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PREVALENCE AND DIVERSITY OF ZOOPLANKTON IN DIFFERENT FRESH WATER SUPPLIES IN ASSIUT GOVERNORATE

AYA A. AHMED[1], AHMED KAMAL DYAB [2 a,b], SARA ABDEL-AAL MOHAMED[3], ASMAA A. E. NASR[4] AND SARA SALAH ABDEL-HAKEEM[5]

[1]Department of Clinical Pathology, Assiut Police Hospital,
[2a]Department of Medical Parasitology, Faculty of Medicine, Assiut University, 71526 Assiut Egypt. And [2b] Department of Parasitology, School of Veterinary Medicine, Badr University in Assiut, New Nasser City, Assiut, Egypt
[3]Department of Parasitology, Faculty of Veterinary Medicine, Assiut University, Assiut 71515, Egypt
[4]Department of Poultry Diseases, Animal Health Research Institute, Assiut 71515, Egypt
[5]Parasitology Laboratory, Zoology and Entomology Department, Faculty of Science, Assiut University, Assiut (71526), Egypt (sara_assiut86@aun.edu.eg), **(ORCID:** https://orcid.org/0000-0003-1069-5806)

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ABSTRACT

The present study aimed to evaluate the diversity of zooplankton including rotifers and cyclops in the various freshwater ecosystems in Assiut Governorate, Egypt. Rotifers can affect the prevalence of different protozoan organisms in water, such as Cryptosporidium species. Cyclops act as intermediate host of some helminthic parasites. Microscopic analysis of the collected samples identified 3 rotifer genera and one copepod in different freshwater supplies including poultry farms, animal farms, human tanks, and fish farms. In the present study, 59 out of 136 (43.3%) water samples were contaminated with plankton. Poultry freshwater showed the highest plankton diversity and prevalence with 29 samples (21.3%), whereas the least prevalence was in human drinking water, 3 (2.2%). In the fishpond, Cyclops were observed in 3 (2.2%) of total samples. The prevalence of rotifers and Cyclops is strongly related to the summer season. Our results enrich the diversity of zooplankton in different freshwater supplies as a bioindicator of water pollution.

Keywords: *Zooplankton; water supplies; Assiut and Rotifers.*

INTRODUCTION

Rotifers "wheel animals" belong to the smallest group of metazoans (Chhaba 2022). They are microscopic multicellular, aquatic invertebrates that can account for over 50% of zooplankton productivity and have wide ecological importance (Herzig 1987).

Currently, there are over 2,200 recognized Rotifers species and 120 genera varying in shape, size, and hardness (Segers 2008). The phylum Rotifer is classified into three classes: *Bdelloidea, Monogononta, and Seisonidea* (Wallace *et al.,* 2015).

Despite their small size, Rotifers are fully functional organisms with multiple specialized organ systems. Their body consists of a head equipped with two ciliated coronae, a trunk containing the organs, and a foot that serves as an anchor for swimming (Wallace *et al.,* 2015). They can be either free-living or parasitic. Most Rotifers are freewheeling and represent an important part of nutrient recycling in freshwater ecosystems (Lawrence *et al.,* 2012). The

Corresponding author: AHMED K. Dyab
E-mail address: ahmed2015@aun.edu.eg
Present address: Department of Parasitology, Faculty of Medicine, Assiut University, Assiut.

rotifer species can be found in all kinds of water bodies worldwide due to their high adaptability to a wide range of environmental conditions and their ability to quickly occupy open niches. They inhabit many freshwater environments (stagnant water) and humid environments in mild to warm temperatures, such as pond bottoms, flowing water (rivers or streams), and moist soil or leaf litter in rain gutters. Some species of Rotifers even live in saltwater environments (Savard 2023). Rotifers can form a colony with their feet attached (Wallace *et al.,* 2015). They feed on dead or decomposing organic materials and microorganisms (Roy and Amzad 2019). Gutkowska *et al.* (2013) reported that the rotifer diversity, total abundance, and species composition serve as important indicators of water quality.

Rotifers, such as *Rotaria rotatoria* and *Philodina acuiicornis*, have been utilized as biological control agents against snail eggs in aquariums (Abou Zaid *et al.*, 2002). In addition to this, they serve as indicators of environmental toxicity (Colvin *et al.,* 2021; Dabrowski and Miller, 2018; Stelzer, 2009; Xu and Niu, 2021) and help assess the quality of freshwater sources (dos Santos Picapedra *et al.,* 2021). Moreover, could be an effective way to control mosquitoes burden (Ranasinghe and Amarasinghe 2020). Moreover, freshwater *rotifer* species may ingest and digest waterborne oocysts such as *Cryptosporidium parvum* that fall within the size range of particles ingested by rotifers. Experimentally, rotifers were exposed to *C. parvum* oocysts, however their ability to ingest oocysts in nature is unknown (Fayer *et al.,* 2000). Although it is unknown whether oocysts are degraded in any way after ingestion by rotifers, it was observed that oocysts were excreted by some rotifers in boluses containing a mixture of other ingested materials. Since these boluses are 20-30 times larger than individual oocysts, they may still contain infectious oocysts (Fayer *et al.,* 2000).

Spontaneous colonization of rotifers on the shells of schistosome-infected *Biomphalaria glabrata* schistosomes reduced both the cercarial output and the motility and infectivity of the cercariae. Their effect was observed in rotifer conditioned water from which the rotifers themselves had been removed by purification. The significance of the rotifer effect to laboratory maintenance of *Schistosoma mansoni* is considerable. It may be one of many factors that reduce the infective index under field conditions (Stirewalt and Lewis 1981). Moreover, the rotifers are potentially controlling the intermediate host of *Scistosoma mansoni* by affecting its fertility and life span (Lin *et al.,* 2021).

Small crustaceans are sensitive to pollution as they represent the nutrient-rich condition of water bodies. This condition is also obvious in fishponds by the presence of *Cyclops* spp. The amount of natural food in the ponds is the most important parameter determining the efficiency of supplementary feed intake by fish for growth (Park and Shin 2007). However, Cyclops can adapt to a parasitic lifestyle on fish and undergo transformation when they attached to the infected fish. They also act as intermediate hosts and vectors for some parasitic diseases, such as *Triaenophorus nodulosus*, *Diphyllobothrium latum* (Piasecki *et al.,* 2004).

Despite recent efforts, there is still lack of information on the distribution of zooplankton species in tropical freshwater ecosystems, either globally or particularly in Egypt. Moreover, less attention has been given to the relationship between zooplankton diversity and seasonal variation. This study aims to investigate the prevalence and diversity of rotifer and cyclops in different freshwater supplies in Assiut Governorate and its relationship with seasonal variation.

MATERIALS AND METHODS

Study area

The study was carried out in Assiut Governorate during October 2022 to April 2024. Assiut climate is a desert climate (1°C to 45°C; annual mean = 23 °C), almost minimal rainfall occurs throughout the year "https://en.climate-data.org/location/612/".

Sample processing
A total of 136 water samples were collected from different water supplies including 59 poultry farms (PDW), 40 animal dwellings (ADW), 27 human dwellings (HDW), and 10 fishponds. Qualitative samples of rotifers were collected by dragging a 50 µm mesh size cast-net at a depth of 15–20 cm and preserved in 4% formaldehyde. Each sample was stored in 25 mL wide mouth, capped sterile plastic bottles at the consumption points for parasitological analysis.

Data was collected using forms to record information on some characteristics that can affect the prevalence of protozoa in water, including water distribution networks, and different water sources (rivers, animal farms, poultry farms, hospital taps, home tanks, and clubs)

Microscopic examination
Water samples were concentrated using flotation and sedimentation concentration techniques according to Zajac *et al.* (2012). The concentrated samples were examined by "Wet mount method" as recommended by Garcia (2001). Samples were pipetted and observed under light microscope at different magnifications (10x and 40x) for identification. Images and movies were easily captured using a Microscope Digital Camera (Olympus EP50) extant in the parasitology laboratory, Faculty of Veterinary Medicine, Assiut University. The morphological identification of plankton genera and species were done according to Wallace *et al.* (2015).

Data analysis
The gathered data was meticulously entered into a spreadsheet in Microsoft Excel 2010 and subsequently analyzed using SPSS software (version 20) for Windows 10 (Abdel-Hakeem *et al.,* 2021). Frequencies, proportions, and standard deviations (SD) for non-parametric quantitative variables were estimated. *P*-value of less than 0.05 was indicative of statistical significance.

RESULTS

Prevalence of plankton in the different water supplies
Of the total water samples, 59 samples were positive for rotifers (43.3%, 59/136). Three rotifer genera and one copepod were identified across four freshwater supplies including poultry farms, animal dwellings, human dwellings, and fishponds. The poultry drinking water (PDW) showed the highest plankton diversity, with a prevalence rate of 49 % (29/59), followed by animal drinking water (ADW) (62.5 %; 25/40). Whereas in human drinking water, only 3 (2.2%) positive samples were observed. In fishponds, the lowest number of rotifers (20%; 2/10) was observed. *Cyclops* were exclusively recorded in fishponds, with a prevalence rate of 30% (3/10) (Table 1 and Fig. 1A).

Table (2) illustrates the seasonal variation of plankton throughout the study. The highest prevalence rate of rotifers was observed in spring (55.5%) and summer (52%), followed by autumn (33.3%). The least diversity was observed in winter (19.2%) (Fig. 1B).

Table 1: Diversity of plankton in the different water supplies

	PDW (n=59)	ADW (n=40)	HDW (n= 27)	Fishponds (n= 10)	Total (n=136)	P value[(b)]
Rotifer	29	25	3	2	59	0.00***
Cyclops	0	0	0	3	3	0.00***
Total	29	25	3	5	62	0.00***
***P* value[(a)]**	0.00**	0.00**	0.0*	0.606	0.00***	

n= number of examined samples; (a) difference in prevalence between rotifers and *Cyclops* in the same water source; (b) difference in prevalence of rotifers and *Cyclops* in the different water sources. (*, *P*<0.05; **, *P*<0.01; **, *P*<0.001).

Table 2: Seasonal variation in plankton diversity

	Summer (n=50)	Autumn (n=24)	Winter (n=26)	Spring (n=36)	Total (n=136)	P value(b)
Rotifer	26	8	5	20	59	0.013*
Cyclops	1	0	2	0	3	0.172
***P* value(a)**	0.00***	0.002**	0.057	0.00***	0.00***	

n= number of examined samples; (a) difference in prevalence between rotifers and *Cyclops* in the same water source; (b) difference in prevalence of rotifers and *Cyclops* in the different water sources. (*, $P<0.05$; **, $P<0.01$; **, $P<0.001$).

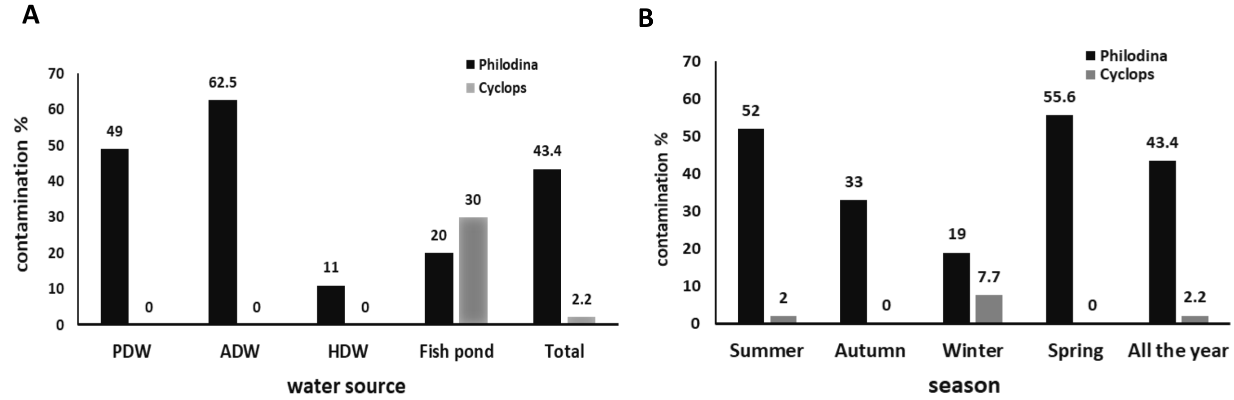

Fig. 1: **(A)** Diversity of Rotifer dispersion in different water supply in Assiut. PDW: poultry drinking water, ADW: animal drinking water, HDW: human drinking water. **(B)** Seasonal Variation in Rotifer Contamination of Water Supply. Data were presented as percentage.

Microscopic identification of rotifers

Microscopically, three rotifer genera were recorded including *Philodina*, *Rotaria*, *Brachionus spp.* All rotifers consist of a head, trunk and foot. The head bears the corona, which carries the cilia (trochus), while the trunk contains a visible stomach (mastax). The jointed feet end in toes and spurs.

Philodina sp. is characterized by a yellowish-brown mastax. The head features a two-jointed rostrum which bears two semicircular rostral lamellae. The trunk tapers into a noticeably slender rump, which transitions into a short, broad, four-jointed foot ending in toes and spurs (Fig. 2 A - E).

Rotaria neptunia is slender, narrow, and elongated, with a white, nearly opaque appearance. The wheel-organ is very narrow and small, while the rostrum is short (Fig. 2 F). The foot is slender, very long, five-jointed and is telescopically projected. It constitutes half the total body length. The foot is very long and thin, and the corona is small and fine.

Brachionus sp. has a structure similar to other rotifers but is distinguished by short, broad trunk and wide corona. The foot is ring type, non-segmented, and short (Fig. 2 G and H).

The body of Cyclops is divided into a head, thorax, and abdomen. The head and first thoracic segment are united to form a cephalothorax. The last abdominal segment has a pair of caudal forks, with 5 plumose bristles at their tip. The cephalothorax has paired antennae and antennules and a single median eye near its tip (Fig. 2 J).

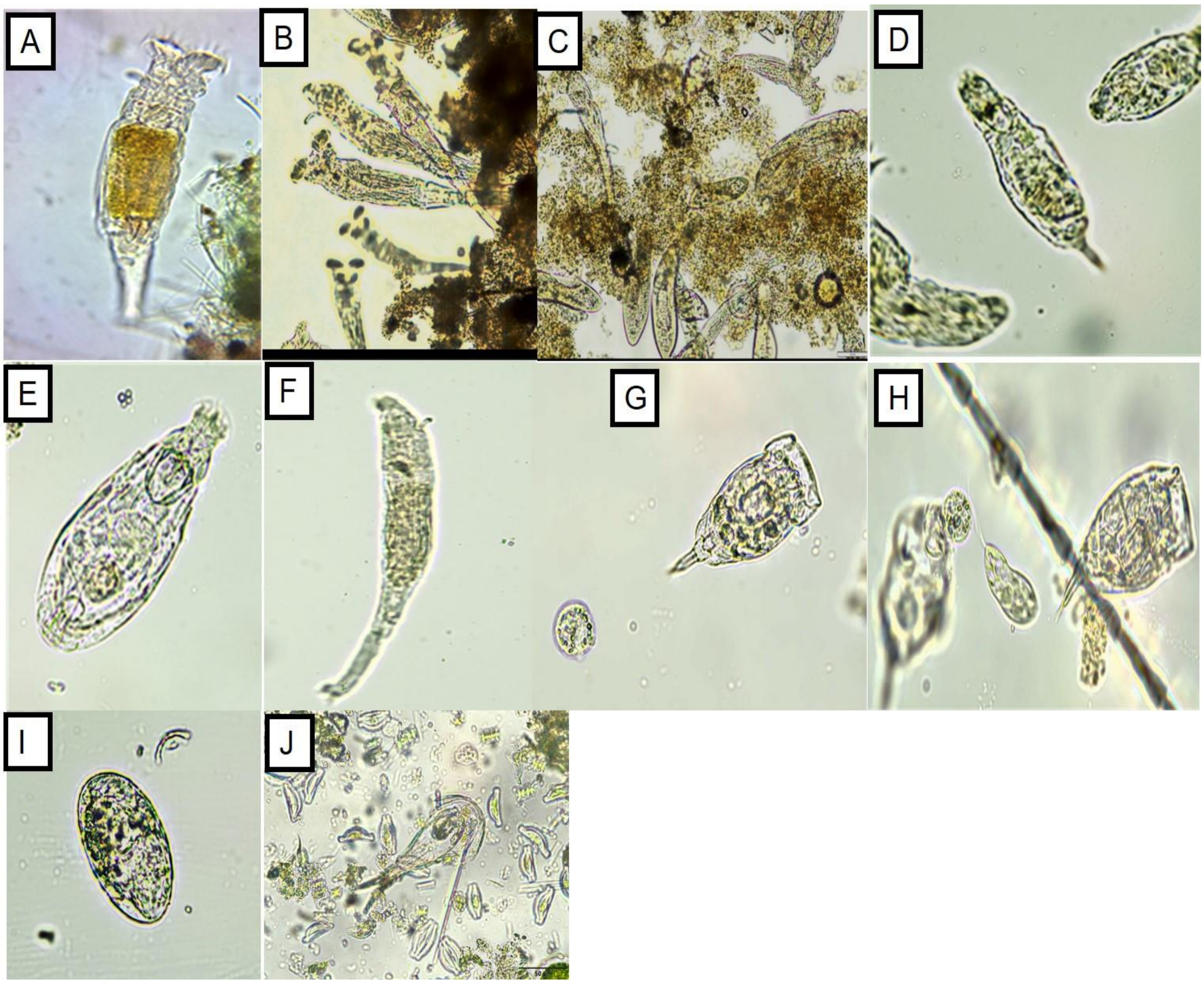

Fig. 2: Showing Zooplankton in Assiut Governorate water supplies, A-E: *Philodina spp.* A: Adults have corona with trochus, B & C: aggregation of *Philodina* spp. with feet attached, D & E: *Philodina* spp with retracted corona, F: *Rotaria neptuna,* G& H: *Brachionus spp.*, I: Rotifers egg, J: Cyclops.

DISCUSSION

Our study highlights the value of routine detection of zooplankton organisms as indicators of contamination in animal, poultry, and human water supplies. In the present study, the total population of rotifers was 43.3%, and Cyclops was 2.2% with the highest diversity in the poultry water supplies (21.3%) and the least diversity in human drinking water (2.2%). Majewska *et al.* (2003) demonstrated that rotifers can serve as an easily accessible bioindicators for detecting biological contamination in surface waters with various stages of protozoan parasites. Rotifers survival is influenced by dissolved oxygen, which is abundant in polluted, alkaline waters (Dahms *et al.,* 2011). However, Ahangar *et al.* (2012) found that various species of *Brachionus* are more abundant in polluted waters. In Egypt, rotifers dominated other zooplanktonic groups, compromising 72.4% of the total zooplankton population, while Copepoda made up 7.2% in El Rahway area (Abdel-Halim *et al.,* 2013). In Upper Egypt (Aswan, KomOmbo, Edfu), *Rotifera* and *Copepoda* accounted for 66.7% and 8.3% of the total species (El-Otify and Iskaros 2015). In the River Nile at El-Qanater El-Khiria region, Galal (2018) demonstrated that zooplankton communities were dominated by rotifers, contributing 96.77%, followed by copepods at 1.88%. On the other hand, lower diversity of Rotifer (14.8%) and Copepods (29.9%) was recorded in the

Damietta Branch (S El-Tohamy and N Abdel-Baki 2019).

In Egypt, there are no literature reports on biotic interactions between zooplankton and waterborne parasites. thus, to validate this hypothesis we evaluated the parasite oocysts/eggs concurred with plankton samples. Our observations might support the deeply intricate biotic interactions that we observed in plankton communities because parasite oocysts/egg were consumed as prey by zooplankton. Fayer *et al.* (2000) demonstrated that rotifers may act as intermediate hosts for *Cryptosporidium* species, the most important newly recognized contaminant in drinking water. though it remains unclear whether oocysts are degraded in any way after ingestion by rotifers. Previous research has also shown seasonality in zooplankton infection prevalence (Duffy *et al.,* 2005), which is linked to the parasite's sporulation, which is probably influenced by declining temperatures (Shields 1994).

Our results indicate that *Cryptosporidium* oocysts were detected in most rotifer samples, across all water supplies, with a high prevalence during spring and summer seasons. This suggests low host-specificity and explain the association of this group with all zooplankton. These results concurred with those of Nowosad *et al.* (2007), who demonstrated that most of the *Cryptosporidium*-positive samples recovered were observed in spring, consistent with hydrological seasonality and the addition of allochthonous matter, such as dung or feces, into lakes. The world's worst case of waterborne human cryptosporidiosis happened in the spring of 1993, especially affecting immunosuppressed individuals (Mac Kenzie *et al.,* 1995). This evidence supports the notion that zooplankton play a role in the transmission of parasitic oocysts/eggs within the freshwater food chain. Furthermore, rotifers may be one of many factors which reduce the infective index of *Shistosoma mansoni* (Stirewalt and Lewis 1981) and may act as a biological control agent against snail eggs.

In this study, the predominance of rotifers was observed during autumn and spring seasons. Several factors affecting the abundance of rotifers and copepods in the Nile River, including their simple parthenogenic reproduction (Herzig 1987). Under favorable conditions, this reproductive strategy leads to high production rates, and high population densities. Additionally, their relatively short generation time compared to larger crustacean zooplankton further contributes to their abundance (Ahmad *et al.,* 2011).

On the other hand, eutrophication affects the composition of zooplankton, by shifting dominance from larger species, such as Copepoda, to smaller species like Rotifer, The observed decrease in rotifer density in favor of copepods suggests an increase in eutrophication (Fishar *et al.,* 2019).

In a study conducted in Malaysia, a high incidence rate of rotifer species of 38.0% was found dominant in Sembrong Lake, comprising 41.2% of the total rotifer species (Umi *et al.,* 2018). This dominance was linked to the lake's health status. The abundance was linked to the eutrophic condition, including turbidity, total dissolved solids (TDS), chlorophyll, and nutrient concentrations. The variations in species distribution were related to environmental factors, especially water temperature, conductivity, and nitrate. The degree of anthropogenic disturbance determines the quality and quantity of plankton in the study area. Consequently, the discharged post-cooling waters should be controlled to protect zooplankton fauna and the planktivorous fish of the study area (Wang *et al.,* 2019).

In conclusion, the routine monitoring of plankton is an easier and sensitive methodology for detecting biological contamination in surface water. However, further investigations utilizing advanced techniques, such as immunoassay and fluorescence in situ hybridization, is necessary to provide visual evidence of these potential interactions.

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انتشار وتنوع العوالق الحيوانية فى مصادر المياه العذبة المختلفة بمحافظة اسيوط

آية عادل أحمد ، احمد كمال دياب ، سارة عبد العال محمد ، أسماء ناصر ، سارة صلاح عبد الحكيم

Email: ahmed2015@aun.edu.eg Assiut University web-site: www.aun.edu.eg

يدرس البحث الحالي تنوع العوالق الحيوانية التي تتضمن الدوارات والقشريات (السايكلوب) في انواع المياه العذبة المختلفة بمحافظة أسيوط، مصر. تستطيع الدوارات أن تؤثرعلي انتشار الاوليات في المياه مثل الكريبتوسبوريديام .القشريات (السايكلوب) يعمل كعائل وسيط لبعض انواع الديدان الطفيلية. أوضح الفحص الميكروسكوبي للعوالق وجود ثلاثة انواع من الدوارات ونوع واحد من القشريات في المياه العذبة باسيوط. انواع المياه التي فحصت في البحث هي المياه المستخدمة لمزارع الدواجن، المياه المستخدمة في مزارع الحيوانات، خزانات المياه اتي يستخدمها الانسان وأخيرا مياه مزارع الأسماك. ٥٩ من ١٣٦ (٤٣,٣ %) مياه كانت ملوثة بالعوالق الحيوانية ، أكثر العينات تلوثا كانت تلك التي جمعت من مزارع الدواجن (٢١,٣%) وأقل انتشار للعوالق في المياه العذبة كان في المياه الصالحة للانسان (٢,٢%). في مياه الأسماك القشريات تمثل (٢,٢%) فقط. انتشار العوالق يرتبط بقوة بفصل الصيف. هذا البحث يشير الي تنوع العوالق الحيوانية في مصادر المياه المختلفة والتي يمكن ان تؤثر في التوازن البيئي، كما يمكن الاستدلال بها كمؤشر حيوي.