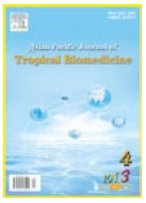
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(13)60061-1

Experimental evaluation of *Candonocypris novaezelandiae* (Crustacea: Ostracoda) in the biocontrol of *Schistosomiasis mansoni* transmission

Fouad Yousif[1*], Sherif Hafez[2], Samia El Bardicy[1], Menerva Tadros[1], Hoda Abu Taleb[1]

[1]*Department of Environmental Research and Medical Malacology, Theodor Bilharz Research Institute, Warrak El Hadar, Imbaba, Egypt*
[2]*Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*

PEER REVIEW

Peer reviewer

Dr. Lim Boon Huat, Biomedicine Programme, School of Health Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
Tel: +6 09767 7619
Fax: +6 09767 7515
E-mail: limbh@kb.usm.my

Comments

In general, this is a well designed and laboratory-based study on the effectiveness of *C. novaezelandiae* in reducing the populations of both the intermediate snail stage and the free-swimming larvae of *S. mansoni*. It would be interesting to know the effect in a field study.

(Details on Page 272)

ABSTRACT

Objective: To test *Candonocypris novaezelandiae* (Baird) (*C. novaezelandiae*), sub-class Ostracoda, obtained from the Nile, Egypt for its predatory activity on snail, *Biomphalaria alexandrina* (*B. alexandrina*), intermediate host of *Schistosoma mansoni* (*S. mansoni*) and on the free-living larval stages of this parasite (miracidia and cercariae). **Methods:** The predatory activity of *C. novaezelandiae* was determined on *B. alexandrina* snail (several densities of eggs, newly hatched and juveniles). This activity was also determined on *S. mansoni* miracidia and cercariae using different volumes of water and different numbers of larvae. *C. novaezelandiae* was also tested for its effect on infection of snails and on the cercarial production. **Results:** *C. novaezelandiae* was found to feed on the eggs, newly hatched and juvenile snails, but with significant reduction in the consumption in the presence of other diet like the blue green algae (*Nostoc muscorum*). This ostracod also showed considerable predatory activity on the free-living larval stages of *S. mansoni* which was affected by certain environmental factors such as volume of water, density of *C. novaezelandiae* and number of larvae of the parasite. **Conclusions:** The presence of this ostracod in the aquatic habitat led to significant reduction of snail population, infection rate of snails with schistosme miracidia as well as of cercarial production from the infected snails. This may suggest that introducing *C. novaezelandiae* into the habitat at schistosome risky sites could suppress the transmission of the disease.

KEYWORDS

Candonocypris novaezelandiae, *Schistosoma mansoni*, *Biomphalaria alexandrina*, Biological control, Schistosomiasis

1. Introduction

Schistosomiasis is a serious public health problem in Egypt and several other countries in the tropical and subtropical regions of Africa, Asia and South America[1]. Planorbid snails play a major role as intermediate hosts of the schistosome parasites and consequently in the transmission of this disease. It was claimed that current population-based schistosomiasis treatment programs are the first step to reduce the global burden of *Schistosoma*-related disease[2]; however, they might not dramatically reduce parasite transmission in highly endemic areas. The real health benefits of transmission control are needed to be reconsidered and attention should be given to more aggressive and, ultimately, more affordable parasite elimination strategies. The next generation of schistosomiasis control can be optimized using new monitoring tools and effective transmission containment.

Snail control strategies are considered a priority for the reduction of transmission. One of the main methods of snail control comprises the application of chemical molluscicides which could give only a temporary relief[3]. Another method is the biological control using specific organisms to eliminate or minimize the population of the

*Corresponding author: Fouad Yousif, Department of Environmental Research and Medical Malacology, Theodor Bilharz Research Institute, Warrak El Hadar, Imbaba, Egypt.
Tel: +2/035406656
Fax: +2/035406656
E-mail: fouadyousif@hotmail.com

Fundation Project: Supported by the Academy of scientific Research and Technology in Egypt, Program of the National Strategy for Biotechnology and Genetic Engineering (Grant No. 41/2005).

Article history:
Received 10 Feb 2013
Received in revised form 25 Feb, 2nd revised form 28 Feb, 3rd revised form 7 Mar 2013
Accepted 18 Mar 2013
Available online 28 Apr 2013

vector snails without negative impact on the environment. In this respect, many organisms were reported to be variably successful biocontrol agents such as viruses, fungi, bacteria and protozoa. Predators belonging to almost all groups of animal kingdom such as leeches, insects and their larvae, crustaceans, fish and mammals are effective biocontrol agents[4-7]. Microcrustaceans and fish have been observed to eat cercariae of some species, although the possibility that predation represents a significant source of mortality for cercariae has been largely unexplored[8]. Although such variety of organisms could be considered as potential biocontrol agents especially under certain conditions in habitats, the ideal one is still missing.

Ostracods are one of the most successful crustacean groups with approximately 8 000 living species. They are found in practically every aquatic environment including freshwater lakes, ponds and streams. *C. novaezelandiae* is found in the River Nile and several streams in Egypt. It is found in many cases associated with aquatic plants like *Eichhornia crassipes*. At the same time, aquatic plants furnish a good habitat for the schistosome vector snails. A review of the literature shows that evaluation of any ostracod as a biocontrol agent in *Schistosoma* transmission is not found. Ostracods naturally feed on dead organic material, suspended particles and microscopic plants, and some are predators. *Cypris* spp. are not known to be specifically able to feed on parasites or snails. In the present study, an ostracod, namely, *Candonocypris novaezelandiae* (Baird) (*C. novaezelandiae*) was tested as a potential bicontrol agent against schistosomiasis transmission in Egypt by its potential predation on the snail intermediate host *Biomphalaria alexandrina* (*B. alexandrina*) and against the free-living larval stages of the parasites as well as reducing the snail infection with the parasite.

2. Materials and methods

2.1. Ostracod, snail and schistosome

The materials used in this study were large numbers of the ostracod *C. novaezelandiae* (Baird) (sub-class Ostracoda, class Maxillopoda, phylum Arthropoda), the schistosome vector snail *B. alexandrina* Ehrenberg (Planorbidae) and the free larvae (miracidia and cercariae) of *Schistosoma mansoni* Sambon (Schistosomatidae) (*S. mansoni*). The original sample of *C. novaezelandiae* individuals (about 200) was obtained from the Nile in Giza Governorate and maintained in the laboratory. They were found to belong to two species of ostracods. About 50 individuals of C. novaezelandiae were picked and carried to a special aquarium where they were maintained under suitable laboratory conditions of temperature, light and algae food to reproduce forming a large colony. The snails, their egg masses and the free larval stages of the parasite (miracidia and cercariae) were obtained from the big colonies at the Schistosome Biological Supply Centre of Theodor Bilharz Research Institute.

2.2. Predatory test for snail eggs

To study the effect of eggs density on the predatory activity of *C. novaezelandiae*, a number of Petri dishes containing 50 mL dechlorinated water were provided each with 100 *C. novaezelandiae* and different numbers of *B. alexandrina* eggs (1-20 egg masses containing 22-371 eggs). Three replicates were set up and all Petri dishes were maintained at (25±2) °C in the laboratory and examined after 24 h using a stereomicroscope. The number of consumed or injured eggs was counted and recorded in each case.

2.3. Predatory test for snails

The predatory activity of *C. novaezelandiae* on *B. alexandrina* snails was tested on both newly hatched and juvenile snails (1 and 3 mm shell height respectively). In the first case, 11 Petri dishes with each containing 50 mL dechlorinated water and 100 *C. novaezelandiae* were provided with different numbers of snails (5-50) and three replicates were set up. The Petri dishes were examined after 24 h, and the number of consumed or injured snails was counted and recorded in each case. The predatory activity on juvenile snails was studied using 50 snails in Petri dish and three replicates were set up. Dead snails were counted daily for 5 d and the mortality rate was calculated in each case.

To elucidate the effect of presence of other diet on the predatory activity of *C. novaezelandiae*, 9 Petri dishes were set up, each containing 50 mL dechlorinated water, 100 *C. novaezelandiae* and 100 *B. alexandrina* eggs. Among them, 3 Petri dishes were provided with excess of blue green algae (*Nostoc muscorum*), another 3 with excess of boiled lettuce leaves and the third group (3 Petri dishes) was left as control. This means that 3 replicates were set up and the eggs were examined daily for 3 d using a stereomicroscope and the consumed eggs were counted in each case.

2.4. Predatory test for the free-living larval stages of S. mansoni

The effect of *C. novaezelandiae* on the survival of *S. mansoni* miracidia and cercariae was tested using three different combinations. Thus, in one combination, different volumes of dechlorinated water (50, 100 and 500 mL) with 100 miracidia or cercariae and 10 *C. novaezelandiae* were used. In another combination, different numbers of free larvae (100, 200 and 300) in the same volume of water (50 mL) and 10 *C. novaezelandiae* were set up. In the third combination, different numbers of *C. novaezelandiae* (10-70) in the same volume of water (50 mL) and the same number of larvae (100) were used. In all cases, *C. novaezelandiae* were maintained with the free larvae (miracidia and cercariae) for 1 h after which the survived organisms were counted using a stereomicroscope. The miracidia and cercariae were used within 1 h after hatching or shedding respectively. Three replicates were performed in each case.

2.5. Predatory test for snails infected by Schistosoma miracidia

Four Petri dishes were provided, each with 25 *B. alexandrina* snails of close size (4-5 mm shell height)

and 10 mL dechlorinated water. The snails were exposed in mass to 10-12 miracidia/snail for 2 h. Three of the Petri dishes were provided with 10, 50 and 100 *C. novaezelandiae* and the 4th was maintained without *C. novaezelandiae* as control. Three replicates were performed in each case and all groups were maintained under the same laboratory conditions. All miracidially exposed snails were maintained and fed according to the routine standards in the laboratory. Starting from day 30 post miracidial exposure, the surviving snails were examined individually for cercarial shedding by exposing them for 1 h to 40 W florescent lamp, 40 cm far, at (25±2) °C. Snails that proved to be positive were isolated. Mortality and infection rates of snails during the incubation period were determined.

Six small beakers each containing 10 mL dechlorinated tap water and five cercarially shedding snails were set up. Fifity *C. novaezelandiae* were added to each one of three beakers and the other three were maintained without *C. novaezelandiae* as control. The emerged cercarial suspension was poured each hour in a graduated Petri dish and replaced by an equal volume of water and equal numbers of *C. novaezelandiae*. A few drops of Bouin fluid were added to each cercarial suspension to kill, fix and colour them and cercariae were counted using a stereomicroscope. The hourly cercarial production was determined for 6 h in the presence or absence of the ostracod to elucidate the effect on shedding pattern.

2.6. Statistical analysis

Results are expressed as mean±SD or number of snails. Comparison between the three different groups was analyzed using the ANOVA test followed by the *post-Hoc* Sheffe test. The data were considered significant if $P<0.05$, highly significant if $P<0.01$ and very highly significant if $P<0.001$. Results of two groups were compared using the non-parametric Wilcoxon-Mann-Whitney *U*-test, while the *Chi*-square test was used to compare categorical data. Statistical analysis was performed with the aid of the SPSS computer program (version 18 for windows).

A logistic regression model was constructed to evaluate the effects of *C. novaezelandiae* ($r=0.57$).

Logistic regression model:

y=exp[-4.185 818-(0.024 9×Number of cercariae)-(0.746 4×Number of miracidia)-(1.003 9×Number of eggs)]

3. Results

3.1. Predatory activity of C. novaezelandiae on B. alexandrina snail eggs

C. novaezelandiae individuals were found to feed on snail egg masses and were observed to gather to feed on a single egg mass. The median numbers of eggs tested and consumed were 354 and 111, respectively, showing a consumption rate of 31.4% (Figure 1). The daily number of eggs consumed by 100 *C. novaezelandiae* maintained in the same volume of water (50 mL) was found to vary with the availability of eggs. However, the rate of consumed eggs was significantly less ($P<0.01$) if the number of eggs was larger (Figure 2).

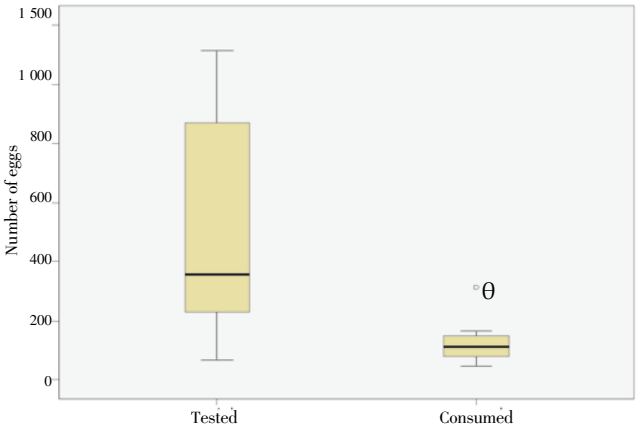


Figure 1. Box plots of the total number of eggs tested and that of eggs consumed.
The horizontal line inside each box represents the median, while the top and bottom of boxes represent the 25th and 75th percentiles, respectively. Vertical lines from the ends of the box encompass the extreme data points. θ represents the outliers.

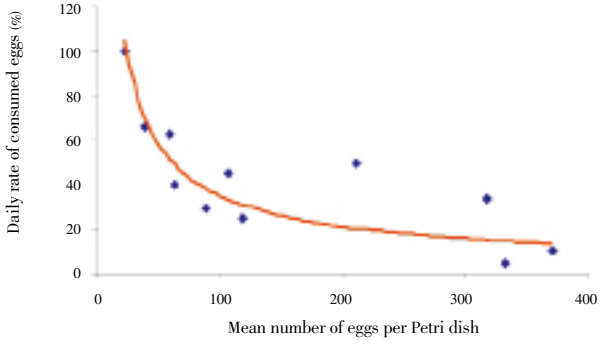


Figure 2. Effect of condensation of *B. alexandrina* eggs on predatory capacity of *C. novaezelandiae*.
Daily rate of consumed eggs was calculated using number of eggs consumed by 100 *C. novaezelandiae*.

3.2. Predatory activity of C. novaezelandiae on B. alexandrina snails

The results showed that predation of *C. novaezelandiae* on hatched snails had a similar pattern to that on eggs. The number of newly hatched snails consumed by 100 *C. novaezelandiae* after 1 d was 2-15. The rate of predation (Figures 3 and 4) was significantly ($P=0.001$) high when the number of available snails was low, and it decreased gradually with the increase of snails. Moreover, it was observed that *C. novaezelandiae* could prey on juvenile snails and almost all available snails were consumed in the duration of the experiment. It was also found that the cumulative rate of consumption of juvenile snails by 100 *C. novaezelandiae* was 16.70%, 46.70%, 60.83% and 96.70% in 5 successive days. The daily predation was non-significant.

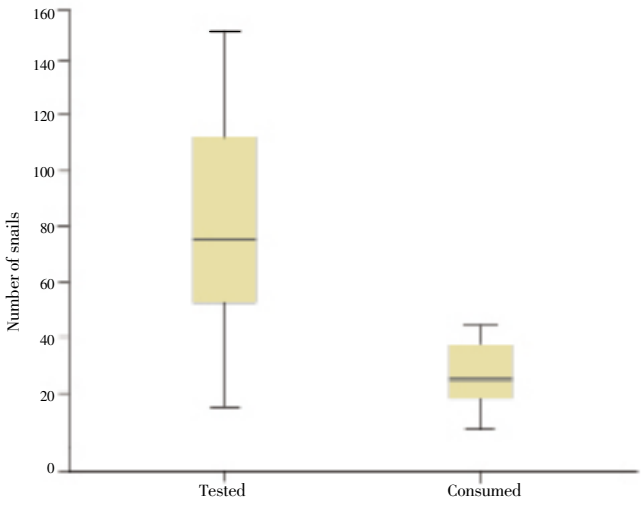


Figure 3. Box plots of the total number of *B. alexandrina* snails tested and that of snails consumed.
The horizontal line inside each box represents the median, while the top and bottom of boxes represent the 25th and 75th percentiles, respectively. Vertical lines from the ends of the box encompass the extreme data points.

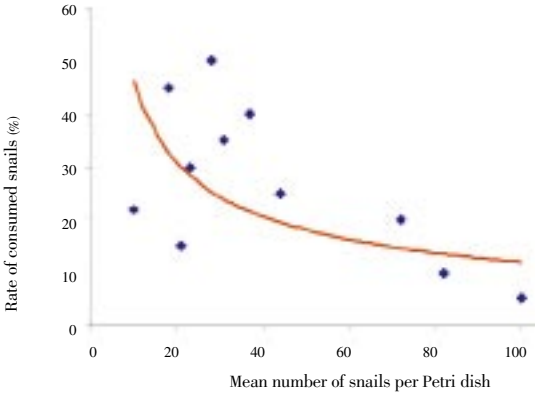


Figure 4. Effect of density of newly hatched *B. alexandrina* snails on predatory activity of *C. novaezelandiae*.

3.3. Effect of presence of other diet on the predatory activity of C. novaezelandiae on snail eggs

During the 3-day observation, both lettuce and algae showed significant reduction ($P<0.01$) in predation of *C. novaezelandiae* on snail eggs. Comparing both types of food, the algae made more slight reduction than the lettuce ($P<0.05$) (Figure 5).

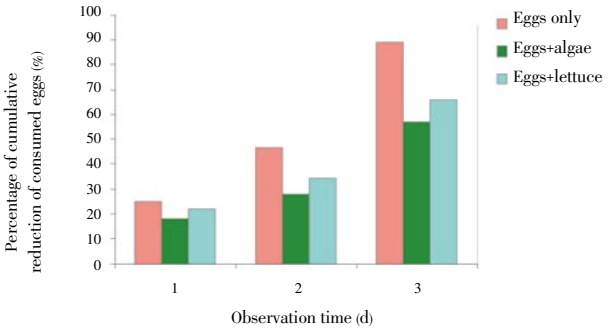


Figure 5. Predatory activity of *C. novaezelandiae* on *B. alexandrina* eggs in presence of diet.

3.4. Predatory effect of C. novaezelandiae on the free larval stages of S. mansoni

The present results showed that *C. novaezelandiae* could prey on the free-living larvae of *S. mansoni* (miracidia or cercariae), an activity that was found to be dependent on water volume, number of available schistosome organisms and number of *C. novaezelandiae*. The consumption decreased with increase of water volume and increased with increase of *C. novaezelandiae* or schistosome larval organisms, statistically significant ($F=20.227$, $P<0.001$). *C. novaezelandiae* were found sufficient to consume all cercariae and miracidia in 50 mL water in 1 h under the present conditions respectively (Table 1). Comparing the predatory effect on miracidia *vs.* that on cercariae, it was found that increase of water volume and number of schistosome organisms were more significantly effective on miracidia than on cercariae ($P<0.01$), while increase in number of *C. novaezelandiae* had almost the same effect on both schistosome larval stages.

Table 1
Predatory activity of *C. novaezelandiae* on free larvae of *S. mansoni*.

Parameter	Value	Miracidia	Cercariae
Volume of water[a] (mL)	50	44.3±8.5	50.0±7.0
	100	31.3±3.8	54.3±9.3
	500	11.3±1.2	33.3±6.5
Number of organisms (miracidia or cercariae)[b]	100	42.0±13.1	42.0±6.2
	200	80.7±9.3	58.7±16.3
	300	94.0±12.8	72.3±8.7
Number of *C. novaezelandiae*[c]	10	35.0±7.9	56.7±4.2
	20	58.7±6.5	72.3±3.5
	30	89.7±6.8	90.0±2.6
	40	82.3±3.5	90.7±2.5
	50	88.3±1.5	95.3±1.5
	60	96.0±2.0	100.0
	70	100.0	–

[a]: using 10 *C. novaezelandiae* and 100 organisms; [b]: using 10 *C. novaezelandiae* and 50 mL water; [c]: using 100 organisms and 50 mL water.

3.5. Effect of C. novaezelandiae on infection of B. alexandrina snails with S. mansoni miracidia

The results (Table 2) showed that snail mortality increased significantly ($P<0.05$) in the presence of larger numbers of *C. novaezelandiae* during exposure period in comparison with snails not associated with *C. novaezelandiae* (maybe because of possible damage exerted on snails) as well as in comparison with snails exposed to miracidia in presence of few number of *C. novaezelandiae*. Regarding the infection of snails, the presence of 10 *C. novaezelandiae* only with 25 *B. alexandrina* snails in 10 mL of water during the miracidial exposure did not affect the infection rate significantly. However, with the increasing number of *C. novaezelandiae* individuals, the infection rate of snails significantly decreased ($P<0.001$). This reducing effect was almost similar for 50 and 100 *C. novaezelandiae*.

Table 2
Effect of different numbers of *C. novaezelandiae* on infection of *B. alexandrina* snails with *S. mansoni* miracidia.

Number of *C. novaezelandiae*	Mortality of snails (%)	Survival of snails (%)	Infection percentage of snails (%)
10	28.0±6.1	54	55.5±8.0
50	40.0±4.6	45	20.0±3.6
100	56.0±12.2	33	18.2±3.0
Control	24.0±6.1	57	63.2±8.9

3.6. Effect of C. novaezelandiae on cercarial shedding

In the control group, the average number of released cercariae/infected *B. alexandrina* snail was 1 392.5 during the 1st hour of exposure to light. This number considerably gradually decreased in the following hours and became distinctly lower starting from the 4th hour to reach about 212.5 cercariae/snail in the 6th hour. The presence of *C. novaezelandiae* in the medium of the shedding snails proved to cause significant reducing effect on the cercariae production throughout the observation period (Figures 6 and 7), being more significant after 6 h (F=10.354, P<0.001). Using the Scheffe test to compare cercarial production in presence and absence of *C. novaezelandiae,* this result was confirmed (P<0.01).

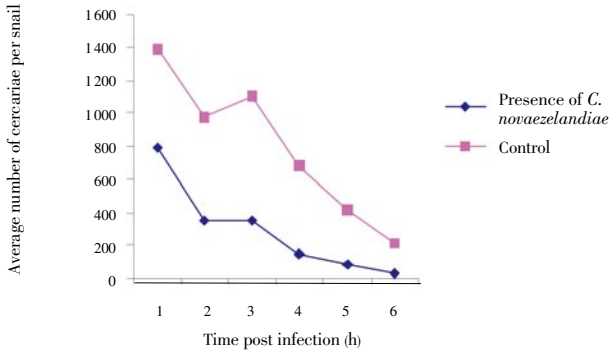


Figure 6. Effect of *C. novaezelandiae* on pattern of cercarial shedding of infected *B. alexandrina*.

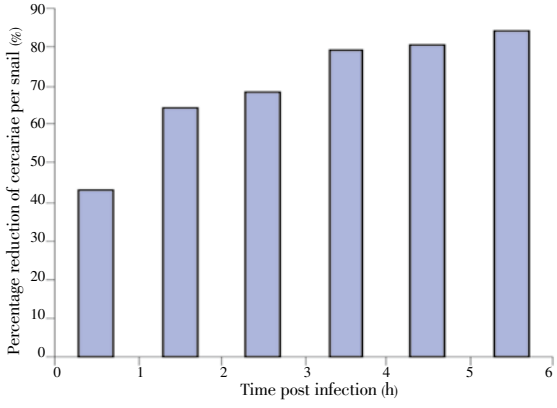


Figure 7. Effect of *C. novaezelandiae* on cercarial production of infected *B. alexandrina*.

4. Discussion

The present study demonstrated variable predatory activities of the ostracod *C. novaezelandiae* on *B. alexandrina* snails, the intermediate host of *S. mansoni* in Egypt, as well as on the free living stages of the parasite. Such effect was found to depend on certain environmental factors such as water volume, density of snails, larval stages of the parasite as well as density of *C. novaezelandiae* individuals. However, the availability of other diet in the habitat like blue green algae or vegetation (lettuce in the study) slightly reduced consumption of snail eggs. Moreover, the presence of *C. novaezelandiae* in the habitat during the exposure of snails to schistosome miracidia significantly reduced mortality and infection rate of snails and consequently the following production of cercariae. Ostracod has not been evaluated as a possible biocontrol agent in *Schistosoma* transmission. Therefore, the present study on *C. novaezelandiae* should draw attraction to a new method of biocontrol of schistosomiasis transmission. It is thought that this ostracod does not affect the natural habitats, since high densities are not associated with significant negative effects. The application of this ostracod would be achieved by introducing large numbers in schistosome risky sites. This organism could be produced in large scale by cultivation, which should be carried out under controlled optimal conditions. It could be introduced into the natural system at the proper time, considering the water volume, temperature and season. However, final evaluation of the present ostracod as a biocontrol agent against schistosomiasis will be arrived at after testing under simulated natural conditions.

Other crustacea like *Daphnia magna* (Copepoda) was found to consume miracidia and consequently reduce the infection rate of vector snails as well as production of cercariae[9]. Previously, Christensen *et al* found that *Daphnia pulex* significantly protected *Lymnaea truncatula* snails from infection with *Fasciola hepatica*[10].

Similarly, predation of snail eggs by sciomyzid larvae (Diptera: Sciomyzidae) was claimed by the researchers[11-13]. Recently, it is reported on larval feeding preference in the case of the sciomyzid fly *Sepedon scapularis*[5]. The present results showed that predation was affected by certain environmental factors, which is agreed with the results of scholars who claimed that snail predation by *Psychoda alternata* (Diptera: Psychodidae) larvae is influenced by the morphology and behavior of the mollusc which makes it more or less vulnerable[14]. The predation by *Psychoda* larvae on juvenile *B. alexandrina* snails showed that this activity on the free-swimming schistosome stages (miracidia and cercariae) depends on some factors such as water volume and density of larvae. They also found that experimental infection of *Biomphalaria glabrata* (*B. glabrata*) snails with schistosome miracidia and the total periodic cercarial production were significantly reduced by *Psychoda* in comparison with the control group. The prevalence of infection by *S. mansoni* in *B. glabrata* was significantly higher in snails that were devoid of *Chaetogaster limnaei* (Oligochaeta: Annelida), indicating that such oligochaete may protect the host from trematode infection[7,15]. The reduction of the cercarial production from infected snails by *C. novaezelandiae* may be explained by the predatory effect of this organism on the cercariae. Another explanation may be that this is due to the mechanical effect *C. novaezelandiae*

may exert on body wall of infected snails.

In conclusion and as universally agreed, the best possible method for schistosomiasis control may be the attack upon the vector snails and free-living larvae of the parasite. Therefore, the present results showing that introduction of *C. novaezelandiae* in a risky transmission sites of schistosomiasis could contribute markedly to this concept.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The work was supported by the Academy of Scientific Research and Technology in Egypt, Program of the National Strategy for Biotechnology and Genetic Engineering (Grant No. 41/2005). The authors thank Prof. Dr. Farouk R. Melek, Natural Compounds Department, National Research Center, Giza, Egypt, for his advice and help during the performance of this work.

Comments

Background

Schistosomiasis is a serious public health problem in the tropics and subtropical countries, including Egypt. The accepted control measure is to reduce the populations of snail vectors and free-living larvae of *Schistosoma* spp. This original paper based on results obtained from laboratory-based experiment highlighted the potential of utilizing *C. novaezelandiae* as a biological agent to break the transmission of *S. mansoni*.

Research frontiers

The currently used chemical molluscicides are not environment friendly, and they provide only temporary relief in snail control. Since no ideal biological predators have been reported to control the intermediate host for *S. mansoni* transmission, this paper reported the potential of using *C. novaezelandiae* as a biological agent not only for the *B. alexandrina* snail, but also revealed that it predated on the free-living larval stages of *S. mansoni*.

Related reports

Gawish *et al.* (2006) reported the positive effect of another crustacea, *Daphnia magna* in reducing the infection rate of *B. alexandrina* snail and also the production of free-swimming stages of *S. mansoni*. This study tested similar effect but utilized a different predator, namely, *C. novaezelandiae*.

Innovations and breakthroughs

Reports on biological control of snail populations are scarce and new ones are unavailable. This is the first paper that reported the potential of an ostracod, *C. novaezelandiae*, as a biological agent for control of *S. mansoni* transmission.

Applications

Chemical molluscicides are not environment friendly. If *C. novaezelandiae* proves to be effective in field study, then the potential would be huge, especially because the predator also feed on the free-swimming larvae of *S. mansoni*.

Peer review

In general, this is a well designed and laboratory-based study on the effectiveness of *C. novaezelandiae* in reducing the populations of both the intermediate snail stage and the free-swimming larvae of *S. mansoni*. It would be interesting to know the effect in a field study.

References

[1] WHO Expert Committee. Prevention and control of schistosomiasis and soil transmitted helminthiasis. *World Health Organ Tech Rep Ser* 2002; **912**: 1-57.

[2] King CH, Sturrock RF, Kariuki C, Hamburger J. Transmission control for schistosomiasis-why it matters now. *Trends Parasitol* 2006; **22**: 575-582.

[3] Aditya G, Raut SK. Predation potential of the water bugs *Sphaerodema rusticum* on the sewage snails *Physa acuta*. *Mem Inst Oswaldo Cruz, Rio de Janeiro* 2002; **97**: 531-534.

[4] Ismail M. The miracidiophagic and cercariophagic activity of the fish *Gambusia affinis* and *Oreochromis niloticus* and their effect on the infection of *Biomphalaria alexandrina* by *Schistosoma mansoni*. *Egypt J Aqu Biol* 2003; **7**: 87-98.

[5] Maharaj R, Naidoo I, Appleton CC. Susceptibility of schistosome host snails to predation by sciomyzid flies (Diptera: Sciomyzidae) in South Africa. *African J Aqu Sci* 2005; **30**: 175-178.

[6] Yousif F, Nashid N, Roushdy MZ, Tadros M, Ayoub M. Factors affecting predation of the freshwater leech *Helobdella punctatolineata* (Glossiphoniidae) on the schistosome vector snail *Biomphalaria alexandrina* (Planorbidae) under laboratory conditions. A- Biotic factors. *Egypt J Zool* 2006; **46**: 175-187.

[7] Tadros M, El Bardicy S, Gawish F, Yousif F. Experimental studies on effect of *Chaetogaster limnaei limnaei* (Oligochaeta: Annelida) on *Schistosoma mansoni* transmission in aquatic habitats. *New Egypt J Med* 2009; **40**: 198-204

[8] Schotthoefer AM, Labak KM, Beasley VR. *Ribeiroia ondatrae* are consumed by aquatic invertebrate predators. *J Parasitol* 2007; **93**: 1240-1243.

[9] Gawish F, El-Khayat H, Ismail N. Predatory activity of *Daphnia magna* on the free swimming stages of *Schistosoma mansoni* and on infection of *Biomphalaria alexandrina* with *Schistosoma mansoni* miracidia. *Egypt J Zool* 2006; **47**: 119-129.

[10] Christensen NO, Nansen P, Frandsen F. Interference with *Fasciola hepatica* snail finding by various aquatic organisms. *Parasitol* 1977; **74**: 285-290.

[11] Manguin S, Vala JC. Prey consumption by larvae of *Tetanocera ferruginea* (Diptera: Sciomyzidae) in relation to number of snail prey species available. *Entomol Soc Am* 1989; **82**: 588-592.

[12] Maharaj R, Appleton CC, Miller RM. Snail predation by larvae of *Sepedon scapularis* Adams (Diptera: Siomyzidae), a potential biocontrol agent of snail intermediate hosts of schistosomiasis in South Africa. *Med Veterin Entomol* 1992; **6**: 183-187.

[13] Foote BA, Keiper JB. The snail killing flies if Ohio (Insecta: Diptera: Sciomyzidae). *Kirtlandia* 2004; **54**: 43-88.

[14] El Bardicy S, Tadros M, Yousif F, Hafez S. Predatory activity of *Psychoda alternata* Say (Diptera: Psychodidae) larvae on *Biomphalaria glabrata* and *Lymnaea natalensis* snails and the free- living larval stages of *Schistosoma mansoni*. *Australian Bas Appl Sci J* 2009; **3**: 4503-4509.

[15] Rodgers JK, Sandland GJ, Joyce SR, Minchella DJ. Multi-species interactions among a commensal (*Chaetogaster limnaei limnaei*), a parasite (*Schistosoma mansoni*), and an aquatic snail host (*Biomphalaria* glabrata). *Parasitol J* 2005; **91**: 709-712.