Divergent Effects of *Schistosoma haematobium* Exposure on Intermediate-Host Snail Species *Bulinus nasutus* and *Bulinus globosus* from Coastal Kenya

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Abstract.

Schistosoma haematobium infection causes urogenital schistosomiasis, a chronic inflammatory disease that is highly prevalent in many parts of sub-Saharan Africa. Bulinid snails are the obligate intermediate hosts in the transmission of this parasite. In the present study, *Bulinus globosus* and *Bulinus nasutus* snails from coastal Kenya were raised in the laboratory and exposed to miracidia derived from sympatric *S. haematobium* specimens to assess the species-specific impact of parasite contact and infection. The snails' subsequent patterns of survival, cercarial shedding, and reproduction were monitored for up to 3 months postexposure. *Schistosoma haematobium* exposure significantly decreased the survival of *B. globosus*, but not of *B. nasutus*. Although both species were capable of transmitting *S. haematobium*, the *B. globosus* study population had a greater cumulative incidence of cercarial shedders and a higher average number of cercariae shed per snail than did the *B. nasutus* population. The effects of prior parasite exposure on snail reproduction were different between the two species. These included more numerous production of egg masses by exposed *B. nasutus* (as compared with unexposed snails), contrasted to decreased overall egg mass production by parasite-exposed *B. globosus*. The interspecies differences in the response to and transmission of *S. haematobium* reflect clear differences in life histories for the two bulinid species when they interact with the parasite, which should be taken into account when planning control interventions aimed at reducing each host snails' contribution to local transmission of *Schistosoma* infection.

INTRODUCTION

The trematode blood fluke *Schistosoma haematobium* is responsible for urogenital schistosomiasis, a chronic inflammatory disease that results in fibrotic or obstructive pathology of the human genitourinary organs.[1] Manifestations of this disease include hematuria, pelvic pain, bladder cancer, subfertility, renal dysfunction, and anemia.[1,2] *Schistosoma haematobium* infection currently affects over 100 million people, with the greatest prevalence occurring in rural or less-developed areas of sub-Saharan Africa.[1] A thorough understanding of the ecology of *S. haematobium* transmission in these areas will be essential to halting its continued transmission.

Local *S. haematobium* transmission requires the presence of particular species of bulinid snails as obligate intermediate parasite hosts.[1,3] In contaminated freshwater bodies, *S. haematobium* miracidia infect the snails, and these go on to develop as parasite sporocysts that reproduce asexually inside their host snail.[1] After approximately 4–6 weeks, snails release

cercariae, the form of *S. haematobium* infectious for humans, which then penetrates the skin of people who enter contaminated water, thus causing new human infection.[1]

Bulinus spp. host snails may be found in a variety of tropical and subtropical freshwater habitats including ponds, rivers, and dams.[3–6] Their population density fluctuates seasonally, following changes in temperature and rainfall.[4–6] These snails are hermaphroditic and can thus reproduce either via self- or cross-fertilization.[3] They typically lay egg masses containing between 5 and 40 embryos, which hatch after about 6–8 days.[3] The snails mature in 4–7 weeks, and their life span is variable, ranging from a just few weeks to a few months depending on local conditions.[6,7] As a result of fluctuations in snail numbers and variations in the timing and efficiency of within-snail parasite reproduction, the local force of infection for *S. haematobium* transmission to humans may vary dramatically over time.

Our present study focused on snail-related factors related to *S. haematobium* persistence in endemic sites in coastal Kenya, where human prevalence ranges from 25% to 90%.[4] *Bulinus globosus* and *Bulinus nasutus* both serve as intermediate hosts for *S. haematobium* in this region, but their habitats tend not to overlap, and their relative contributions toward local transmission are not fully understood.[4,5]

Previous studies, both experimental and field based, have shown that bulinid snails have variable susceptibility to *S. haematobium*. For example, *B. nasutus* has been found to be a permissive host for *S. haematobium* in Msambweni, Kenya, but not in Unguja, Zanzibar, where *B. nasutus* appears to be refractory to the parasite.[8–10] The snail-species-specific effects of *Schistosoma* infection on snail growth, survival, and reproductive success have also been shown to be variable in previous studies.[11–13] In the present study, we aimed to use a controlled laboratory setting to further clarify the relationship between *S. haematobium* exposure and the life histories of its Kenyan *B. globosus* and *B. nasutus* intermediate snail hosts. Postexposure survival, cercarial output, and reproduction were evaluated to investigate the different roles these two snails might play in natural transmission scenarios.

MATERIALS AND METHODS

Snail cultivation.

Exposed and unexposed *B. globosus* and *B. nasutus* snails were kept in separate groups in glass aquaria measuring 25 cm depth × 22 cm width × 40 cm length. All of the aquaria were kept under the same conditions in the same room. There was no air-conditioning, but fans were running throughout the study period. Ambient noontime room temperatures ranged from 27.5 to 31.8°C during the experiment. Water collected from ponds where snails were known to breed well was heated to above 50°C to kill any living organisms, allowed to cool, and then used in the aquaria. Water pH varied from 7.6 to 8.3. Small stones were also introduced into each aquarium to provide additional calcium. The snails were fed every day with boiled (then dried) lettuce supplemented with nutrient-rich cereals meant for humans (Weetabix Limited) and fish food. Water was changed weekly, and in the process, it was filtered through a fine sieve of less than 1-mm mesh size in order not to discard any young snail hatchlings. Aeration was aided by electric pumps similar to those used in fish aquaria. Strips of transparent plastic sheet of hard consistency were placed in the aquaria as egg-laying surfaces.

To establish the respective *B. globosus* and *B. nasutus* colonies, field-collected snails were incubated as described earlier, and their first-generation offspring were used in the experiments

reported in this study. All of the progenitor field specimens were collected from remote rural sites on the coast of Kenya. *Bulinus nasutus* were collected in August 2003 from Kanana dam II and Ramisi drains, south of Msambweni sub-location. *Bulinus globosus* were collected in August and September 2003 at the Kwatelengo Mwamdulli Timboni quarry, between Mazeras and Mariakani, inland from Mombasa. Preliminary species identification of these colonies was made based on shell morphology, based on standard reference guidelines of the Danish Bilharziasis Laboratory.[14] Species ID was then confirmed by molecular mitochondrial DNA barcode testing, as previously described.[13,15] After hatching, the young snails were allowed attain a minimum size of 5 mm before they were subjected to infection via exposure to *S. haematobium* miracidia.

Snail exposure.

Schistosoma haematobium eggs were collected from pooled urine specimens discarded after community surveys of school-age children residing in the Msambweni sub-location of Kwale County, Kenya.[16] These surveys were performed under ethical review and approval of the University Hospitals of Cleveland Institutional Review Board (protocol 03-88-84), and of the Kenya Medical Research Institute's Ethical Review Committee (KEMRI/RES/7/3/1). Miracidia were obtained by washing and filtering the eggs in normal saline, then hatching them via exposure to fresh water, as described by Hamburger and others.[13]

Each snail was exposed to five miracidia in an individual tube that contained filtered pond water maintained under fluorescent light. When no more living miracidia were detected in the incubation tubes, the initial stage of infection was presumed to be complete.

Cercarial shedding.

Each day, snails were put in individual 3×1 inch flat-bottomed specimen bottles with a known volume of filtered pond water and were exposed to fluorescent light for a maximum of 2 hours. The cercariae shed by each individual snail were estimated using a micropipette to sample a known quantity of water from the vial. Total daily shedding for that snail was then calculated based on the total volume of water present in the vial.

On the day snails began to shed, they were marked with uniquely coded visible identifiers for each day of the study to monitor their duration of shedding and time to mortality.

Snail reproduction.

For each species and each infection exposure status, total egg masses in their respective aquaria were counted each week. To estimate the mean number of embryos per egg mass, five egg masses were randomly selected and their embryos were counted.

Statistical analysis.

Daily survival and shedding data and weekly egg mass data were entered into Excel spreadsheets and analyzed using SPSS software (v.22; IBM, Armonk, NY). Differences between survivals over time were tested using log-rank and Wilcoxon tests. Between-species differences in weekly shedding rates and egg mass production over time were compared using the Mann–Whitney U test. Egg mass embryo data were compared using Student's t test. Two-sided P values less than 0.05 were considered statistically significant.

RESULTS

Species identification of laboratory snail colonies.

The studies reported here were performed on first-generation, laboratory-bred offspring of field-caught *B. nasutus* and *B. globosus* from coastal Kenya. Initial species identification was made based on shell morphology and later confirmed by molecular "barcode" polymerase chain reaction testing of mitochondrial DNA sequence variants for cytochrome oxidase I for four or more snail specimens from each sampling location. The Kanana/Ramisi locations had only *B. nasutus* recovered. The Kwatelengo quarry location yielded only *B. globosus*.

Effect of *S. haematobium* miracidial exposure on survival of *B. globosus* and *B. nasutus*.

As shown in Figure 1A, the survival of *B. globosus* snails was significantly reduced with *S. haematobium* exposure (log-rank $\chi^2 = 131$, df = 1, $P < 0.001$). The median survival of the exposed *B. globosus* group was 6.0 weeks (95% confidence interval [$CI_{95\%}$] = 5.6, 6.4), compared with 12.0 weeks ($CI_{95\%} = 11.1, 12.9$) for the unexposed control group. By contrast, as shown in Figure 1B, the survival of *B. nasutus* was not significantly altered by exposure (log-rank $\chi^2 = 0.14$, df = 1, nonsignificant [NS]), the results showing a median of 5.0 weeks ($CI_{95\%} = 4.1, 5.9$) survival for control snails compared with a median of 6.0 ($CI_{95\%} = 5.1, 6.9$) weeks for exposed snails.

Between the two species, survival of unexposed *B. globosus* was significantly longer than that of unexposed *B. nasutus* (log-rank $\chi^2 = 123$, df = 1, $P < 0.001$), but the survival of exposed *B. globosus* was not significantly different from that of exposed *B. nasutus* (log-rank $\chi^2 = 0.31$, df = 1, NS). Notably, though, a small number of exposed *B. globosus* snails survived into week 20, whereas the exposed *B. nasutus* snails had all died by the end of week 12.

Cercarial shedding patterns of infected *B. globosus* and *B. nasutus*.

Number of snails shedding cercariae.

We next observed the capacity of *B. globosus* and *B. nasutus* to shed *S. haematobium* cercariae. As summarized in Figure 2, the two species of snails had a similar timing for onset and peak periods of cercarial shedding. For each, snails first began to shed *S. haematobium* cercariae on day 21. The peak number of snails shedding occurred on day 28 (week 4) for *B. globosus* and, comparably, on day 30 (week 5) for *B. nasutus*. However, the duration of shedding was appreciably less for *B. nasutus*. The *B. nasutus* population had snails shedding only until day 53 (week 8), whereas the *B. globosus* population had snails shedding until day 79 (week 12).

Day to day, *B. globosus* shedders consistently outnumbered *B. nasutus* shedders, and over the entire study period, 86% of exposed *B. globosus* snails visibly shed cercariae, as compared with just 20% of exposed *B. nasutus* snails.

Number of cercariae produced.

As shown in Figure 3, median weekly cercarial production per snail was substantially greater for surviving *B. globosus* snails (median = 581, interquartile range [IQR] = 91, 2,743) than for *B. nasutus* snails (median = 5, IQR = 0, 41; $U = 217.5$, $P < 0.001$). Across the full study period, which included 4 weeks when only *B. globosus* snails were shedding, the 100 miracidia-exposed

B. globosus snails ultimately shed 90,228 cercariae, whereas the 100 exposed *B. nasutus* snails shed 1,582 cercariae.

Reproductive patterns of *B. globosus* and *B. nasutus*.

Average egg mass production per snail.

Among surviving snails, weekly egg mass production/snail was similar between unexposed and exposed *B. globosus* snails until reproduction by exposed snails ceased at week 20 (Figure 4A). Unexposed snail production ranged from 0.1 to 6.4 masses/snail/week, whereas exposed snail production ranged from 0 to 9 masses/snail/week. The unexposed control snails continued to reproduce throughout the rest of the study observation period. Cumulatively, the egg mass production per exposed *B. globosus* snail plateaued at 38.6 masses, whereas production per control *B. globosus* snail rose to a final value of 52.5 masses/snail. Total aggregate egg mass production was highest for the control group of *B. globosus* (1,645 masses), compared with that of the exposed group of *B. globosus* (232 masses), reflecting, in part, the much longer survival of control snails.

In the case of *B. nasutus*, exposed snails initially produced more egg masses (0.5–2.1 masses/snail/week) than control *B. nasutus* snails (0.2–1.7 masses/snail/week), but their reproduction faded earlier than that of control snails (Figure 4B). Cumulatively, overall individual egg mass production was higher for exposed *B. nasutus* (355 masses) than that for the control unexposed *B. nasutus* group (223 masses). Unlike for *B. globosus*, because *B. nasutus* survival was not affected by exposure, aggregate egg mass production by the two populations of *B. nasutus* snails reflected their average individual egg mass production, with exposed snails ultimately producing more total egg masses.

Embryos per egg mass.

Within each snail species, the number of embryos per egg mass remained fairly consistent between the exposed and unexposed groups (Table 1). For *B. globosus*, the mean number of embryos per mass was 8.85 in the control snail population and 8.81 in the exposed snail population. For *B. nasutus*, the mean was 17.06 for control snails and 17.03 for exposed ones. Between species, the *B. nasutus* snails produced significantly more embryos per egg mass, on average, than that of *B. globosus* snails, both with (Student's $t = 21.6$, $P < 0.001$) and without (Student's $t = 27.6$, $P < 0.001$) *S. haematobium* exposure.

Hatchling production.

All snail populations in this study were able to produce viable hatchlings (Table 2). In the case of *B. globosus*, exposed snails produced more total hatchlings (339) over the course of the study than did control snails (281 total hatchlings). The reverse was true for *B. nasutus*, whose unexposed control population produced 131 hatchlings, while the exposed population only produced 75 hatchlings in total.

DISCUSSION

In the present study, survival, reproduction, and cercarial shedding were monitored for *B. globosus* and *B. nasutus* snails exposed at 4–6 weeks of age to *S. haematobium* miracidia under laboratory conditions. Our results suggest that the survival of *B. globosus* snails was significantly

reduced with *S. haematobium* exposure, but the survival of *B. nasutus* snails was unaffected. Additionally, although both snail species in our study were capable of transmitting *S. haematobium*, *B. globosus* appeared to be the more efficient intermediate host, having a greater percentage of exposed snails that shed cercariae and a much higher average number of cercariae shed per snail. *Schistosoma haematobium* exposure had a variable effect on the reproductive success of each snail species. As measured by the cumulative number of egg masses produced per snail, reproductive success was slightly reduced in exposed *B. globosus* snails; this result appears to mainly be a reflection of the exposed snails' shortened life span. In contrast, exposed *B. nasutus* snails produced, on average, more egg masses than unexposed controls, which may reflect fecundity compensation in response to exposure.[11] Actual reproductive success, as measured by the number of viable hatchlings produced, yielded a different story, with exposed *B. globosus* snails producing more viable hatchlings than their unexposed counterparts, and exposed *B. nasutus* snails producing fewer.

Previous studies have provided inconsistent data on the impact of *S. haematobium* infection on survival, reproductive capability, and transmission potential in *Bulinus* snails. Although decreases in the survival of *B. globosus* in response to *S. haematobium* have been previously demonstrated, other researchers have shown unaffected survival of *Bulinus senegalensis* and *Bulinus truncatus* in response to *S. haematobium*.[11] Laboratory studies using different incubation systems have documented decreased survival of host snail *Biomphalaria glabrata* in response to *Schistosoma mansoni* infection,[12] and decreased survival of *B. nasutus* snails in response to *S. haematobium* exposure, in contrast to the results of the current study.[13]

The enhanced transmission potential of *B. globosus* compared with that of *B. nasutus* in our laboratory correlates with previous findings in field studies. In Zanzibar, Tanzania, an island location not far from our study sites, *B. nasutus* snails collected from local water bodies are not naturally infected with *S. haematobium*, nor are they susceptible to infection in the laboratory.[8–10] However, *B. globosus* snails were infected with *S. haematobium* in Zanzibar, and a strong correlation emerged there between the prevalence of *B. globosus* and the prevalence of urinary schistosomiasis.[8,10] In the present study's environment, the coastal area of Kenya, *B. nasutus* is often the only *S. haematobium*-shedding snail identified in highly endemic areas. In the coastal region, *B. nasutus* occurs in a geographic belt closer to the Indian Ocean than does *B. globosus*, which is found at distances of 30 km or more away from the sea in areas like Mariakani and Kinango. Pringle and others have reported similar allopatric distributions for *B. globosus* and *B. nasutus* in the northeast part of mainland Tanzania, adjacent to our study zone.[17] They similarly found that, after laboratory exposure to *S. haematobium* miracidia, *B. globosus* more frequently developed patent infection than did *B. nasutus*. It is thought that partiality of *B. nasutus* for shallow coastal pools may reflect a greater tolerance to salinity than that of *B. globosus*. Thus, despite the present data showing apparently poor transmission efficacy by *B. nasutus*, this snail's colonization, abundance, and persistence in local waterbodies is nevertheless associated with effective transmission of *S. haematobium* to humans.[4,13] Not measured in our study, it is possible that estivation of more hardy, older, but infected *B. nasutus* in a dried seasonal habitat may result in significantly more rapid resumption of local *S. haematobium* transmission once the monsoonal rains return.[18] It is important, then, not to dismiss *B. nasutus* as an unimportant intermediary of transmission, particularly if it may survive under conditions where *B. globosus* cannot. Enhancement of the relative resistance of *B. nasutus* to patent *S. haematobium* infection or successful blocking of its estivation might prove beneficial in efforts to achieve local elimination of *S. haematobium* transmission.

The enhanced reproductive efforts of our *B. nasutus* snails exposed to *S. haematobium* may represent fecundity compensation, in which snails enhance their reproductive efforts in the face of increased parasite-associated mortality. This response has been observed in a laboratory study of prepatent *B. glabrata* snails, which are infected with, but not shedding *S. mansoni*.[12] Of note, however, despite their increased egg mass production, these *B. glabrata* snails, like our exposed/infected *B. nasutus* snails, did not produce significantly more hatchlings than unexposed controls. The authors suggested that a trade-off is likely to occur in infected snails between the number of eggs produced and the quality of resources allocated to each egg.[12]

Our study is limited in that laboratory results in a highly controlled setting may not necessarily reflect what happens in the wild.[19] Snails in the laboratory were not exposed to changing seasonal climates, predators, and other infections. This may have important implications when drawing conclusions about transmission potential. In particular, the average survival of a bulinid snail in the wild has been reported to be ~3.6–5.7 weeks, and yet it takes 4 weeks for a patent *S. haematobium* infection to develop in a snail.[5] On average, then, fewer than 10% of *B. globosus* snails in field populations are observed to be shedding cercariae, which differs considerably from the 80% shedding that we observed in the laboratory.[5] In our study, longitudinal data were not gathered on individual snails; thus, we were unable to evaluate the interplay of survival, shedding, and reproduction in individual snails. We also did not record snail increase in size over time, though growth measurements could have helped clarify the effects of *S. haematobium* exposure or patent infection on snail maturation, beyond what our survival curves have shown.[19]

Although the study setting was not a natural one, keeping the snails in a laboratory allowed us to accurately track survival, cercarial shedding, and reproduction for defined exposure groups of each species of snail. Of note, survival of *B. nasutus* hatchlings in aquaria is known to be relatively poor,[20] and this may account for the reduced number of *B. nasutus* hatchlings we observed relative to their numbers of egg masses and their embryo numbers per egg mass. To reflect local transmission as closely as possible, the *S. haematobium* strains used in our study were local clinical parasites rather than attenuated, laboratory-bred parasite strains.[11] The snails studied were also low passage from wild snails, thus minimizing the disturbance of genetic factors that might play a role in snail-schistosome compatibility. Finally, in comparison to other studies that raised snails in isolated containers,[12] we raised groups of 100 snails together. This allowed for the expression of density-dependent factors that, based on an Allee effect, have been shown to increase snail growth and survival.[21] Proposed factors that might apply in this incubation setting include increased success in finding a mate and increased, density-dependent release of growth-promoting physicochemical factors.[21]

Overall, management of snail populations will be an essential intermediate phase in achieving the World Health Organization's goal of eliminating urogenital schistosomiasis.[22,23] The data presented here demonstrate significant differences between two species of snails that can serve as *S. haematobium* hosts. By defining these differences and applying them to snail control interventions in the field, we can more effectively target these disease vectors and thus move one step closer to lifting the burden of schistosomiasis.

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FIGURE 1. Kaplan–Meier survival curves for 100 unexposed (control) and 100 *Schistosoma haematobium*-exposed snail populations from each of two bulinid species: (**A**) *Bulinus globosus* and (**B**) *Bulinus nasutus*. Daily survival was monitored over 26 weeks; the indicated percent survival indicates the number of remaining snails. The separate curves show respective control (■) and *S. haematobium*-exposed (■) groups for each species. Relative to the survival of unexposed snails of the same species, survival of exposed *B. globosus* was significantly reduced ($P < 0.001$ by log-rank test), whereas survival of exposed *B. nasutus* was not. This figure appears in color at www.ajtmh.org.

FIGURE 2. Numbers of *Bulinus globosus* and *Bulinus nasutus* snails shedding *Schistosoma haematobium* cercariae each day over the course of the experiment. The *x* axis indicates days since exposure to miracidia. The *y* axis indicates the number of snails shedding each day. ■ = *B. globosus*; ■ = *B. nasutus*.

FIGURE 3. Average daily cercarial production per snail is shown for each week over the first 14 weeks of the study. The total number of *Schistosoma haematobium* cercariae produced each day by exposed (**A**) *Bulinus globosus* and (**B**) *Bulinus nasutus* snails was divided by the respective number of surviving snails. The daily rate shown was the mean daily rate for each study week. ■ = *B. globosus*; ■ = *B. nasutus*.

FIGURE 4. Average egg mass production per *Schistosoma haematobium*-exposed and unexposed snails. The number of egg masses produced by exposed and control groups of (**A**) *Bulinus globosus* and (**B**) *Bulinus nasutus* snails was counted each week and averaged over the number of surviving snails per group. ■ = control snails; ■ = exposed snails; bars = average egg masses per snail; lines = cumulative mean egg masses per snail over the period of observation. The two panels use different scales for the *x* axis and the *y* axis.
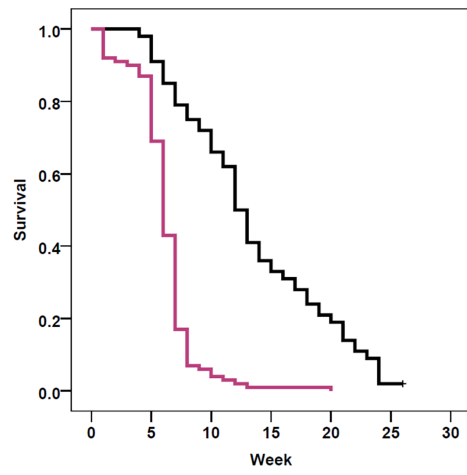
TABLE 1

Average number of embryos per egg mass (mean ± SD)

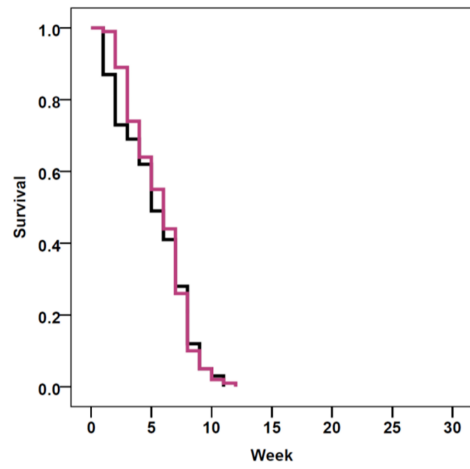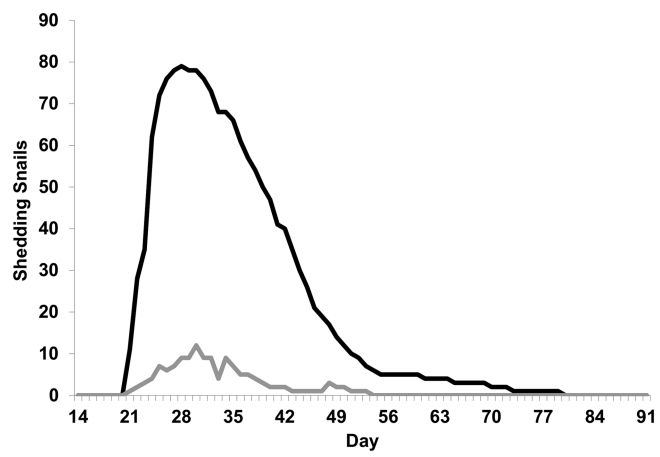| Species	Control	Exposed
Bulinus globosus	8.85 ± 0.77	8.81 ± 1.7
Bulinus nasutus	17.03 ± 1.23	17.06 ± 0.79

SD = standard deviation.

Figure 1
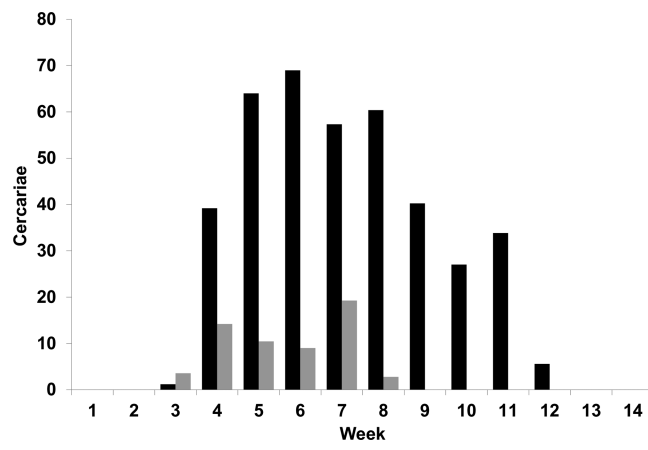

B. globosus

B. nasutus

Figure 2

Figure 3

Figure 4