Human schistosomiasis remains an intractable problem in much of the developing world. In Kenya, both the intestinal and urinary forms of the disease, caused by Schistosoma mansoni and S. haematobium, respectively, affect an estimated 3.5 million individuals, with 12 million more at risk for infection (Ministry of Health, Kenya, unpublished data). Traditionally, control efforts have relied heavily on anthelmintics such as praziquantel and/or the use of molluscsides to control the gastropod intermediate hosts of the schistosome. Such strategies, however, require sustained effort to prevent a rapid return to pre-intervention infection rates. Chemotherapy, for instance, must be repeated regularly and by itself cannot prevent reinfection. Likewise, molluscsides, inevitably short term in their effects, must be reapplied frequently to maintain effective control and pose obvious environmental concerns.

As an alternative to chemical intervention, the use of natural enemies of snails has frequently been mentioned as a sustainable means of schistosomiasis control. Over the past decade we have investigated the potential of several such putative control agents in Kenya. Our results indicate that of the organisms evaluated, the Louisiana red swamp crayfish, Procambarus clarkii, was the most effective in terms of controlling snail populations.

Procambarus clarkii was initially introduced into Kenya for aquacultural purposes, probably in the 1960s, but possibly as early as the late 1950s. Since that time, this exotic crustacean has dispersed widely, and is now present within all major drainage basins in the country. As part of a snail survey in Kenya, we observed that P. clarkii rarely if ever coexisted with known molluscan intermediate hosts of human schistosomes. Consequently, we examined the ability of this crayfish to affect pulmonate snails in the genera Biomphalaria and Bulinus, known to transmit S. mansoni and S. haematobium, respectively. Crayfish eradicated or greatly reduced snail populations in both laboratory and in field enclosures, as well as in small-scale field trials. In addition to being voracious snail predators, crayfish also compete with snails by consuming aquatic plants used by snails as refuge, oviposition sites, and food.

Although we believe that P. clarkii can significantly reduce or eliminate populations of medically important pulmonate snails, this does not necessarily translate into reduced human infection rates. In this report, we investigate whether or not crayfish introduced into natural foci of S. haematobium transmission significantly reduced such transmission. Our results indicate that under certain conditions, the presence of P. clarkii can dramatically reduce S. haematobium prevalence in primary school children. Although important questions exist regarding the impact of P. clarkii on Kenyan freshwater ecosystems, this introduced species is likely to remain well-established in Kenya. Consequently, the epidemiology of schistosomiasis in east Africa may be significantly altered by the presence of P. clarkii.

MATERIALS AND METHODS

Study area and site selection. The study was conducted in the Machakos-Kitui area of Kenya, within the Athi River drainage basin. Both S. mansoni and S. haematobium are endemic to this area, with members of the Bulinus africanus species group serving as intermediate hosts for S. hae-
Study sites. In the Machakos district, B refers to Kwandoo and Kisukioni. P. clarkii geographic range of B. africanus. Snails refer to matobium. Except where noted, all further references to snails refer to B. africanus. The area lies within the known geographic range of P. clarkii in Kenya, but crayfish had never been introduced into the snail habitats used for the present study.

To be considered, a potential study site needed to have 1) a local school, 2) high enough disease prevalence for a crayfish effect to be detected, 3) at least one human-made water impoundment harboring snails, 4) no other ongoing schistosomiasis study, and 5) agreement of local people and authorities. Ultimately, the 6 sites with the highest prevalence that met the above criteria were selected for study. Impoundments were selected as study habitats because they are usually isolated and, once introduced, crayfish would remain confined within the habitats. Also, crayfish in Kenya appear to be well-adapted to such habitats. The study schools selected were allocated to experimental or control treatments on a pairwise basis, based, as far as possible, on similar initial S. haematobium prevalences and geographic proximity (see Figure 1 for general school locations). Thus, Kataluni (experimental, approximately 10 km north of Tala township) with an initial prevalence of 61% was paired with Matuu (control, about 45 km east of Nairobi) with a prevalence 62%. Kwandoo (experimental, approximately 70 km east of Nairobi) was paired with Kisanji (control, approximately 50 km east of Nairobi), where prevalences were 27% and 37%, respectively. Ikitomwithe (experimental, located about 35 km south of Kitui town) with a prevalence of 65% was paired with Nzangathi (control, 20 km south of Kitui) with a prevalence of 33%. The study was discussed with local administrators, teachers, and residents in a series of village meetings. Approval from villagers was granted in all cases.

Study subjects. The subjects at each school were children 7–16 years old. Since all schools included in the study were located in a part of Kenya inhabited by the Kamba people, these children were relatively homogeneous in terms of ethnicity and cultural practices. Ethical approval for the study was granted by the Ethical Review Committee of the Kenya Medical Research Institute and the Human Research Review Committee of the University of New Mexico (Approval # HRRC 92–163). A signed informed consent form was obtained from all participating children and their parents or guardians. At each school, 150–200 children were selected at random to participate. This followed an initial screening of a single urine sample from each child in January 1994 to determine his or her infection status.

Snail and crayfish sampling. After identifying study sites and selecting study children, monthly snail sampling began in March 1994 at all snail habitats in the 6 localities. Permanent sampling stations, each 10 meters long, were established along the shoreline of each snail habitat at both experimental and control study sites. At monthly intervals, a standard snail scoop was used to sample along the length of each sampling station for 5 min. Total snail counts for each species per habitat per sampling period were then recorded. Immediately after collection, snails of the B. africanus group were isolated in small vials and screened for schistosome cercariae. Snails releasing mammalian schistosome cercariae were noted. All snails were returned to the site from which they had been originally collected. Snail collections continued in this manner until the study was terminated in January 1997.

Between June and July 1994, approximately 4 months after initiation of snail sampling, crayfish collected from a dam near Thika, approximately 25 km northeast of Nairobi, were introduced into the snail habitats of localities designated as experimental sites. Permission for this activity was granted by Kenya’s Department of Fisheries. No crayfish were released into control snail habitats. Stocking density was a low-end density for standard aquacultural stocking between 1 and 2 crayfish/m². After stocking, experimental habitats were sampled monthly for crayfish until the study ended in January 1997. During each sampling session, 20 meat-baited traps were placed in the water body for 1 hr. The traps, constructed of wire and covered with nylon mesh (mesh size 1 × 1 cm), were 45 cm long with a diameter of 20 cm. Traps were checked after 15, 30, and 60 min. Captured crayfish were sized, sexed, and counted, and the total number caught during the 1-hr trapping session period was recorded. All captured crayfish were returned to the habitat. In some cases the habitats received crayfish after the June–July 1994 crayfish introduction period because some habitats dried up shortly after the initial crayfish introduction, and others were dry at the time of the first crayfish introduction. Furthermore, at Ikitomwithe, we did not discover some small ephemeral water bodies until March 1995, at which time crayfish were introduced.

Examination of urine samples. In January 1995, 6 months after crayfish introductions, sampling of study children began. Each child was examined by a physician, and a urine sample was obtained on 2 consecutive days. Samples

Figure 1. Map of Kenya showing the location of the paired study sites. A refers to Ikitomwithe and Nzangathi in the Kitui district. In the Machakos district, B refers to Kataluni and Matuu and C refers to Kwandoo and Kisukioni.
were processed using standard filtration techniques. For each urine sample, two 10-ml volumes were stirred and filtered through a polycarbonate membrane (pore size = 12 μm; Poretics Corp., Livermore, CA), and the filters were placed on a numbered glass microscope slide. Each slide was examined for *S. haematobium* ova at 100× magnification by 2 independent microscopists, with eggs counted on each entire filter. A 10% sample of the slides was also examined by a third microscopist for quality control. The average number of eggs for the 2 consecutive days of sampling was recorded.

Children previously found to be infected with *S. haematobium* or those found to be infected on examination in January 1995 received a curative dose (40 mg/kg of body weight) of praziquantel. Thereafter, urine samples were taken at 3-month intervals until January 1997. In July 1996, another dose of praziquantel was issued to children who had become reinfected or those who had acquired first time infections during the course of the study.

**Statistical analysis.** Initial probabilities of infection, prior to praziquantel treatment in January 1995 (referred to below as the before period), were calculated for the study population in each school by dividing the number of children passing *S. haematobium* eggs by the total number of children. These probabilities were initially compared for each experimental-control school pair using chi-square analysis. Several factors affected the choice of the experimental school in each school pair. It was necessary that we felt confident of knowing all, or at least the vast majority of the local transmission sites near the experimental school. We believe that we were largely successful in this regard, owing to the substantial effort directed toward the identification of transmission sites, including the involvement of the local populace. However, we cannot say with absolute certainty that no transmission sites were overlooked. Such careful identification of transmission foci was not essential at the control school. This principle was used to designate both Kwandoo and Kataluni as experimental schools. Also, if 1 site had significantly higher prevalence than the other, it was chosen for treatment, on the principle that any bias should be against our hypothesis. This led to the selection of Ikotamwithe as an experimental school and Nzangathi as the corresponding control.

Following administration of praziquantel, each child at a particular school was classified as either infected if he or she acquired a patent *S. haematobium* infection at any time in the post-praziquantel treatment period (referred to below as the after period, lasting from March 1995 to January 1997), or non-infected if they did not acquire such infections. A post-treatment probability of infection was then calculated for the study population of each school by dividing the number of infected children by the total number of children for which data were available. Infection status of some children in each study population could not be determined because they were absent from school. These children were not included in this calculation.

Two different methods of comparing *S. haematobium* prevalence in control and experimental schools were used. Method A estimated this difference, with its standard error, using the normal approximation to calculate *P* values with 95% confidence intervals.

It could be argued that the samples from the same school in different time periods are not independent because the same children were usually involved. To allow for the possibility that this dependence might affect the inference, method B was used, which included only children whose infection status was known in both periods. The change in infection rate at a school can be estimated by scoring each of these children as +1 (infected in the before period but not the after period), −1 (not infected in the before period but infected in the after period), or as 0 (infected in the before period and again in the after period or uninfected during both periods). Method B calculates this estimated change, with its standard error, for each school. Then, for each experimental-control pair of schools, it estimates the difference between the changes and its standard error, and uses the *t*-approximation to calculate *P* values and 95% confidence intervals.

Other reasons could explain differential acquisition of schistosome infections such as frequency of water contact or differences in inherent susceptibility. Thus, each child entering the study in January 1995 was classified as either previously infected (group 1) or previously uninfected (group 2). Group 1 children had 1 or more *S. haematobium* eggs in their urine and were treated at the start of the study. At the completion of the study, for each school, the probability that an infection had been acquired was compared for these 2 groups using chi-square analysis.

As a separate test of any protective effect of crayfish, children who had entered either grades 1 or 2 during the study were examined at the end of the study. None of these children were enrolled in school when the study began. Probabilities of infection for these children were determined, and chi-square analysis was used to compare these probabilities at experimental and control school pairs.

Intensity of infection, defined as the mean number of eggs observed in four 10-ml urine samples (2 samples taken on each of 2 successive days), was determined for each child known to be infected in January 1995 (the before period). We also determined the maximum intensity of infection for each child that acquired an infection from March 1995 to January 1997 (the after period). Box plots summarizing the intensity data were prepared for each pair of schools, and an analysis of variance of log-transformed intensity data was performed. We note that our intensity data in the after period are biased by the fact that any child found to become infected during our study was, for ethical reasons, re-treated with praziquantel. This had the effect of lowering intensity in the after period.

**RESULTS**

*Nzangathi/Ikotamwithe.* A summary of the data for snail and crayfish populations and for reinfestation of school children in the paired Kitui district schools, Nzangathi (control) and Ikotamwithe (experimental), is provided in Figure 2. In transmission sites near Nzangathi, snails remained abundant...
 Throughout the study. At Ikotamwithe, there were 2 important transmission sites. In 1, crayfish established well and snail populations decreased sharply, with no snails recovered after December 1994. Crayfish failed to establish at the second site and snails remained present in substantial numbers throughout the study.

The pretreatment prevalence at Nzangathi and Ikotamwithe schools was approximately 33% and 65%, respectively. The significantly higher pretreatment prevalence at Ikotamwithe ($P < 0.004$), resulted in this school’s designation as the experimental site in this pair. The final prevalence at the 2 schools was not significantly different. Likewise, a comparison of the change in probability of infection in the pre-praziquantel and post-praziquantel periods between schools was not significant (Table 1). The probability of infection for grade 1 and 2 students was significantly lower at Nzangathi (Table 2). At Nzangathi, the rate at which children reacquired infection following treatment was significantly higher for group 1 than group 2 children ($P < 0.05$); this difference was not significant at Ikotamwithe ($P > 0.5$; Figure 2). Regarding intensity of infection (Figure 3 and Table 3), not surprisingly, both main effects (school and time period) were significant, but the interaction term between these 2 was not, indicating that the experimental treatment (presence of crayfish) did not affect intensity of infection for this pair of schools.

Kisukioni/Kwandoo. At the single transmission site near Kisukioni (control), snails remained numerous throughout
the study (Figure 4). At Kwandoo (experimental), snails were initially abundant at 1 transmission site but disappeared following the successful introduction of P. clarkii (Figure 4). Crayfish did not establish well at a second transmission site, and snails continued to be recovered sporadically. Pre-chemotherapy prevalence rates of 37% and 27% at Kisukioni and Kwandoo, respectively, were not significantly different \((P > 0.05)\). Likewise, neither final prevalence nor the change in the probability of infection was significantly different for this pair of schools (Table 1). The probability of infection for grade 1 and 2 students was not significantly different at the 2 schools \((P > 0.8; \text{Table 2})\). At either school, the probability of infection for group 1 and 2 children did not differ \((P > 0.8, \text{Figure 4})\). Intensity of infection decreased significantly more at Kwandoo than at Kisukioni \((P < 0.03; \text{Figure 3 and Table 3})\) during the course of the study, indicative of an effect of crayfish on infection intensity.

Matuu/Kataluni. At Matuu (control), the primary transmission site was not discovered until January 1996. Snails were abundant at that time, and remained so (Figure 5). Approximately 1% of the snails recovered were actively shedding S. haematobium cercariae. A second possible transmission site was monitored throughout the study, but no schistosome-transmitting snails were found there. At Kataluni (experimental), P. clarkii established well at both identified transmission sites. Snails were particularly abundant in 1 site, and initially more than 7% of those snails recovered were infected. Following the successful establishment of crayfish, snails disappeared from this habitat (Figure 5). At a second site, snails were never numerous, but once again, no snails were recovered once P. clarkii became well established. The prevalence of infection at Matuu and Kataluni was 62% and 61%, respectively (Figure 5), prior to the administration of praziquantel. Final prevalence, however, was more than 3 times higher at Matuu \((42\%)\) than it was at Kataluni \((12\%)\). This highly significant difference \((P < 0.001)\) was also reflected in a comparison of the change in the probability of infection, which was significantly greater at Kataluni, when calculated by either method A or B \((P < 0.01; \text{Table 1})\). The apparently greater transmission rate at Matuu relative to Kataluni was confirmed when the probability of infection for previously unexamined first- and second-year children was determined at the end of study. This probability was significantly greater at Matuu than it was at Kataluni \((42.3\% \text{ versus } 7.9\%, \text{respectively}; P < 0.001; \text{Table 2})\). At Matuu, both group 1 and 2 children either reacquired or acquired infections at a high rate, with no significant difference \((P > 0.8)\) between the 2 groups. At Kataluni, group 2 children became infected more readily than group 1 children, but the difference was not significant \((P > 0.1; \text{Figure 5})\). Regarding intensity of infection, it decreased significantly more at Kataluni than at Matuu \((P < 0.04; \text{Figure 3 and Table 3})\) during the course of the study, indicative of an effect of crayfish on infection intensity.

**DISCUSSION**

Previous studies have demonstrated that P. clarkii introduced into natural foci of schistosome transmission can reduce the abundance of, or even eradicate, the molluscan intermediate hosts of schistosomes.\(^8\) The purpose of this current study was to determine if such crayfish-related effects on snails can translate into reduced transmission to, and consequently lower prevalence and intensity in, school-age children living in areas where crayfish have been introduced.

In this present study we assessed the impact of P. clarkii introductions on S. haematobium transmission. Our underlying motivation was to introduce crayfish only into human-made water impoundments within the known geographic range of P. clarkii in Kenya. These impoundments are used
**Table 3**
Summary of analysis of variance for intensity of infection in each of the 3 pairs of schools*

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F</th>
<th>Pr (F)</th>
</tr>
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<tr>
<td><strong>Nzangathi-Ikotamwithe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Period</td>
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<td>236.3457</td>
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<td>0.3667148</td>
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<tr>
<td>Residuals</td>
<td>96</td>
<td>244.0198</td>
<td>2.5419</td>
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<td></td>
</tr>
<tr>
<td><strong>Kisukioni-Kwandoo</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period</td>
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<td>270.6035</td>
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<td><strong>Matuu-Kataluni</strong></td>
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<td>2.8462</td>
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</table>

* Intensity data were log-transformed. Significance in the interaction term is indicative of a significant difference between experimental and control schools over the interval of the study (a crayfish effect). df = degrees of freedom.

by local residents to meet domestic and livestock water needs and because Bulinus snails are well-adapted to such stationary water bodies, they are often important sources of *S. haematobium.* Because the full ecologic impact of *P. clarkii* on African ecosystems has not yet been assessed, we avoided introductions into river or stream systems that might allow crayfish to spread to new areas outside their current Kenyan geographic range. The sites selected were also deliberately far-removed from the study sites for other ongoing schistosomiasis projects.21–23

An additional consideration was the fact that *P. clarkii* is well-adapted to ponds,24,25 increasing the likelihood of successful crayfish establishment. However, the types of habitats in which we introduced crayfish are often subject to prolonged periods of drying, and although *P. clarkii* is able to burrow into the substrate and survive periods of drought,15,24 extremely dry conditions would certainly diminish the ability of crayfish to establish and affect snails. Bulinus snails are able to estivate, and rapidly re-colonize aquatic habitats, even following periods of prolonged...
drought. Thus, by studying the capacity of crayfish to reduce snail numbers and schistosome transmission under such conditions, we set up a worst case scenario for *P. clarkii*, biasing the study against our hypothesis.

At the paired Kitui district schools, crayfish introduction did not result in either lower prevalence or intensity of infection. However, because crayfish failed to establish in 3 of the 4 identified transmission sites, little can be concluded here about their ability to reduce schistosome transmission. Indeed, at the 1 experimental transmission site where crayfish did establish, snails were rapidly eradicated. We attribute the failure of crayfish to establish to the severe drought conditions that were prevalent at the time of crayfish introductions.

At the second pair of schools, Kisukioni and Kwandoo, we were again unable to demonstrate an effect of crayfish in lowering prevalence. *Procambarus clarkii* did not establish at 1 of the 2 identified transmission sites. At the second transmission site, snails were initially abundant, but disappeared following the successful introduction of *P. clarkii*. However, in spite of the fact that prevalence decreased at Kwandoo over the course of the study (from approximately 30% to 10%), reinfection rates at both schools were low throughout the study, and final prevalence at the Kisukioni control school was similarly low. The low rates at which children became infected at either school is probably because snail habitats at both sites were dry during long stretches of the study, and such low rates make it difficult to show a crayfish effect. The significantly lower intensity rate at Kwandoo, however, is indicative of a crayfish effect, suggesting that even if snails are not eradicated at all transmission sites, crayfish may reduce schistosome transmission when overall rates of infection are low.

At the third school pair, Matuu and Kataluni, a strong effect of crayfish was evident. Any effects of drought, which may have impacted crayfish introductions and transmission at other sites, were not apparent here since snail habitats near these schools retained water throughout the study. At the control school, snails persisted in transmission sites throughout the study, and this was reflected in both measures of prevalence as well as intensity in the post-praziquantel period. At the experimental school, crayfish established well at both transmission sites, and snails which were initially abundant, essentially disappeared following crayfish introduction. Children at the experimental school were more than 3 times less likely to acquire infections in the post-chemotherapy period, and those that were infected had significantly lower intensities. We further note the reduced prevalence evident in young, first-, and second-year students at Kataluni that were not part of the original study design. It will be of interest to revisit the Kataluni school in the future to determine how long crayfish might keep transmission below pre-intervention rates.

It is interesting to note that a stream running adjacent to Kataluni school harbors *Biomphalaria pfeifferi* snails that serve as intermediate hosts for *S. mansoni*. When children at this school were initially screened for *S. haematobium*, we also performed fecal examinations on these children and found that the prevalence of *S. mansoni* infection was approximately 25%. No crayfish were placed in this stream, but all children harboring patent *S. mansoni* infections were treated in January 1995, at the same time children infected
with *S. haematobium* were treated. At the conclusion of the study, in January 1997, approximately 12% of the children at Katuluni had either acquired or reacquired *S. mansoni* infections. Thus, following chemotherapy, *S. mansoni* prevalence increased to almost 50% of its pretreatment level. In contrast, in the case of *S. haematobium*, the post-chemotherapeutic prevalence increased to only 20% of its pretreatment level over the same time period (Figure 5). These results provide additional independent confirmation of our conclusion that crayfish introductions into water bodies near Katuluni school did in fact reduce *S. haematobium* transmission.

Thus, at 1 of the 3 school pairs, the data suggest that the introduction of *P. clarkii* resulted in a significant and dramatic decrease in *S. haematobium* transmission. A second school pair can essentially be considered a failed introduction, about which no conclusions regarding the effects of crayfish can be deduced. At the third pair of schools, although snails were reduced by the presence of crayfish at the experimental school, the overall low transmission at either school makes it difficult to identify a crayfish effect. Intensity data, however, do suggest such an effect at Kwando school.

Based on these results, it appears that under some conditions, crayfish have a significant impact on schistosome-transmitting snails, and consequently on prevalence and intensity levels in children. In our study, this effect was most assured in those habitats least prone to drying. The design of our study required us to introduce crayfish at a time that happened to be when habitats were already drying, and were probably among the least favorable times for initiating and establishing crayfish populations. It is in permanent stationary habitats where the greatest impact of crayfish on snail populations might be expected since the impact of aquatic predators on prey is often directly related to degree of ecological stability.

One factor to consider with respect to the interactions between crayfish and African snails is the possibility that in time, African snails will evolve measures to avoid crayfish predation. Such mechanisms have been detected in North American snails commonly preyed upon by crayfish. Likewise, the only gastropod to be found regularly in Kenyan water bodies with *P. clarkii* is the North American exotic *Physa acuta*, which has coevolved with crayfish. In Kenya, we have observed repeatedly that although freshwater crabs of the genus *Potomonautes* are voracious snail predators, such crabs are frequently found coexisting with snails, suggesting that African snails have adapted to this indigenous predator. Whether such adaptation will occur in response to *P. clarkii* remains to be seen.

Finally, because *P. clarkii* is an introduced species in Africa, obvious concerns exist regarding its ecologic impacts, including its possible impact on other aquatic species. One of the realities regarding crayfish in Africa is that they are likely to continue to spread, either naturally or assisted by human activities. As crayfish colonize new habitats, it is likely that schistosome-transmitting snails may be reduced in abundance or even eliminated from some habitats. This phenomenon may be already under way. For example, a strong negative correlation between the presence of *P. clarkii* and schistosome-transmitting snails has been documented in Kenyan water bodies. Similar outcomes may be likely in places like the irrigation canals of the Nile Delta where crayfish have also been introduced. Thus, the epidemiology of schistosomiasis may be modified considerably by the continued spread of crayfish. Further study of crayfish in African freshwater habitats is clearly warranted to better assess both the overall ecologic impact of this decapod, as well as its specific effects on freshwater snails transmitting schistosomes and other parasites of medical or veterinary significance.

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