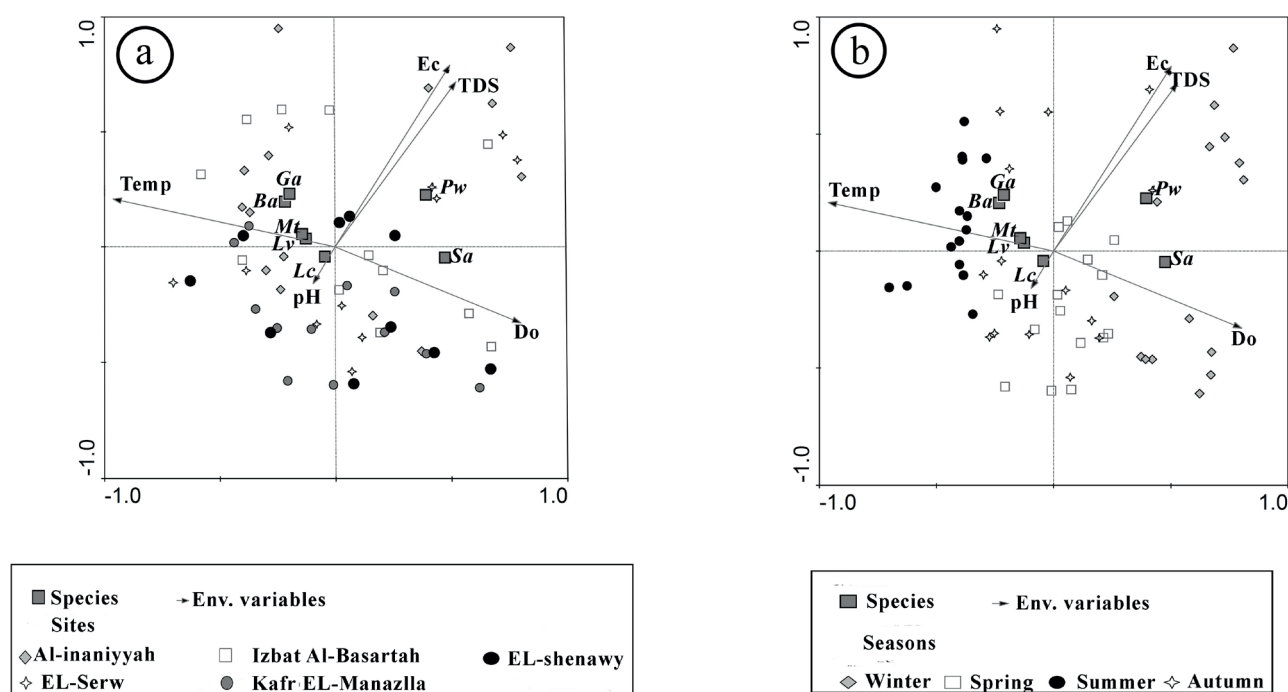


Fig. 4. Ordination diagram representing Canonical Correspondence Analysis of snail species and environmental variables in Damietta Governorate. a: Distribution of sampling sites and snail species along the gradients of environmental variables and b: Seasonal changes and snail species distributed along the gradients of environmental variables. (Ba: *Biomphalaria alexandrina*; Do: Dissolved oxygen; Ec: Electrical conductivity; Ga: *Gabiella senoriansis*; Lc: *Lanistes carinatus*; Lr: *Lanistes varicus*; Mt: *Melanoides tuberculata*; pH: Water pH; Pw: *Pila wernei*; Sa: *Segmentorbis angustus*; Temp: Water temperature and TDS: Total dissolved salts).

correlated with DO with a high significance ( $p=0.001$ ). The abundance of the snail *Pila wernei* was positively correlated with DO with a high significance ( $p=0.01$ ). The abundance of the snail *Lanistes carinatus*, *Lanistes varicus*, and *Gabiella senoriansis* was negatively correlated with DO with a high significance ( $p\leq 0.001$ ). Also, the abundance of the snails *Melanoides tuberculata* and *Biomphalaria alexandrina* was negatively correlated with DO ( $p=0.572$  and  $0.188$ , respectively). The prevalence of gymnocephalus cercariae was positively correlated with Do ( $p=0.267$ ), while the prevalence of xiphidiocercariae was negatively correlated with Do with a high significance ( $p\leq 0.001$ ). The abundance of the snail *Pila wernei* was positively correlated with TDS with a high significance ( $p\leq 0.001$ ). However, the abundance of the snails *Segmentorbis angustus*, *Biomphalaria*

*alexandrina*, and *Gabiella senoriansis* was positively correlated with TDS ( $p=0.148$ ,  $0.8$ , and  $0.83$ , respectively). While, the abundance of the snails *Lanistes carinatus* was negatively correlated with TDS with a high significance ( $p\leq 0.001$ ). Moreover, the abundance of the snails *Lanistes varicus* and *Melanoides tuberculata* was negatively correlated with TDS ( $p=0.57$ , and  $0.95$ , respectively). The prevalence of gymnocephalus cercariae and xiphidiocercariae was negatively correlated with TDS ( $p=0.4$  and  $0.8$ , respectively).

The abundance of the snail *Pila wernei* was positively correlated with water EC with a high significance ( $p\leq 0.001$ ). The abundance of the snails *Segmentorbis angustus* and *Biomphalaria alexandrina* were positively but non-significantly correlated with water EC ( $p=0.195$ , and  $0.575$ , respectively). While, the abundance of the

Table 4. Variability of water physicochemical parameters among the sites of snail collection in Damietta Governorate.

Sites	Temp. °C	Do. mg/L	TDS Ppm	EC µS/cm	pH
Al-Inaniyyah	21.95±6.20	6.74±0.95	823.58±439.28	1380±650	7.59±0.30
Izbat Al-Basartah village	22.08±6.52	6.44±0.95	669.75±368.12	850±320	7.47±0.29
EL-Shenawy village	21.83±6.90	6.61±0.92	433.08±126.44	640±180	7.74±0.35
EL-Serw	21.75±6.48	6.66±1	624.08±362.63	920±530	7.58±0.40
Kafr EL-Manazlla village	22.29±6.01	7.02±0.61	385.50±88.93	570±1300	7.59±0.30

Table 5. Correlation analysis of the relationship between the physicochemical parameters of water and both of the snail abundance and cercaria prevalence in Damietta Governorate.

Snail species	Temp. °C	Do. mg/L	TDS ppm	EC $\mu$ S/cm	pH
<i>Lanistes carinatus</i>	0.653** (0.00)	-0.587** (0.00)	-0.651** (0.00)	-0.428** (0.001)	-0.074 (0.576)
<i>Lanistes varicus</i>	0.874** (0.00)	-0.715** (0.00)	-0.247 (0.057)	-0.155 (0.236)	-0.111 (0.397)
<i>Pila wernei</i>	-0.415** (0.001)	0.329* (0.01)	0.454** (0.00)	0.507** (0.00)	-0.125 (0.343)
<i>Segmentorbis angustus</i>	-0.496** (0.00)	0.425** (0.001)	0.189 (0.148)	0.170 (0.195)	-0.043 (0.747)
<i>Melanoides tuberculata</i>	0.124 (0.346)	-0.074 (0.572)	-0.008 (0.95)	-0.030 (0.818)	-0.057 (0.665)
<i>Biomphalaria alexandrina</i>	0.158 (0.227)	-0.172 (0.188)	0.032 (0.808)	0.074 (0.575)	0.040 (0.761)
<i>Gabbiella senoriansis</i>	0.383** (0.003)	-0.450** (0.00)	0.028 (0.83)	-0.071 (0.588)	-0.072 (0.582)
<b>Cercaria type</b>					
<b>Gymnocephalus cercariae</b>	0.203 (0.119)	0.146 (0.267)	-0.11 (0.404)	-0.112 (0.395)	0.080 (0.542)
<b>Xiphidiocercariae</b>	0.494** (0.00)	-0.522** (0.00)	-0.031 (0.813)	-0.140 (0.284)	-0.121 (0.356)

P values (between brackets) were considered significant if  $\leq 0.05$ ; \*, \*\* refer to high significance (0.01 and 0.001, respectively)

snail *Lanistes carinatus* was negatively correlated with EC with a high significance ( $p=0.001$ ). The abundance of the snails *Lanistes varicus*, *Gabbiella senoriansis*, and *Melanoides tuberculata* were negatively but non-significantly correlated with water EC ( $p=0.236$ , 0.588, and 0.818, respectively). The prevalence of gymnocephalus cercariae and xiphidiocercariae was negatively correlated with EC ( $p=0.39$  and 0.28, respectively).

The abundance of the snail *Biomphalaria alexandrina* was positively but non-significantly correlated with water pH ( $p=0.76$ ). While, the abundance of the snails *Lanistes carinatus*, *Lanistes varicus*, *Pila wernei*, *Gabbiella senoriansis*, *Melanoides tuberculata*, and *Segmentorbis angustus* was negatively correlated with water pH ( $p>0.05$ ). The prevalence of gymnocephalus cercariae was positively but non-significantly correlated with pH ( $p=0.54$ ), while the prevalence of xiphidiocercariae was negatively correlated with pH ( $p=0.35$ ).

#### Multivariate analysis of the snails distribution

The Canonical Correspondence Analysis (CCA) was performed to investigate the response of snail species to the environmental parameters (Fig. 4). The results indicate that the environmental parameters had significant influences on the snail species

distribution ( $P=0.002$ , F-ratio = 14.2). The Monte Carlo test revealed that only water temperature had a significant influence on the distribution of snail species (Table 6). According to CCA analysis, the abundance of *Pila wernei* and *Segmentorbis angustus* was associated with the lowest values of water temperature and the highest TDS, EC and DO concentrations. While, the abundance of *Lanistes carinatus*, *Lanistes varicus*, *Biomphalaria alexandrina*, *Melanoides tuberculata*, and *Gabbiella senoriansis* was associated with the highest value of water temperature and the lowest values of pH and DO.

#### Discussion

In the present study, seven different freshwater snail species were identified and their distribution was evaluated at 5 different collection sites. The relation between the abundance of these snail species and the physico-chemical water quality was also studied. The Shannon diversity index status of the present investigation at 5 the study sites ranged from poor to bad status. The snails at Al-Inaniyyah and Izbat Al-Basartah villages were almost similarly distributed with high snail diversity and richness. While, the snails at EL-shenawy village, EL-Serw, and Kafr EL-Manazlla village

Table 6. Monte Carlo test for evaluating the significance of environmental parameters in Damietta Governorate.

Variables	Variance explained	F-ratio	P-value
Temp.	0.23	14.05	0.002
Ec	0.04	2.26	0.06
pH	0.01	0.73	0.596
Do	0	0.36	0.832
TDS	0.01	0.19	0.812

P values were considered significant if  $\leq 0.05$

were distributed with low diversity and richness. The same results were also confirmed by the multivariate analysis (CCA). In a recent study, Mahmoud & Sayed (2018) recorded 13 species of snails at 5 sites in Damietta governorate with a diversity Index (H') status ranging from poor to bad status. Although the study was conducted in the same governorate but at different study sites its results coincides with our data. This may give an indication of stable snail coexistence in the same ecosystem. According to Dung *et al.* (2010), Mohammed *et al.* (2016), and El-deeb *et al.* (2017), many factors affect the density and distribution of snail species. These factors include, but not limited to, the use of agriculture's chemicals, degree of pollutants, and the speed of the water that may be high during the initial period after an irrigation cycle.

*Lanistes carinatus* was the most predominant snail species in the study with 56.4 % abundance. Consistent with this result, *Lanistes carinatus* was reported as the most abundant snail species at three Egyptian governorates; Giza, Damietta, and Minya (Mahmoud & Sayed, 2018). Inconsistent with these reports, Hussein *et al.* (2011) found that the majority of the collected snails at El-Kelabia irrigation canal and the River Nile at Qena Governorate in the south of Egypt was of the species *Cleopatra bulimoides* (1704 specimens, 25.17 %). In Africa, Igbinosa *et al.* (2015) reported the abundance of the snail species *Lanistes varicus*, which constituted 44.6 % of the collected snails at Ovia, Edo state in Nigeria. Outside Africa, Chantima *et al.* (2018) reported that about half of all snails collected from August 2016 to October 2017 in Mae Lao agricultural basin, Chiang Rai in Thailand, were of the species *Bithynia siamensis* (54.6 %).

In the current study, only two cercarial types (gymnocephalus cercariae and xiphidiocercariae) were detected in *Lanistes carinatus* at the 5 different study sites. The external features of the gymnocephalus cercaria in the present investigation were consistent with the characteristics of the gymnocephalus cercariae emerging from *Bellamya aeruginosa* (Xiao *et al.*, 2005). The morphological features of the xiphidiocercariae in the current study were also consistent with the characteristics of the xiphidiocercaria harvested from *Asolene platae* (Dellagnola *et al.*, 2019). Based on our results, only *Lanistes carinatus* was intermediate host for transmitting trematodes larvae at Damietta

Governorate. On the contrary of this, Yousif *et al.* (2010) recorded four main cercarial types (xiphidiocercariae, furcocercous cercariae, pleurolophocercous, and gymnocephalus cercariae) in the snail *Melanoides tuberculata* that was the most infected snail at the irrigation canal and the River Nile at Giza Governorate in Egypt. In the present study, the highest prevalence of cercariae was detected at the Nile's Damietta Branch in Kafr EL-Manazlla village, (4.53 %). Similar to our results, Mohammed *et al.* (2016) reported xiphidiocercariae as the most common type of cercariae (44.3 %) but from the snail *Bulinus truncates* at the irrigation canal in the East Nile, Khartoum, Sudan. In addition, Mereta *et al.* (2019) reported the highest infection rate of xiphidiocercariae (1.5 %) emerging from *Lymnaea natalensis* at wet land of Omo-Gibe River Basin, southwest Ethiopia. According to Mereta *et al.* (2019), human activities such as open field defecation, urination, livestock grazing, farming, and swimming were highly correlated with trematode infection. Being industrial and agricultural governorate, it is unlikely that the latter factors only determine trematode prevalence and diversity in Damietta Governorate.

Our results showed that the prevalence of snail species varied during the four seasons. The highest snail abundance was recorded during summer while the lowest abundance was during winter. CCA analysis revealed that the density of the most snail species was high during summer and spring except *Pila wernei*, which was high during winter. The feeding habits of each snail species could affect its seasonal occurrence. The higher density of snails recorded in the dry season was thought to be due to the occurrence of microflora (food supply) and aquatic macrophytes (Salawu & Odaibo 2014). According to Kazibwe *et al.* (2006) and Oloyede *et al.* (2017), some freshwater snails are herbivores and more abundant during the late rainy seasons due to increase in light intensity and hence, the increased photosynthesis and the more abundance of the aquatic macrophytes. Similar to our results, Igbinosa *et al.* (2015) recorded an increase in the abundance of *Lanistes varicus* from August to September at Ovia, Edo state, Nigeria. These authors believed that the feeding habits and light are important factors in the seasonal fluctuations of the snail prevalence. Based on a study in the south of Egypt, Hussein *et al.* (2011) reported varied snail densities during the different

seasons, with the highest seasonal peak recorded during autumn while the lowest was during winter. It is noteworthy to report the considerable increase in the temperature in the south of Egypt compared to the north. According to Hussein *et al.* (2011), *Lanistes carinatus* showed a maximum reproduction during summer, the highest number of *Gabbiella senaariensis* was recorded during winter, while *Biomphalaria alexandrina* showed March – May peak and reached the highest number in April. Our findings are in consistence with the report of Marie *et al.* (2015) that the snails *Biomphalaria alexandrina* and *Lanistes carinatus* showed the highest peak in spring at four northern Egyptian governorates; Giza, Ismailia, Menoufia, and Gharbia. Pointier *et al.* (1993) recorded a maximum reproduction of *Melanoides tuberculata* between June and November. Beyond the feeding habits, Islam *et al.* (2015) reported that late summer provides an optimum temperature for the breeding and reproduction of snails. According to our results the gymnocephalus cercariae disappeared during winter and the highest prevalence of these cercariae was recorded during spring. While, the xiphidiocercariae were detected during the four seasons with the highest prevalence was during summer. The effect of climatic changes on trematode occurrence has been documented in several studies. Islam *et al.* (2015) revealed that the prevalence of the gymnocephalus cercariae emerging from *Lymnaea* sp. varied significantly with seasonal changes and reached a peak in April – October then decreased in February – March, and disappeared in November – January. The higher abundance of snails during summer, as recorded in the current study, and dry seasons in general might lead to increased cercarial prevalence as it will be easier for the miracidia to find new snails for infection. The mean value of water temperature in the current study was slightly similar between the 5 sites and ranged from 21.75 – 22.29°C. This temperature is thought to be suitable for increasing the population density of snails and the emergence of cercariae from snails. According to Yirenya-Tawiah *et al.* (2011), the temperature ranging from 27.4 – 33°C is suitable for the survival of snails. Marie *et al.* (2015) revealed that the mean values of water temperature ranged from 23.5 – 25.7°C at seven sites in the four northern Egyptian governorates; Giza, Ismailia, Menoufia, and Gharbia. The highest occurrence of the snail *Biomphalaria alexandrina* was recorded at 28°C and 31°C in two northern Egyptian governorates; Giza and Kafr El Shiekh (El-deeb *et al.*, 2017). Salawu & Odaibo (2014) reported the suitability of the water temperature range 21.7 – 29°C in all river bodies at Ogun state, Nigeria, for the survival of aquatic snail species. Islam *et al.* (2015) revealed that the suitable water temperature needed for the development of gymnocephalus cercariae in *Lymnaea* sp. was 18 – 34 °C, while Mohammed *et al.* (2016) suggested that the proper mean value of water temperature needed for the development of xiphidiocercariae in *Lymnaea natalensis* and *Bulinus truncatus* was 16 – 28 °C. The distribution of most snail species (except *Pila wernei* and *Segmentorbis angustus*) and the two types of cercariae in the current study were positively correlated with water temperature. Our findings

are consistence with Hussein *et al.* (2011) study, which revealed a positive correlation between water temperature and the abundance of *Lanistes carinatus*, *Melanoides tuberculata*, and *Biomphalaria alexandrina*. For the snails *Segmentorbis angustus*, Salawu & Odaibo (2014) observed a negative correlation between the water temperature and the abundance of the snail. Sunita *et al.* (2012) reported a direct correlation between the prevalence of gymnocephalus cercariae emerging from *Lymnaea* snails were and the water temperature. Also, Mohammed *et al.* (2016) reported a direct correlation between the distribution of xiphidiocercariae emerging from *Lymnaea natalensis* and *Bulinus truncatus* and water temperature. In addition to its influence on the abundance of aquatic snails, water temperature is thought to influence the rate of parasite development in the snail host, and probably the distribution of the related disease (McCreesh & Booth 2014).

In the current study, the mean value of water dissolved oxygen ranged from 6.44 – 7.02 mg/l. Several reports have addressed the importance of a suitable range of oxygen concentration for the snail activity, feeding, and reproduction. Salawu & Odaibo (2014) recorded mean dissolved oxygen of 6.1±4.6 mg/L at Ogun state, Nigeria. According to our results, the distribution of most snails, except *Pila wernei* and *Segmentorbis angustus*, were negatively correlated with the value of dissolved oxygen. These results are supported by Hussein *et al.* (2011) who revealed that the water DO was negatively correlated with the abundance of *Lanistes carinatus*, *Melanoides tuberculata*, and *Gabbiella senariensis*, and that DO was positively correlated with the abundance of *Biomphalaria alexandrina*. According to Sharma (1986), some snails could survive in very low oxygen conditions. However, Ofoezie (1999) reported that the density of some pulmonate snails, e.g. *Bulinus* sp. and *Lymnaea natalensis*, increased with the increase in DO value. Our results showed a positive correlation between the distribution of gymnocephalus cercariae and the value of DO, while xiphidiocercariae distribution was negatively correlated with DO. Similar to our results, Mereta *et al.* (2019) reported a negative correlation between the prevalence of the xiphidiocercariae emerging from *Lymnaea natalensis* and water DO. However, Sunita *et al.* (2012) recorded a negative correlation between the prevalence of gymnocephalus cercariae and water DO ranging (1 – 6.02 mg/l). This discrepancy could be due to the difference in concentration and range; in the study of Sunita *et al.* (2012) the DO ranged from 1 to 6.02 mg/L.

According to our results the mean values of TDS and EC of the collected water samples ranged from 385.5 – 823.58 ppm and 570 – 1380 µS/cm, respectively. Close to our results, Marie *et al.* (2015) revealed that the values of TDS and EC ranged from 369.5 – 1221 ppm and 530 – 2155 µS/cm, respectively, at eight sites in the northern Egyptian governorates Giza, Ismailia, Menoufia, and Gharbia. However, Hussein *et al.* (2011) indicated that the values of TDS and EC ranged from 145 – 638 ppm and 240 – 930 µS/cm, respectively, at six sites in Qena Governorate in the south of Egypt. The latter values reflect the different nature

of water at the southern governorates (e.g. Qena) in Egypt, where the river Nile starts its journey in Egypt and the water quality is considerably different. Rowel *et al.* (2015) observed that the values of TDS and EC ranging from 58 – 568 ppm and 112 – 1081  $\mu\text{S}/\text{cm}$ , respectively, are suitable for the survival of *Biomphalaria* at six locations in Ugandan shores of Lake Albert and Lake Victoria. Yirenya-Tawiah *et al.* (2011) revealed that a total EC range from 70 – 122  $\mu\text{S}/\text{cm}$  is suitable for the survival of the snail intermediate host for schistosomiasis. Based on our results, the distribution of most snail species *Lanistes carinatus*, *Lanistes varicus*, and *Melanoides tuberculata* was negatively correlated with the mean values of TDS and EC. These snail types might be detected at a wide range of water TDS and EC. Similar to our findings, Yigezu *et al.* (2018) observed an association of the distribution of snail species with the highest EC values. Hussein *et al.* (2011) observed a negative correlation between the abundance of *Lanistes carinatus*, *Gabbiella senaariensis*, *Melanoides tuberculata*, and *Biomphalaria alexandrina* and TDS and EC. Also, Salawu & Odaibo (2014) observed a negative correlation between the abundance of *Lanistes lybicus* and *Melanoides tuberculata* and TDS and EC. Moreover, Rowel *et al.* (2015) and Marie *et al.* (2015) detected a negative correlation between the abundance of *Biomphalaria alexandrina* and EC and suggested that this snail species might have a limit range of water conductivity permitting it to live whatever in the flowing or stagnant water. The distribution of gymnocephalus cercariae and xiphidiocercariae was negatively correlated with the values of TDS and EC. Similarly, Mereta *et al.* (2019) revealed that the prevalence of the xiphidiocercariae emerging from *Lymnaea natalensis* was negatively correlated with water conductivity and were detected at 159  $\mu\text{S}/\text{cm}$ , while echinostome cercariae were found at 181  $\mu\text{S}/\text{cm}$  of water conductivity.

The mean value of pH in the current study ranged from 7.47 – 7.74. This range is slightly alkaline and might be suitable for the existence of the different snail and cercariae species. El-deeb *et al.* (2017) revealed that *Biomphalaria alexandrina* survived in almost the same range of water pH 7.22 – 7.33 in the two northern Egyptian governorates Giza and Kafr El Shiekh. Hussein *et al.* (2011) recorded pH values ranging from 6.4 – 7.9 at six sites in Qena Governorate in the south of Egypt. In addition, Opisa *et al.* (2011) found that snails survive at a wide range of water pH (6.7 – 11). In the present study, the abundance of most snail species was negatively correlated with pH except *Biomphalaria alexandrina*. Our findings are in consistence with Hussein *et al.* (2011) who reported a negative correlation between the water pH and the abundance of *Lanistes carinatus*, *Gabbiella senaariensis*, and *Biomphalaria alexandrina*. Marie *et al.* (2015) recorded a negative correlation between the abundance of *Biomphalaria alexandrina* and pH levels. Salawu & Odaibo (2014) reported positive correlation between the water pH and the abundance of the snail *Segmentorbis angustus*. Opisa *et al.* (2011) and Salawu & Odaibo (2014) suggested that snail abundance is independent of water pH value fluctuations. Because of the conflicting reports, it

seems that the effect of water pH on snail abundance is limited. Our results showed that the distribution of gymnocephalus cercariae is positively correlated with water pH, while the xiphidiocercariae distribution is negatively correlated with water pH. Sunita *et al.* (2012), Yigezu *et al.* (2018), and Mereta *et al.* (2019) reported a direct correlation between the prevalence of both of echinostome cercariae and xiphidiocercariae and the pH. It worthy to report that, the pH ranges in all sites of the current study were very close and the range was very narrow (7.47 – 7.74).

According to the current results, only two types of trematode cercariae; gymnocephalus cercariae and xiphidiocercariae, were harvested from the same snail, *Lanistes carinatus* and the total prevalence of xiphidiocercariae was higher than that of gymnocephalus cercariae. The relatively low prevalence of gymnocephalus cercariae that emerged from this snail may be due to the antagonism by xiphidiocercariae. Mohammed *et al.* (2016) suggested that the low prevalence of furcocercous cercariae emerging from *Bulinus truncatus* could be due to antagonism by xiphidiocercariae, which were found with high rates. Antagonism involves complex interactions amongst larval trematode species and it has predictable outcomes that can influence patterns of abundance among these species. Interspecific competition for resources and space represents a potentially strong selection pressure for trematodes infecting snail hosts (Hechinger *et al.*, 2011). Laidemitt *et al.* (2019) observed an obligatory dependence of some digenetic trematode species on *Biomphalaria* snails for their larval development and they reported that the larval stages of these species might strongly interact with the larvae of *Schistosoma mansoni* for access to the resources offered by these snails. There is a possibility that xiphidiocercariae in the current study might be competed with other trematode larvae (gymnocephalus cercariae) for infecting *Lanistes carinatus*. The definitive hosts for xiphidiocercariae and gymnocephalus cercariae are different (Insectivorous bats and fish-eating birds or mammals, respectively) and, also, the secondary intermediate hosts for these two cercarial types are different (Damson or dragon fly nymphs and fish, respectively). Although it is unlikely, it is possible that the difference in the existence and abundance of these hosts could affect the life cycle and the natural occurrence of xiphidiocercariae and gymnocephalus cercariae.

According to our findings, the apple snails *Lanistes carinatus* and *Lanistes varicus* were detected at the 5 studied sites in Damietta governorate. Although, these snails might act as pests for agriculture fields, they may serve as medically and ecologically important factors by consuming aquatic weeds and reducing schistosomiasis. Anto *et al.* (2005) reported that *Lanistes varicus* has the ability to control the intermediate host snail of intestinal schistosomiasis. Also, Anto & Bimi (2017) revealed that *Lanistes varicus* snail feeds on the egg masses and juveniles of *Bulinus truncatus* snails. Our findings, therefore, suggest that the invasion of some Ampularidae freshwater snails (*Lanistes carinatus* and *Lanistes varicus*) in Damietta governorate may be the cause of

the low abundance of *Biomphalaria alexandrina* and the absence of *Bulinus* and *Lymnaea* genera, which were not detected at all in the current study in spite of the extensive search. The invasion of *Lanistes carinatus* by xiphidiocercariae in Damietta governorate might be the cause of the low infection of this snail with gymnocephalus cercariae, and the absence of *Schistosoma* larval stages at all sites in the present investigation.

To conclude, the abundance of snail species varied between the five locations in Damietta governorate and was correlated positively or negatively with some physico-chemical parameters. The invasion of the freshwater snails *Lanistes carinatus* and *Lanistes varicus* in Damietta governorate may be the cause of the low abundance of *Schistosoma* intermediate hosts. The invasion of *Lanistes carinatus* by xiphidiocercariae may be the cause of the low prevalence of gymnocephalus cercariae and the complete absence of *Schistosoma* larval stages at all the studied sites. Our study recommends further characterization of the snail distribution and the snail interrelationships and suggests a controlled use of the Ampularidae freshwater snails in the biological control of parasite intermediate hosts.

### Conflict of interest

The authors declare no conflict of interest.

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