Life cycle, population dynamics and schistosome infection in *Bulinus* spp from a crater lake in the South West Province of Cameroon

Ambrose F. ATEMNKENG, Kenneth J.N. NDAMUKONG*, Nelson N. NTONIFOR and Judith MBUH
Faculty of Science, University of Buea, Cameroon.

ABSTRACT

The study was designed to investigate the life cycle and seasonal variation in population of *Bulinus* spp, and their importance as intermediate hosts of *Schistosoma haematobium.* The population of these snails at frequently used man-water contact sites of Lake Barombi-Kotto was determined once monthly by hand picking the snails from the water surface near the lake border. The snails were examined for infection with schistosomes within 24hrs of collection by exposing them to artificial light for 4hrs and then observing for cercariae using the stereomicroscope. Eggs laid by *Bulinus camerunensis* and *B. truncatus* adults were used to study the life cycle in the laboratory. The snail population in the lake increased gradually from May to a peak in September, with a drop in August and October, before gradually rising to another peak in January. The snail population was significantly higher ($P < 0.05$) on the island side than on the mainland side with averages of 38 snails and 16 snails/10mins of sampling respectively. *B. camerunensis* was more abundant than *B. truncatus.* The population was higher in the dry than in the rainy season, but the difference was insignificant ($P > 0.05$). Infection rate of the snails with schistosomes increased significantly ($P < 0.001$) from November to a peak in February. The rate was significantly higher ($P < 0.001$) during the dry season (51.8%) than the rainy season (32.9%). The average duration from hatching of eggs to sexual maturity in *B. camerunensis* was 28 days and egg lay occurred at intervals of 10-15 days. In *B. truncatus* , newly hatched snails took 21 days to attain sexual maturity, and the duration between successive egg lay was 10 days.

Key words: *Bulinus,* population, snails, *Schistosoma,* cercariae, eggs, infection

RÉSUMÉ

L'étude a été conçue pour enquêter sur le cycle de vie et la variation saisonnière de la population de *Bulinus* spp, ainsi que leur importance comme hôtes intermédiaires du *Schistosoma haematobium.* Dans les sites du lac Barombi-Kotto, la population de ces escargots était déterminée une fois par mois. Ces derniers étaient examinés afin de déceler une probable infection par des schistosomes, dans les 24 heures suivant le ramassage. Cette opération consistait à les exposer à la lumière artificielle pendant 4 heures, ensuite à observer les cercariae sous le steréomicrosope. Les oeufs obtenus des escargots adultes des 2 espèces (*Bulinus camerunensis* et *B. truncatus*) servaient d' échantillon pour observer le cycle de vie au laboratoire. Le nombre d'escargots dans le lac augmentait progressivement du mois de Mai, et atteignait un pic en Septembre, connaissant une chute en Août et Octobre, pour s'élever de nouveau et atteindre un nouveau pic en Janvier. La population des escargots était considérablement plus élevée dans l'île ($p < 0,05$) avec une moyenne par échantillon de 38 escargots/10min que dans le continent avec une moyenne qui s'élevait a 16 escargots/10min. Les *B. camerunensis* étaient plus abondants que les *B. truncatus.* La population était plus élevée en saison sèche qu'en saison pluvieuse; néanmoins, la différence était insignifiante. Le taux d'infection des escargots par les schistosomes augmentait considérablement ($p < 0,001$) à partir de Novembre pour atteindre un pic en Février. Le taux était considérablement plus élevé en saison sèche ($p < 0,001$, 51,8%) qu'en saison pluvieuse (32,9%). Pour *Bulinus camerunensis*, la durée moyenne entre l'éclosion des oeufs et la maturité sexuelle était de 28 jours, la ponte avait lieu dans un intervalle de 10 à 15 jours tandis que pour *B. truncatus*, la période allant de l'éclosion des oeufs à la maturité sexuelle était de 21 jours et la ponte durait 10 jours.

Mots clés: *Bulinus,population,* escargots, schistosome, cercariae, oeufs, infection.

* Corresponding author

INTRODUCTION

Schistosomiasis is endemic in 74 tropical countries, and estimates show that over 200 million people are infected (WHO, 1997).About 85% of all schistosomiasis cases and all of the most severely affected, are in sub-Saharan Africa (Chitsulo, 2000). *Bulinus camerunensis* and *B. truncatus* (*B. rohlfsi*) are the intermediate hosts of *Schistosoma haematobium* in Lake Barombi-Kotto, South West Province of Cameroon (Duke & Moore, 1971; Moyou *et al.*, 1984). Ndamukong *et al.* (2001) reported that the prevalence of *S. haematobium* in Barombi-Kotto village was 75.9% while the infection rate on the Kotto island was 93.5%.

The present study investigated the life cycle and seasonal variation in the population density of *Bulinus* spp. and their importance in the transmission of schistosomiasis due to *S. haematobium.*

MATERIALS AND METHODS

Determination of the population of Bulinus snails

Sample collection was done at Lake Barombi-Kotto in Meme Division of the South West of Cameroon. Specific man-water contact sites were identified on the lakeside bordering Barombi-Kotto mainland and that bordering the Kotto island. An estimate of the population of *Bulinus* spp snails at these frequently used sites of the lake was carried out by using protected hand to pick the snails from the water surface near the lake border within 10 minutes at each sampling site. Sampling was done on the 15th of every month between 10am and midday, from June 2004 to May 2005. The snails were transported to the laboratory in labeled wide-mouth polyethylene containers containing a small amount of water from the collection sites, and used for further studies.

Determination of infection rate in snails

Snails brought to the laboratory were examined for infection with schistosomes within 24hrs of collection. They were thoroughly washed to avoid false positives from contamination with cercariae from infected snails. Individual snails were placed in glass tubes containing spring water and exposed to artificial light for 4hrs to induce them to discharge cercariae. Thereafter, the water in each tube was examined for cercariae using a stereomicroscope. The cercariae in the water were immobilized before examination using iodine solution. Snails that did not shed cercariae on the first exposure were re-exposed on the second day (Curtis *et al.*, 2004).

Life cycle of Bulinus species in the laboratory

Snails used in the study of the life cycle were *B. camerunensis* obtained from Lake Barombi-Kotto and *B. truncatus* collected from a stream in a village located about 16km from Barombi-Kotto. *B. truncatus* brought from the field more easily acclimatized to laboratory conditions than *B. camerunensis.* Eggs of *B. camerunensis* were collected on leaves from Lake Barombi-Kotto and incubated in aquaria at the laboratory. Adult snails that developed from these eggs were then used for life cycle studies. Adults of each species were kept in separate 5L aquaria, 10 snails per aquarium. Small pieces of thin glass sheets (15x8cm) were submerged in each aquarium at an inclination along the glass wall of the tank for the snails to lay their eggs. The snails were reared at room temperature and fed with fresh lettuce leaves supplemented with bone meal. Fresh food was provided on alternative days while water was changed every five days.

The tanks containing the snails were provided adequate light through a fluorescent bulb suspended above the aquaria in order to induce egg laying and hatching. The snails were observed daily. Two days after the first appearance of egg masses on the glass sheet or wall of the tank, the snails were transferred to other tanks and the eggs allowed to hatch. The young snails were fed on boiled or fresh lettuce supplemented with bone meal. The snails were subsequently used as parent stock to follow up the life cycle. There were 15 successive follow up experiments to record the egg incubation period, duration from hatching to egg laying, and frequency of egg laying. Records on temperature, rainfall, water depth and current velocity of the lake were kept during the period of the study.

Statistical analysis

Schistosome infection rates of the snails on both sides of the lake were compared using chi-square test. Snail population densities on both sides of the lake were compared using Mann-Whitney U test.

RESULTS

Temperature of the water in the lake taken between 10am and midday on each sampling day varied between 23°C and 29°C during the rainy season (28.0±2.6°C), and between 26°C and 32°C during

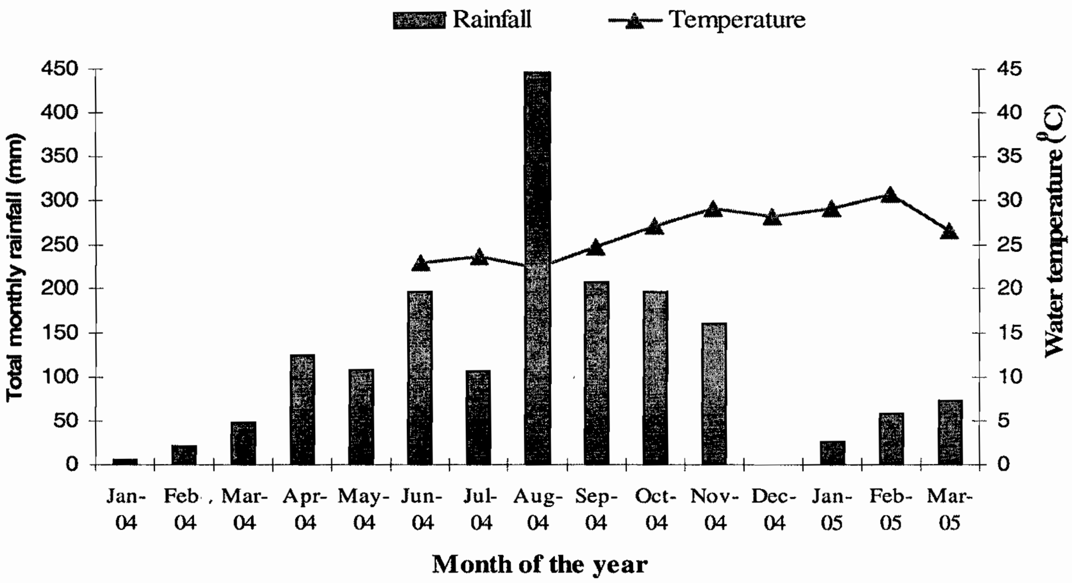

Fig. 1: Water temperature in Lake Barombi-Kotto and total monthly rainfall in Barombi area during the study period.

the dry season (29.8±1.9°C) (Fig. 1). For *B. truncatus*, there was a significant correlation between its abundance and temperature ($r=0.796$, $P<0.005$) as well as between its infection rate and temperature ($r=0.873$, $P<0.001$) on both sides of the lake. Rainfall data from the nearest meteorological station to Lake Barombi-Kotto located at Barombi Kang showed a gradual increase from March to a peak in August, then dropped to zero in December (Fig. 1). Water level (depth) near the shore (Fig. 2) increased from June to September, with the highest level recorded in August. As the dry season approached, water level reduced steadily from October to February before starting to rise in March when the rains started. Current velocity (Fig. 2) was closely related to seasonal changes. Water flow rate was comparatively fast during the rainy months with a peak in August, and very slow during the dry months of the year.

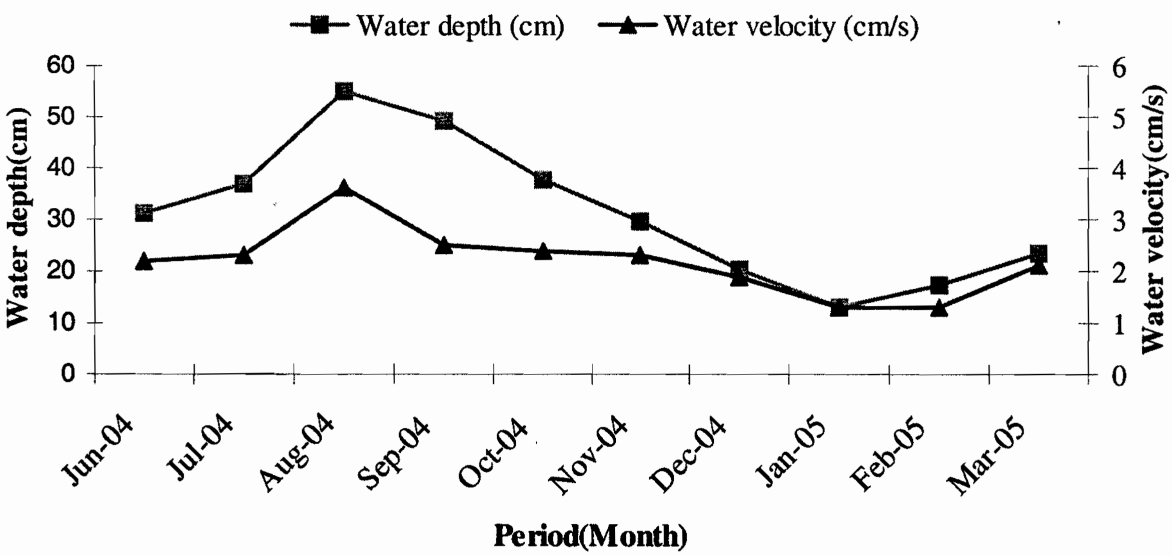

Fig. 2: Changes in water depth and current velocity recorded during sampling on the island side of Lake Barombi- Kotto.

Variation in population density of Bulinus species

The variation in population of *Bulinus* species in Lake Barombi-Kotto between June 2004 and May 2005 is shown in Tables 1 & 2. Overall, the snail population on the island side of the lake (Table 1) increased gradually from April to a peak in September (46 snails/10min sampling), but dropped in May and August. The drastic drop in August (24 snails/10min sampling) coincided with the period of heavy rains. It also dropped slightly in October before rising gradually to a peak (46 snails/10min sampling) in January after which it decreased gradually to a low population (31 snails/10min sampling) in May. A similar trend, though with lower abundance, was observed on the mainland side of the lake (Table 2). The snails were significantly more abundant ($P < 0.05$) on the island side, with an average of 38 snails/10min sampling, than on the mainland side, with 16 snails/10min sampling. At both the island and mainland sampling sites, *B. camerunensis* was comparatively more abundant (34 and 15 snails/10min sampling respectively) than *B. truncatus* ($P < 0.05$) (Tables 1 & 2).

Snails of *Bulinus* species were more abundant in the dry season on both sides of Lake Barombi-Kotto than during the rainy season (Tables 1 and 2), but the difference was not significant ($P > 0.05$). On the island side, the snail population in the dry season was 42 snails/10min sampling, compared to 36 snails/10min sampling during the rainy season (Table 1). On the mainland side, the population was 19 snails/10min sampling during the dry season, compared to 15 snails/10min sampling during the rainy season (Table 2). A similar trend was observed for the individual species. The population of *B. camerunensis* on the island side during the dry season was 36 snails/10min sampling compared to 33 snails/10min sampling during the rainy season; that of *B. truncatus* was 3 snails/10min sampling during the dry season compared to 1 snail/10min sampling during the rainy season. Similarly on the mainland side, the population of *B. camerunensis* during the dry season was 16 snails/10min sampling compared to 14 snails/10 min sampling during the rainy season, while that of *B truncatus* was 3 snails/10 min sampling during the dry season compared to 1 snail/10 min sampling during the rainy season. These differences were, however, not significant ($P > 0.05$).

Infection of snails

The rate of infection of snails with schistosomes on both sides of the lake is shown in Tables 1 & 2. After an initial drop in May, the infection rate increased gradually from June with a sharp drop in August before rising again in September. Then after, it dropped to a low level in November, before

Table 1: Population abundance of *Bulinus camerunensis* and *B. truncatus* collected from the island side of Lake Barombi-Kotto and their cercariae infection rate

Season	Month 04/05	*Bulinus camerunensis*			*Bulinus truncatus*			Overall		
		No. snails collected/ 10min sampling	No. snails infected	Infection rate (%)	No. snails collected/ 10min sampling	No. snails infected	Infection rate (%)	No. snails collected/ 10min sampling	No. snails infected	Infection rate (%)
Rainy season	April 04	31	13	41.9	3	1	33.3	34	14	41.2
	May 04	29	9	31.0	2	0	0.0	31	10	32.3
	June 04	36	14	38.9	0	0	0.0	36	14	38.9
	July 04	36	16	44.4	2	0	0.0	38	16	42.1
	Aug 04	23	1	4.3	1	0	0.0	24	1	4.2
	Sept 04	41	19	46.3	5	2	40.0	46	21	45.7
	Oct 04	34	11	32.4	3	1	33.3	36	12	33.3
	Nov 04	36	10	27.8	3	1	33.3	39	11	28.2
	Average	**33.3±5.5**	**11.6±5.4**	**33.4±13.5**	**2.4±1.5**	**0.6±0.7**	**17.5±18.8**	**35.5±6.4**	**12.4±5.7**	**33.2±13.1**
Dry season	Dec 04	39	20	51.3	5	2	40.0	44	22	50.0
	Jan 05	39	21	53.8	7	4	57.1	46	25	54.3
	Feb 05	34	19	55.9	7	5	71.4	41	24	58.5
	Mar 05	33	17	51.5	4	2	50.0	37	19	51.4
	Average	**36.3±3.2**	**19.3±1.7**	**53.1±2.2**	**5.8±1.5**	**3.3±1.5**	**54.6±13.2**	**42.0±3.9**	**22.5±2.6**	**53.6±3.8**
Overall		**34.3±4.9**	**14.2±5.8**	**40.0±14.6**	**3.5±2.2**	**1.5±1.6**	**29.9±24.6**	**37.7±6.3**	**15.8±6.9**	**40.0±14.6**

Table 2: Population abundance of *Bulinus camerunensis* and *B. truncatus* collected from the mainland side of Lake Barombi-Kotto and their cercariae infection rate

Season	Month	*Bulinus camerunensis*			*B. truncatus*			Overall		
		No. snails collected/10 min	No. snails infected	Infection rate (%)	No. snails collected/ 10min	No.snails infected	Infection rate (%)	No. snails collected/ 10min	No. snails infected	Infection rate (%)
Rainy season	Apr 04	11	4	36.4	1	0	0.0	12	4	33.3
	May 04	10	2	20.0	0	0	0.0	10	2	20.0
	June 04	15	4	26.7	0	0	0.0	15	4	26.7
	July 04	16	5	33.3	1	0	0.0	17	5	29.4
	Aug 04	7	0	0.0	0	0	0.0	7	0	0.0
	Sept 04	19	7	36.8	3	1	33.3	22	8	36.4
	Oct 04	16	5	31.4	0	0	0.0	16	5	31.3
	Nov 04	17	5	29.4	1	0	0.0	18	5	27.8
	Average	13.9±4.1	4.0±2.1	26.8±12.1	0.8±1.0	0.1±0.4	4.2±11.8	14.6±4.8	4.1±2.4	25.6±11.4
Dry season	Dec 04	18	8	44.4	3	1	33.3	21	9	42.9
	Jan 05	18	9	50.0	4	2	50.0	22	11	50.0
	Feb 05	16	9	56.3	3	2	66.7	19	11	57.9
	Mar 05	13	5	38.5	2	1	50.0	15	6	40.0
	Average	16.3±2.4	7.8±1.9	47.3±7.6	3.0±0.8	1.5±0.6	50.0±13.6	19.3±3.1	9.3±2.4	47.7±8.0
Overall		14.7±3.7	5.3±2.7	33.6±14.5	1.5±1.4	0.6±0.8	19.4±25.5	16.2±4.7	5.8±3.4	33.0±14.8

rising significantly (P < 0.001) to the highest level in February. It was observed that 28.2% (33/117) of the snails sampled on the mainland side of the lake during the rainy season and 48.1% (37/77) of those sampled during the dry season were infected. Similarly, 34.9% (99/284) of the snails sampled on the island side during the rainy season and 53.6% (90/168) of those sampled during the dry season were infected. Overall, the infection rate was significantly higher (P < 0.001) during the dry season (51.8%, 127/245) than during the rainy season (32.9%, 132/401). The highest infection rate on both sides of the lake during the dry season occurred in February (58.5% and 57.9% on the island and mainland sides respectively) while in the rainy season, it occurred in September (45.7% and 36.4% on the island and mainland sides respectively).

The schistosome infection rate in *Bulinus camerunensis* and *B. truncatus* was different on the two sides of the lake, with higher rates (40% and 29.9% for *B. camerunensis* and *B. truncatus* respectively) recorded on the island side, but this was insignificant (P > 0.05). During the dry season months, *B. truncatus* had a higher infection rate with cercariae (54.3%) than *B. camerunensis* (51.4%), even though their abundance was lower. The difference was, however, not significant (P > 0.05).

Life cycle of snails

In an aquarium containing 10 adult snails, 13 egg capsules containing 2-6 eggs each were produced in the first oviposition. The eggs hatched to produce 33 young snails, but only 69.7% survived after one week and these were followed up. The results revealed that *B. camerunensis* eggs required averagely 9 days to hatch in the laboratory. The average duration from hatching to the onset of oviposition was 28 days. Oviposition occurred at intervals of 10-15 days. The frequency of deposition of egg capsules increased with the age of snails, with each capsule containing 6-11 eggs. The average life span was 56 days.

B truncatus had a faster reproductive rate in the laboratory than *B. camerunensis*. Newly hatched snails took 21 days to attain maturity and begin laying eggs. Initially, egg capsules contained 6-10 eggs, but as the snails grew older, the number of eggs per capsule increased to 8-20. The interval between successive ovipositions was 10 days and their average life span was 89 days.

DISCUSSION

The number of snails of *Bulinus* spp. was significantly higher on the island side of the lake than on the mainland side. Similarly, human activities on the lake as well as pollution with domestic refuse and leaves from trees bordering the lake were higher on the island side than on the mainland side. These pollutants must have provided suitable surfaces for snail attachment, feeding, egg laying and growth of hatchlings (Appleton and Brutton, 1979).

Snails of *B. camerunensis* were comparatively more abundant than *B. truncatus* at the human-water contact sites, suggesting that human activities like waste disposal accounted for the higher population. The significant drop in the snail population in August coincided with the peak of the rainy season presumably because the heavy rains washed away many snails, causing them to be stranded in the deeper lake waters. The peak abundance of snails in the lake was attained during the dry season, specifically in the month of February. Factors such as slow flow and/or more stagnant waters at the lake borders, and pollution with domestic refuse that provided food and surface attachment for snails (Brown, 1994) may have accounted for the increase abundance. The main breeding period occurred during the dry season, and this was reflected in the rise in snail population at this period.

Despite the seasonal variation in snail abundance, with slightly higher populations during the dry season, these differences were not statistically significant ($P > 0.05$). This indicates that the population of snails in Lake Barombi-Kotto is fairly constant throughout most of the year, probably due to a staple supply of food (Moyou *et al.*, 1984). However, the reduction of snails during the rainy season may be attributed to high current velocity which might have easily washed away the food substrates on which the snails feed, thus starving many of them to death. It is also likely that the high current velocity during the rainy season carried away some of the hatchlings and even adult snails. The high water level in August flooded the sampling sites and probably dislodged the snails from their attachment sites.

The higher infection rate of snails with cercariae recorded on the lake side bordering the island (40%) compared to that bordering the mainland (33%) agrees with the observations of Ndamukong *et al.* (2001) who reported a schistosome infection rate of 93.5% in the human population of the island compared to 46.2% in the population on the mainland. Most of the activities that brought people in contact with the lake water were on the island (Chandiwana and Woolhouse, 1991).

More snails of *B.camerunensis* than *B. truncatus* were infected, probably because of better adaptation between the parasite and *B .camerunensis.* The higher prevalence rate of infection in snails during the dry season compared to the rainy season may be attributed to the warm weather during the dry season which favours human-water contact, especially swimming by children who pass out urine that contains schistosome eggs (Sturrock *et al.*, 2001). This agrees with Woolhouse and Chandiwana(1990) who reported that peak prevalence of infection of snails occurred late in the hot dry season on the high veld of Zimbabwe. The overall infection rate of snails in Lake Barombi-Kotto ranged from 4.2% in August to 58.5% in February. The application of molluscicides would be most effective if timed to take advantage of seasonal changes in the life cycles of snails and parasites. This implies that control of the snail population in Lake Barombi-Kotto can be most efficiently done towards the end of the rainy season up to the early dry season when the snail population and infection rate are high.

The life cycle of snails in the laboratory was observed to be longer than in their natural environment. Duke and Moore (1976) observed that eggs of *B. camerunensis* took 5 days to hatch and 11 days to mature. The physical environment, including temperature, pH and conductivity of the water in the aquaria, though not measured during the study, might not have been as conducive as in their natural habitat. This might have lengthened the duration of development from one stage to another. Nevertheless, these snails can conveniently serve as laboratory models for studies on schistosomiasis.

It can be concluded from this study that *B. camerunensis* and *B. truncatus* serve as the intermediate hosts of *S. haematobium* at the Barombi-Kotto crater lake. Their population and infection rate with schistosomes are significantly influenced by seasonal changes. The higher survival and reproductive rates of *B. truncatus* than *B.camerunensis* under laboratory conditions make the former species a more suitable model for laboratory studies on schistosomes and their snail intermediate hosts.

REFERENCES

Appleton, C.C. and Brutton, M.N. (1979). The epidemiology of schistosomiasis in the vicinity of Lake Sibaya, with a note of other areas of Togoland. *Annals Trop. Med. Parasitol.*, **73**: 541-561.

Brown, D.S. (1994). *Freshwater snails of Africa and their medical importance.* Revised 2nd Edition. Taylor and Francis Publishers, Hongkong.

Chandiwana, S.K. and Woolhouse, E.J. (1991). Heterogeneities in water contact patterns and epidemiology of *Schisosoma haematobiium. Parasitology*, **103**: 363-370.

Chitsulo, L. (2000). The global status of schistosomiasis and its control. *Acta Tropica*, 77(1): 41-51.

Curtis, H.K., Julie, A.C., Brady, M.S., Kitron, U., Sturrock, R., Ouma, J.H., Ndzovu, S.T.M., Mungai, P., Hoffmann, O., Hamburger, J., Pellegrini, C., Muchiri, E..M. and King, C.H. (2004). Distribution patterns and cercarial shedding of *Bulinus nasutus* and other snails in the Msambweni area, Coast Province, Kenya. *Am. J. Trop. Med. Hyg.* 70(4): 449-456.

Duke, B.O. and Moore, P..J. (1971). The control of *Schistosoma haematobium* in West Cameroon. *Trans. R. Soc. Trop. Med. Hyg.* **65**: 841-843.

Duke, B.O. and Moore, P.J. (1976). The use of molluscicides in conjunction with chemotherapy to control *Schistosoma haematobium* at Barombi Lake foci in Cameroon. Part 1-3, *Tropenmedezin und Parasitologie*, **27**:297-313, 489-504, and 505-508.

Moyou, S.R, Eyong, P.A., Dinga, J.S., Kouamo, J. and Ripert, C. (1984). Controle de la bilharziose urinaire dans la foyer de Barombi-Kotto. Evaluation préliminaire du niveau d'endémicités. *Rev. Sci. Techn. (Sci. Sante)*, **1**:77-86.

Ndamukong, K.J.N., Ayuk, M.A., Dinga, J.S., Akenji, T.N., Ndiforchu, V.A. and Titanji, V.P.K. (2001). Prevalence and intensity of urinary schistosomiasis in primary school children of the Kotto Barombi Health Area, Cameroon. *East Afri. Med. J.*, **78**(6). 287-289.

Sturrock, R.F., Diaw, O.T., Talla, I., Niang, M., Piau, J.P. and Capron, A. (2001). Seasonality in the transmission of schistosomiasis and in populations of its snail intermediate hosts in and around a sugar irrigation scheme at Richard Toll, Senegal. *Parasitology*, **123**:Suppl: 577-589.

WHO (1997). *The use of essential drugs*. Seventh Report of the WHO Expert Committee. WHO Technical Report Series, No. 849, Geneva, World Health Organization.

Woolhouse, M.E.J. and Chandiwana, S.K. (1990). Population biology of the freshwater snail *Bulinus globosus* in the Zimbabwe Highveld. *J.Appl. Ecol.*, **27**:41-59.

Received: 18/01/2006
Accepted:20/08/2006