

Cooccurrence of *Schistosoma haematobium*, other trematode parasites, an annelid (*Chaetogaster limnaei limnaei*), and a nematode parasite (*Daubaylia potomaca*) in *Bulinus globosus*

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Abstract: Cooccurrence of *Schistosoma haematobium*, other trematodes, *Chaetogaster limnaei limnaei*, and *Daubaylia potomaca* was investigated in *Bulinus globosus* collected at Nike Lake from October 2011 to September 2012. The snail sizes as well as the numbers of these organisms were recorded. Single infections of *S. haematobium*, other trematodes, *C. limnaei limnaei*, and *D. potomaca* were 0.3%, 46.38%, 1.03%, and 2.66%. Coinfection of *S. haematobium*-*C. limnaei limnaei* was 0.3% while that of *S. haematobium*-other trematodes was 0.15%. Coinfections of *C. limnaei limnaei*-other trematodes and *D. potomaca*-other trematodes were 2.16% each. There was no cooccurrence of *C. limnaei limnaei*-*D. potomaca* or *S. haematobium*-*D. potomaca*. The mean intensity of *C. limnaei limnaei*-*S. haematobium* was significantly higher than *C. limnaei limnaei* single infection. The mean intensity of single *C. limnaei limnaei* infection and *C. limnaei limnaei*-other trematodes coinfection was comparable. Mean intensity of *D. potomaca*-other trematodes coinfection and *D. potomaca* single infection was similar. In relation to snail size, significant variation was observed in other trematodes infection where the snails ≥ 8 mm had significantly higher infection rates than the snails < 8 mm. The varying prevalence and mean intensities of the various symbionts in *B. globosus* suggest the existence of biotic interactions.

Key words: Cooccurrence, *Chaetogaster*, *Schistosoma haematobium*, *Daubaylia potomaca*, trematodes, prevalence, intensity

Multiple symbiotic interactions are the coexistence of three or more organisms of different taxonomic groups. Such symbioses are common in freshwater snails because they face a variety of biotic interactions (Hunter, 2006). The parasitic symbiotic relationship between freshwater snails and trematode parasites is well documented. There is evidence that trematodes affect growth, reproduction, and survival of their snail host. There are also records of symbiotic association between freshwater snails and an annelid worm, *Chaetogaster* (Buse, 1974; Rodgers et al., 2005; Agbolade et al., 2007; Fried et al., 2008; McKoy et al., 2011; Zimmermann et al., 2011; Stoll et al., 2013). Two subspecies of *Chaetogaster limnaei* are recognized: one subspecies, *C. limnaei limnaei* von Baer, 1827, lives as an ectosymbiont in the mantle cavity or outer surfaces of freshwater snails and occasionally mussels, whereas the other subspecies, *C. limnaei vaghini* Gruffydd, 1965, is parasitic in freshwater snails and feeds mainly on kidney cells (Buse, 1974; McKoy et al., 2011). Smythe et al. (2015) recorded both the ectosymbiotic and parasitic

forms of *C. limnaei* in *Physa gyrina* collected from streams and ponds in central New York. Another symbiont is the parasitic nematode *Daubaylia potomaca* Chitwood & Chitwood, 1934. The nematode inhabits all tissue and blood spaces within the snail host and ultimately leads to death of the host. It is postulated that *C. limnaei limnaei* indirectly increases nematode presence by downregulating trematode parasites, which through competition are negatively associated with the nematode (McCaffrey, 2014). Other symbionts are bacteria, fungi, Ichthyosporea spp., Microsporidia spp., rotifers, and protozoans (Hertel et al., 2004).

The aim of the present study was to investigate the symbiotic interactions within *Bulinus globosus* Morelet, 1866 collected from Nike Lake, southeastern Nigeria.

Nike Lake is situated in the Enugu East Local Government Area (LGA) and lies between latitudes 6°31'N and 6°36'N and longitudes 7°36'E and 7°41'E. The lake is eutrophic with mean depth, pH, water temperature, dissolved oxygen, total hardness, calcium hardness, and

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conductivity of 9 m, 7.75, 28.29 °C, 6.93 mg L⁻¹, 4.43 ppm, 2.84 ppm, and 72.33 µs/cm, respectively (Figure 1).

Snails were collected from October 2011 to September 2012 by fractional sampling method (Okafor and Obiezue, 2004), in which snails were collected for a sampling duration of 30 min from the undersurface of water lilies and at a clear sandy part of the bank of the lake once a month for 12 months. Snails were collected in the morning (0800–1100 hours) before the lake was disturbed by farmers who use the lake water for irrigation and car washers. A total of 1311 snails were collected, with more snails collected in the dry than in the rainy season months. Collected snails were kept in plastic containers with water and transported to the Zoology and Environmental Biology Laboratory, University of Nigeria, Nsukka, where they were sorted and identified using the field guide to West African freshwater snails prepared by the Danish Bilharziasis Laboratory,

Denmark (Brown, 1994). The shell lengths of the identified *B. globosus* were measured.

The 677 *B. globosus* specimens collected were screened for symbionts. Each snail was put into a 50-mL tube containing 20 mL of water and exposed to two 50-W electric bulbs for 5 h. The water was examined for trematodes, the annelid, and the nematode. The snails not shedding cercariae after exposure to light were crushed between two microscope slides and the crushed snails were also examined for trematodes, the nematode, and the external annelids. *Schistosoma haematobium* Bilharz, 1852 and the xiphidiocercaria were identified as described by Frandsen and Christensen (1984), the rat-king cercaria was identified according to Cheng (1999), the nematode was identified as described by Chitwood and Chitwood (1934), and the annelid *C. limnaei limnaei* was identified according to Brinkhurst (1971). The xiphidiocercaria

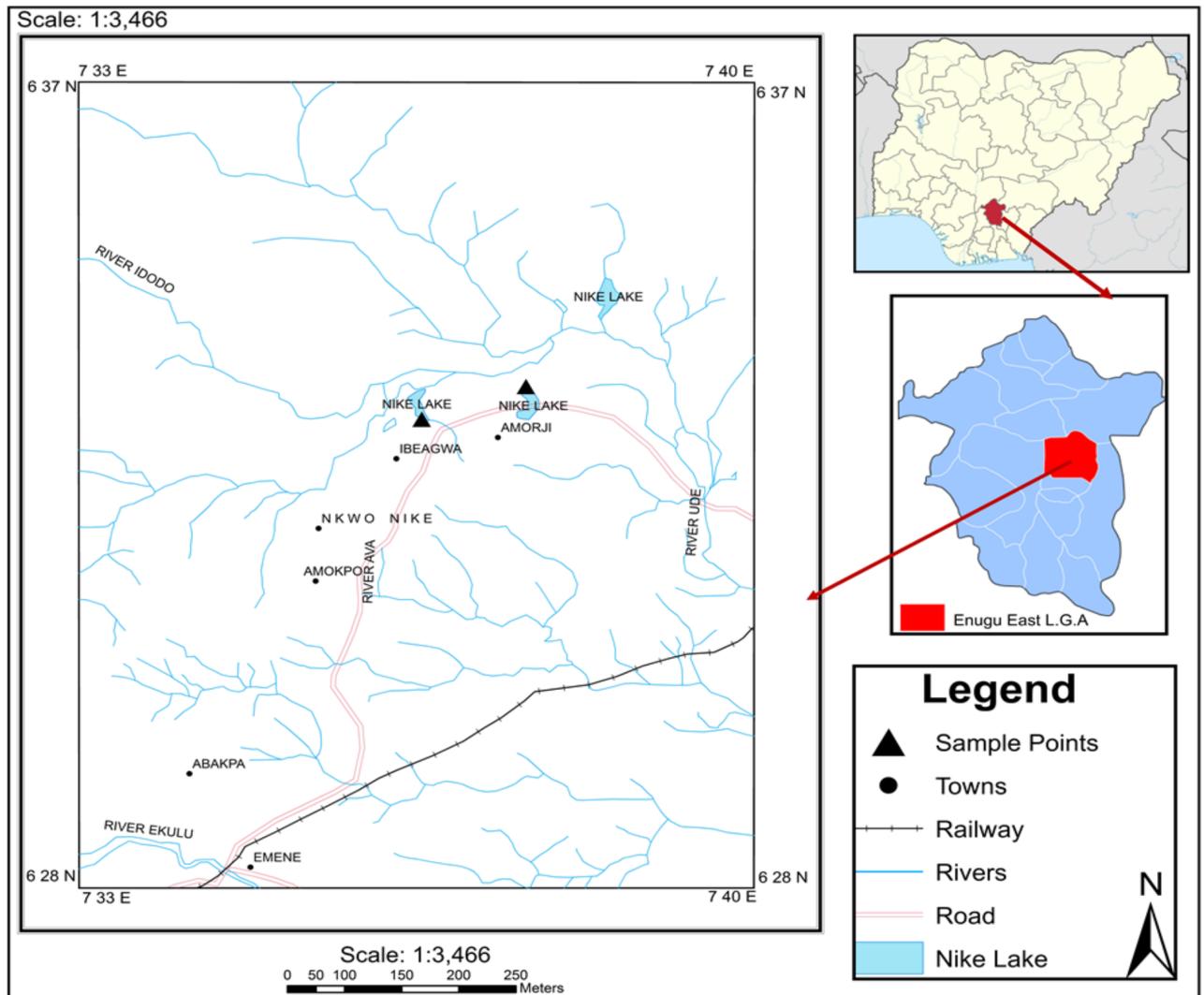


Figure 1. Map showing Nike Lake in Enugu East LGA, Enugu State, Nigeria.

and the rat-king cercaria recorded were categorized as "other trematodes" in the present study. All the organisms identified were viewed under a microscope unstained and counted.

Prevalence and mean intensities of the symbionts were investigated. For the purpose of statistical analysis, shell lengths of the snails were grouped into categories of <8 and \geq 8 mm. A Student t-test was used to compare the intensities of the trematodes, *C. limnaei limnaei*, and *D. potomaca* in both single and coinfections and in relation to snail size. A chi-square test was used to assess differences in size prevalence of the symbionts in the snails. All statistical analysis was performed using SPSS 20.0 at a 5% level of significance.

Lymnaea natalensis Krauss, 1848 (n = 439); *B. senegalensis* Muller, 1781 (n = 180); *B. globosus* (n = 677); *Biomphalaria pfeifferi* Krauss, 1848 (n = 8); and *Melanoides tuberculata* Muller, 1774 (n = 7) were collected from Nike Lake but coinfections of the different organisms were observed only in *B. globosus*. Of the 677 *B. globosus* collected, 329 (48.59%) were infected with one or two symbionts. As shown in Figure 2, the snails infected with *S. haematobium*, other trematodes, *C. limnaei limnaei*, and *D. potomaca* single infections were 2 (0.3%), 314 (46.38%), 7 (1.03%), and 18 (2.66%). No *S. haematobium*-*D. potomaca* or *C. limnaei limnaei*-*D. potomaca* coinfection was found in any of the collected snails. Coinfection of *C. limnaei limnaei*-*S. haematobium* occurred in only two (0.3%) of the snails while coinfection of *S. haematobium*-other trematodes was found in only one (0.15%) *B. globosus*. Coinfections of *C. limnaei limnaei*-other trematodes and *D. potomaca*-other trematodes were found in 15 (2.16%) snails each (Figure 3).

Although there was no significant difference ($P = 0.465$) in the mean intensity of *S. haematobium* when it occurred singly and in coinfection with *C. limnaei limnaei*, the mean intensity of *S. haematobium*-*C. limnaei limnaei* was lower than the mean intensity of *S. haematobium* single infection. There was also no significant difference between the mean intensity of *S. haematobium* single infection and intensity of *S. haematobium*-other trematodes coinfection ($P = 0.386$). Mean intensity of other trematodes single infection was higher than the mean intensity when it cooccurred with *S. haematobium*, but the difference was not significant ($P = 0.981$). Mean intensity of other trematodes-*C. limnaei limnaei* and other trematodes-*D. potomaca* coinfections was 406.87 ± 253.81 each, but these intensities did not differ significantly from the mean intensity in other trematodes single infection ($P > 0.05$). The mean intensity of single *C. limnaei limnaei* infection was significantly lower than that of *C. limnaei limnaei*-*S. haematobium* coinfection ($P < 0.0001$). However, mean intensity of the cooccurrence of *C. limnaei limnaei* with other trematodes was not significantly different from mean intensity in single *C. limnaei limnaei* infection ($P = 0.652$). *Daubaylia potomaca* only cooccurred with other trematodes, with the mean intensity in coinfection being slightly lower than in single infection ($P = 0.87$) (Figure 4).

No *B. globosus* of the size <8 mm was found with *S. haematobium* infection, while prevalence of *S. haematobium* infection was 1% in the snails \geq 8 mm ($P = 0.336$). Prevalence of other trematodes infection in the snails \geq 8 mm was significantly higher than in the snails <8 mm ($P < 0.0001$). There was no significant difference ($P = 0.158$) in prevalence of *C. limnaei limnaei* in the snails <8 mm and \geq 8 mm. Prevalence of *D. potomaca* in the snails

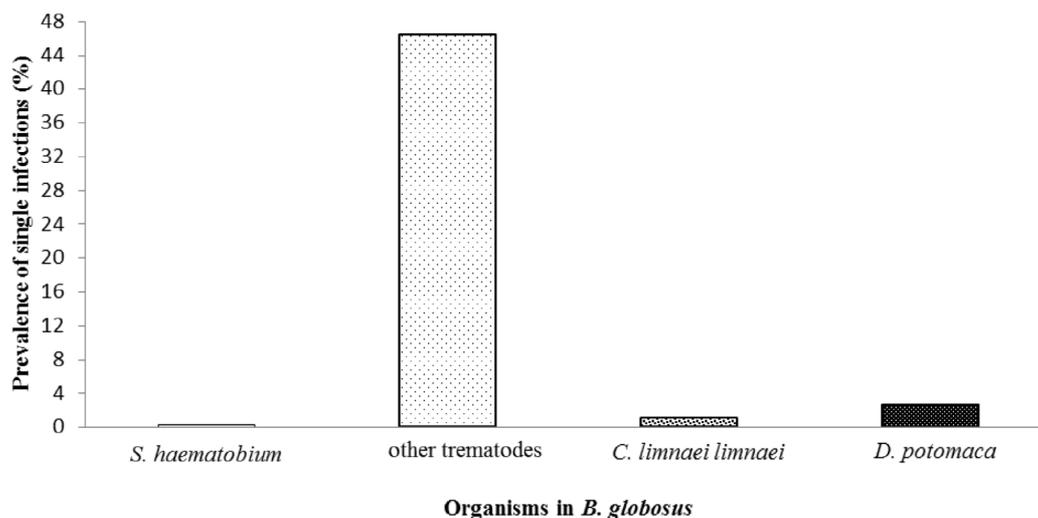
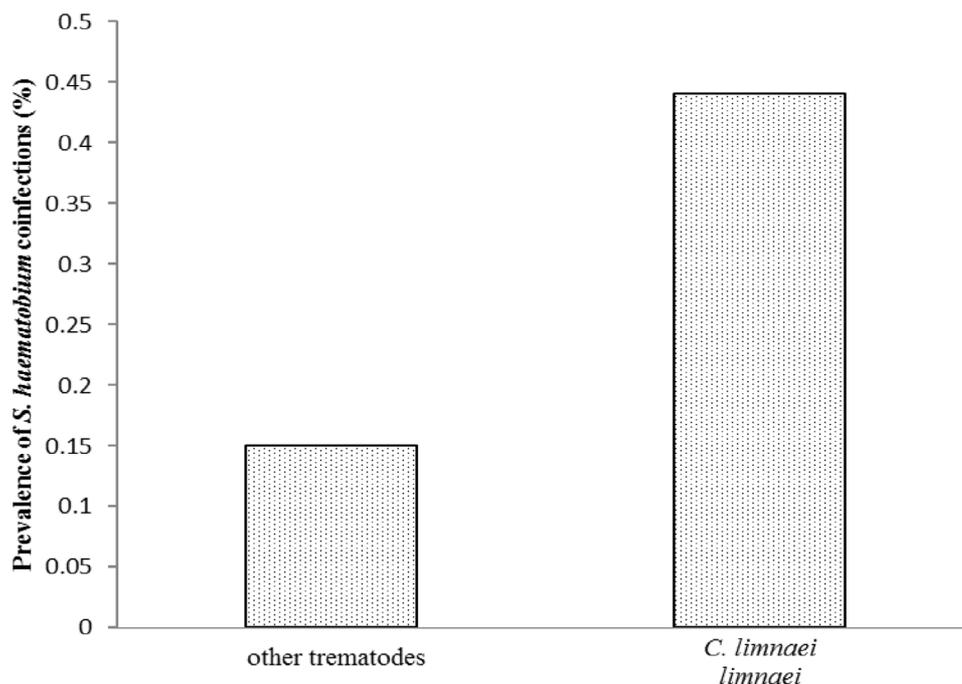
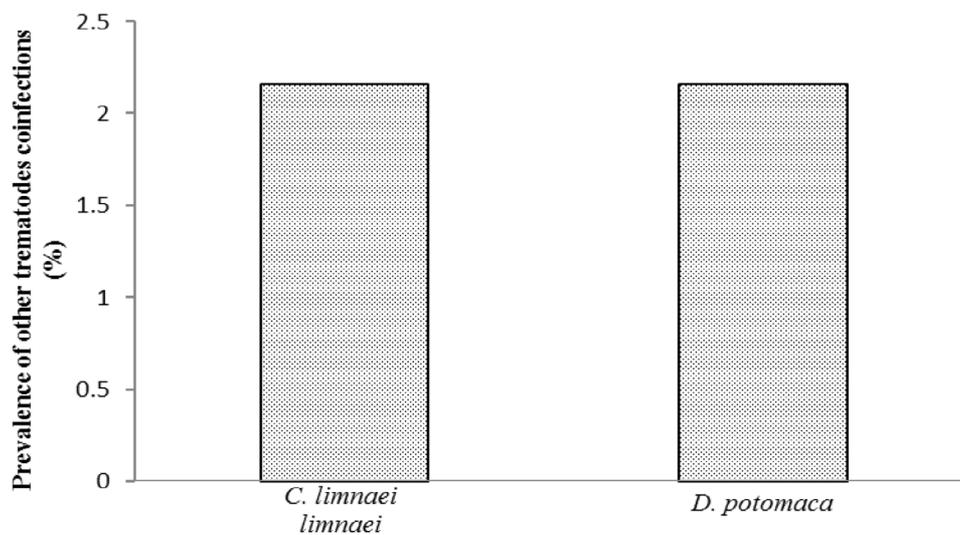


Figure 2. Prevalence of single infections of the symbionts in *Bulinus globosus*.



Cooccurring symbionts in *B. globosus*



Cooccurring symbionts in *B. globosus*

Figure 3. Prevalence of coinfections of the symbionts in *Bulinus globosus*.

<8 mm and ≥8 mm was comparable ($P = 0.844$). The mean intensity of *S. haematobium* in the snails ≥8 mm was 420 ± 258.84 . The snails <8 mm and infected with the other trematodes had significantly higher mean intensity than the ≥8 mm snails ($P = 0.007$). Mean intensity of *C. limnaei limnaei* in the snails <8 mm was not significantly different from that of the snails ≥8 mm ($P = 0.857$). The mean

intensity of *D. potomaca* infection in the snails <8 mm was significantly higher when compared with the snails ≥8 mm ($P = 0.014$) (Table).

Prevalence of *S. haematobium* in both single infection and coinfection was low in the present study. The low prevalence of *S. haematobium* may be attributed to lower proportions of the snails reaching the cercarial shedding

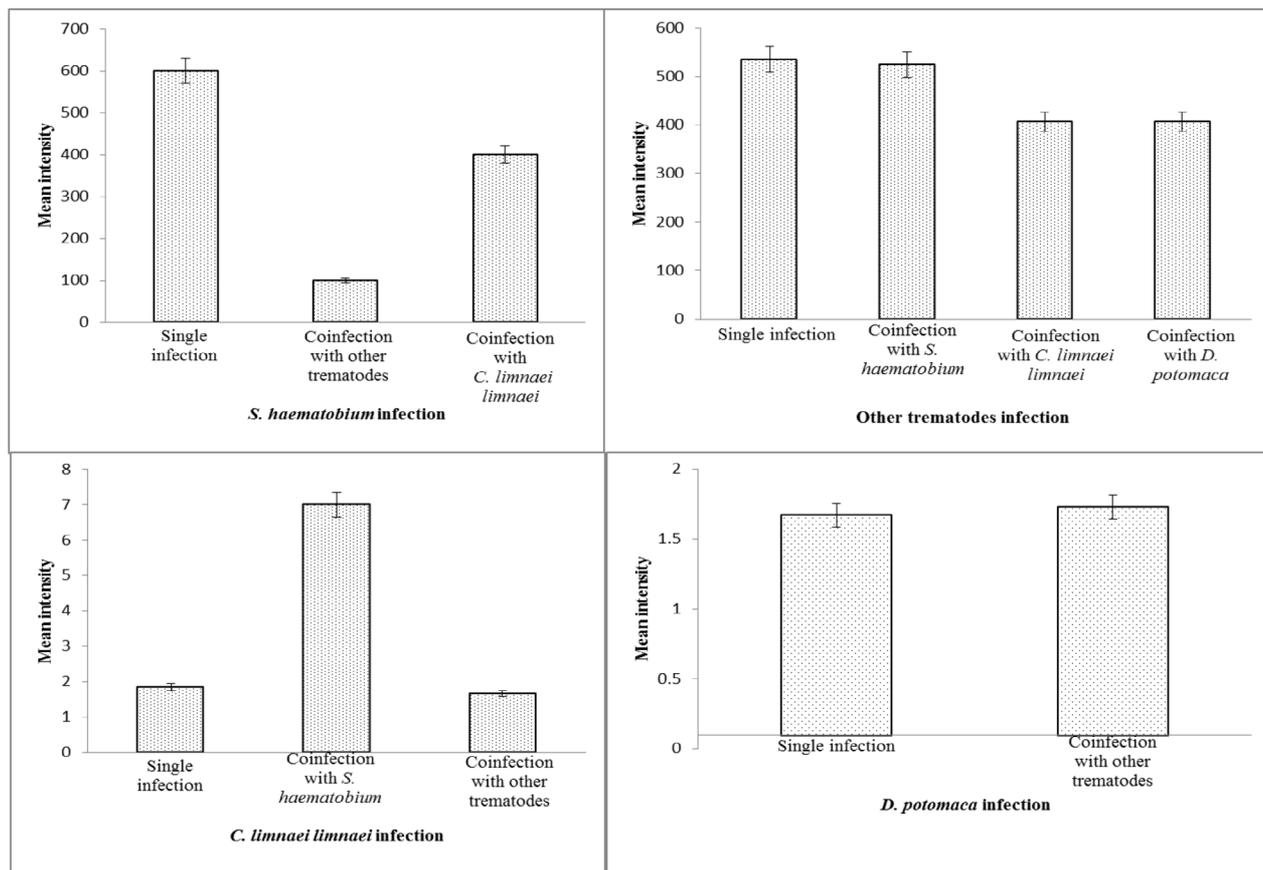


Figure 4. Mean intensity of single and coinfections of the symbionts in *Bulinus globosus*.

Table. Prevalence and mean intensities of the symbionts in the two shell length classes of *Bulinus globosus*.

Symbionts	Prevalence (%)		Intensity (mean ± SD)	
	Size (mm)		Size (mm)	
	<8	≥8	<8	≥8
Trematodes				
<i>S. haematobium</i>	0	1	0	420 ± 115.76
Other trematodes	34.3	53.3*	679.93 ± 82.79	502.32 ± 24.76*
Ectosymbiotic annelid <i>C. limnaei limnaei</i>	1.7	4.2	2.33 ± 0.88	2.14 ± 0.39
Parasitic nematode <i>D. potomaca</i>	4.7	5	2.87 ± 0.58	1.64 ± 1.99*

*Significant difference at P < 0.05.

stage after several weeks of prepatent infection (Opisa et al., 2011). The reduction in the mean intensity of *S. haematobium* when it cooccurred with *C. limnaei limnaei* corroborates results of several studies that have shown that *C. limnaei limnaei* limits prevalence and intensity of trematode parasites through consumption of miracidia and cercariae (Rodgers et al., 2005). In the present study, the

significantly higher mean intensity of *C. limnaei limnaei* in coinfection with *S. haematobium* also gives credence to the established observations. Hopkins et al. (2013) found that in endemic areas, as snail hosts are exposed to pulses of infection, the percentage of parasites successfully infecting the host increases as parasite abundance increases, exceeding the ability of the predators to intercept parasites.

This may explain the lack of significant variation in the mean intensity of *S. haematobium* in single infection and coinfection with *C. limnaei limnaei*.

As shown by the high prevalence, it is plausible that the trematodes categorized as “other trematodes” in the present study are the dominant morphotypes in the lake. However, *C. limnaei limnaei* did not significantly protect the snails from infections of these other trematodes. Hopkins et al. (2013) also found that the numerical strength of predators affects their response to parasite pulses. Consequently, lack of significant protection of the snails from infections by other trematodes may be attributed to the low mean intensity of *C. limnaei limnaei*. Nevertheless, there was a reduction in the mean intensity of the other trematodes when it cooccurred with *C. limnaei limnaei* and *D. potomaca*. According to Cézilly et al. (2014), the reduction may be related to the decreased fitness the other trematodes are subjected to as their host’s manipulative efforts increase. In the work of Zimmermann et al. (2011), trematodes through competition and predation were

negatively associated with the nematode parasite, but in the present study, the other trematodes were unable to prevent establishment of *D. potomaca* infection. The differing sites of infection of *C. limnaei limnaei* and *D. potomaca* in the snail host may explain the absence of cooccurrence between these two species.

The snails ≥ 8 mm had higher prevalence of infection in all organisms studied. The higher prevalence may be ascribed to the very active nature of the larger snails. However, the smaller snails (<8 mm), except for *S. haematobium* infection, had higher mean intensity. The higher mean intensity observed in the smaller snails may be linked to their higher susceptibility due to their weaker defense system, as stated by Namsanor et al. (2015).

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