DOCTOR OF PHILOSOPHY

# Sudies on the epidemiology of a Nigerian strain of Schistosoma haematobium with particular reference to the molluscan hosts

Fryer, Sarah Elizabeth

*Award date:*
1986

*Awarding institution:*
Bangor University

# STUDIES ON THE EPIDEMIOLOGY OF A NIGERIAN STRAIN OF *SCHISTOSOMA HAEMATOBIUM* WITH PARTICULAR REFERENCE TO THE MOLLUSCAN HOSTS

A thesis submitted to the University of Wales

by

SARAH ELIZABETH FRYER (B.Sc. Hons)

in candidature for the Degree of

PHILOSOPHIAE DOCTOR

School of Animal Biology,
University College of North Wales,
Bangor,
Gwynedd.

March 1986.

## DEDICATION

I dedicate this work to the memory of Dr C.A. Wright who first put me in touch with Christine Betterton and then encouraged me to take on this research project.

## ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr Allan Probert, for his patient assistance throughout this study, and Professor E. Naylor for the use of facilities at the School of Animal Biology, U.C.N.W.

I am indebted to Dr Christine Betterton for her enthusiasm in initiating this project, and all her advice throughout both field work and laboratory studies. The staff of the Experimental Taxonomy Unit, British Museum (Natural History) were also a constant source of information and advice concerning culture and other techniques.

I am also grateful to all those who supported and assisted me during my field trips to Nigeria: Chad Basin Development Authority, C.B.D.A. Diagnostic Unit, University of Maiduguri, M.R.T.(Nigeria) Ltd., and the Headmaster and staff at New Marte Secondary School. The second visit to Nigeria was made possible through financial assistance from Sir M. MacDonald and Partners.

Thank you also to all staff and students at the School of Animal Biology who helped me in various ways, particularly Dr Norman Runham for discussions on matters malacological. Finally, thank you to Bob Oswald for his constant patient support.

## SUMMARY

Laboratory infection studies involving Bulinus species from the area of the South Chad Irrigation Project, Borno State, Nigeria, were performed using Schistosoma haematobium obtained during a survey of schistosome and intestinal helminth infections in pupils at a local secondary school. These revealed the presence of both typical truncatus and africanus borne strains of the parasite with the former developing in Bulinus rohlfsi and the latter in one of two populations of B. globosus (the Ngala population). The forskali group snail, B. senegalensis, was susceptible to infection with both parasite strains, while B. globosus Lake Chad failed to become infected with either.

Investigations into the development and productivity of S. haematobium, and the effects of infections on the growth and reproductive biology of intermediate hosts, revealed that although initial establishment of an infection in a given bulinid depended on the strain to which it had been exposed, there was no correlation between parasite strain and subsequent interactions. Observed differences between each bulinid/ schistosome combination depended on the species of host involved, and could be linked with aspects of the mollusc's biology.

B. globosus Ngala exposed to compatible S. haematobium produced long-lived infections. Detailed studies of the changes in cercarial output and host oviposition over the course of the infection in this case showed patterns that were not evident in the other shorter lived species.

Laboratory experiments revealed that immature schistosome infections were capable of surviving in aestivating B. senegalensis for periods of 56 days, developing to release cercariae after snails were revived.

Morphological comparisons of the susceptible B. globosus Ngala and refractory Lake Chad populations showed significant differences in the dimensions of their male copulatory organs. Breeding experiments, however, proved that members of the two populations are capable of successful cross-fertilization.

## CONTENTS

# CHAPTER 1

# INTRODUCTION

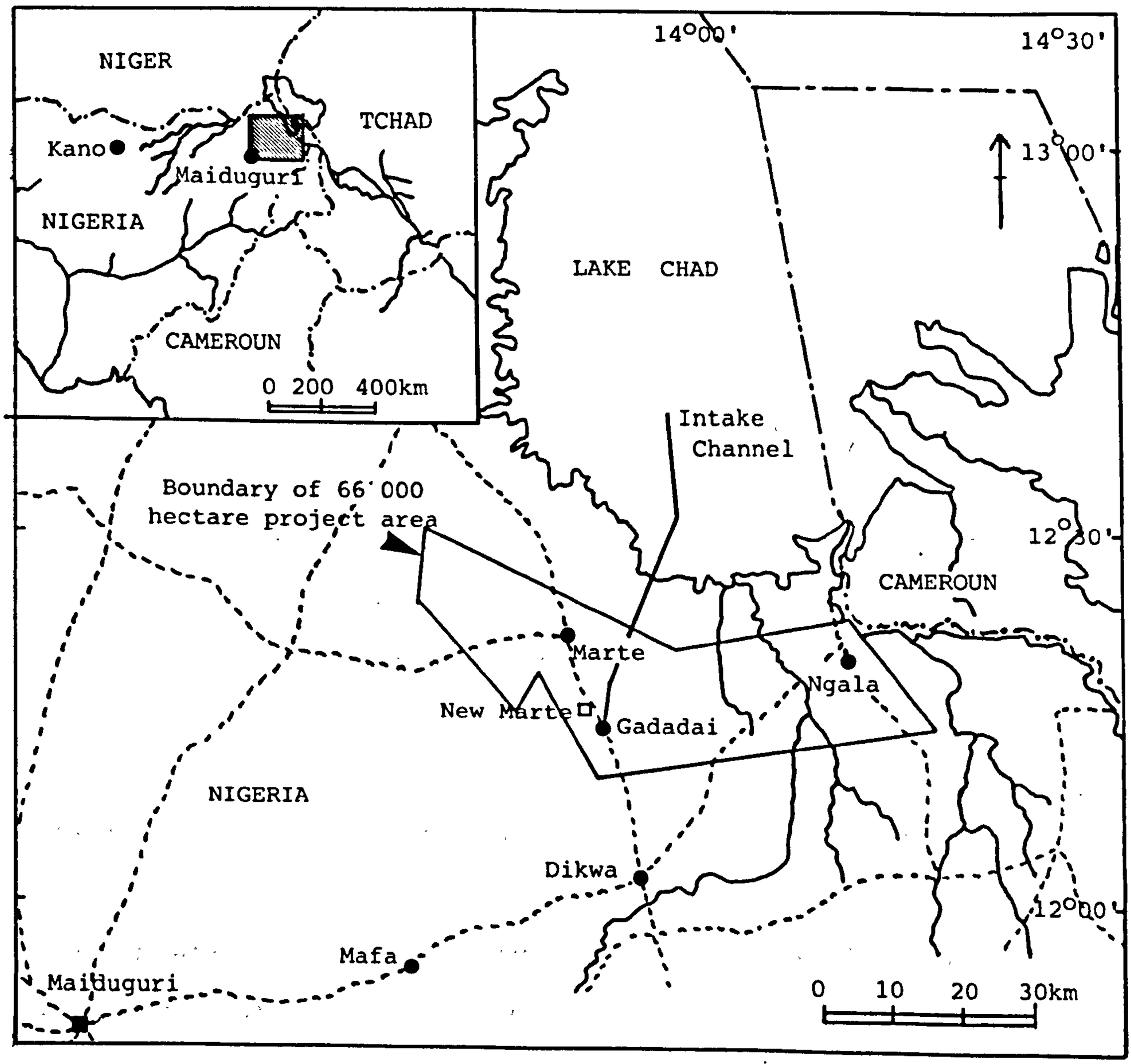
Schistosomiasis is one of the most widespread parasitic diseases of man, with an estimated 500 million people exposed to infection. The disease, caused by trematodes of the genus Schistosoma, is endemic in 73 countries, involving four species of the parasite (Iarotski and Davis, 1981).

The distribution and transmission dynamics of schistosomiasis have been greatly influenced by water developments in the tropics. The introduction of perennial irrigation in areas previously served by seasonal rains has caused considerable disruption of the surrounding environment, often producing ideal conditions for the transmission of this and other water-related diseases. Irrigation channels may provide suitable habitats for the intermediate hosts of many parasites, including the freshwater molluscs involved in the life-cycle of the schistosomes. This alteration of the ecosystem, combined with human population movements associated with such schemes, has already resulted in increased prevalence and intensity of infections in many areas where schistosomiasis is endemic. The continuing extension of existing projects and the building of new schemes is likely to result in an increase in the transmission of these parasites.

The South Chad Irrigation Project (S.C.I.P.) is a major agricultural development in Borno State, northern Nigeria (Fig. 1.1). Detailed descriptions of the climate, topography and

FIGURE 1.1

Location of the South Chad Irrigation Project



layout of the scheme have been published previously (Betterton, 1984). When complete, the scheme will incorporate 66,000 hectares of irrigated land. Limited operations involving approximately 1000ha of the central area were started in May 1979. A further 400ha have been cultivated since 1970 at the small Pilot Project at Ngala.

The project area is situated close to the southern shore of Lake Chad, between latitude 12°N - 13°N and longitude 13°30'E - 14°30'E. It is at an altitude of about 290m above sea level, with a gentle declivity towards the lake. Surface soils generally consist of clays, with occasional 'sand islands' protruding slightly above the plain. The climate is classified as semi-arid tropical with wide seasonal and diurnal changes in temperature. Annual rainfall is highly seasonal, averaging about 690mm with the rainy season extending from late May to late September. The region can be classified as Sudan or sub-Sahelian savanna, characterised by grasses and trees of the genus Acacia.

Both urinary and intestinal schistosomiasis, caused by infection with Schistosoma haematobium (Bilharz) and S. mansoni Sambon respectively, are known to be endemic in Nigeria, with an estimated 15 million Nigerians (19.1% of the population) exposed to infection in 1977 (Iarotski and Davis, 1981). Pre-constructional studies confirmed that S. haematobium was endemic within the S.C.I.P. area, with overall prevalence rates of 27% (Menu, Dove, Noamesi and Dazo, 1973) and 30.25% (Noamesi and Morcos, 1974) reported from two surveys of the local

population. S. mansoni infections appeared to be uncommon, with only two cases among 610 persons examined (Menu et al., 1973) although Noamesi and Morcos (1974) later detected infections in a larger proportion of workers from the Pilot Project at Ngala.

During the feasibility studies for S.C.I.P. a survey of local water bodies identified potential intermediate hosts of both schistosome species. Three members of the genus Bulinus (intermediate hosts of S. haematobium) were reported: Bulinus rohlfsi (Clessin), B. globosus (Morelet) and B. forskali (Ehrenberg). Biomphalaria sudanica (Martens), a possible host of S. mansoni, was also found in the area (Menu et al., 1973; Noamesi and Morcos, 1974; Odei, 1977).

Extensive long-term field studies on the intermediate hosts of schistosomiasis in the S.C.I.P. area have been carried out from shortly after the first commencement of irrigation in the main scheme (Betterton, Fryer and Wright, 1983; Betterton, 1984 a and b). One additional species of bulinid, B. senegalensis (Muller) was reported from both temporary pools in the region and within irrigation channels of the project. A full list of potential intermediate host species from the area, together with their distribution in major habitat categories is shown in Table 1.1.

The genus Bulinus has been divided into four species groups: B. africanus, B. reticulatus, B. forskali and the B. truncatus/tropicus complex (see Brown, 1980). The existence of distinct geographical strains of S. haematobium differing in

**TABLE 1.1**

**Species of snail found in the Project area and their distribution in major habitat categories.**
(From Betterton, 1984 a)

| Species | Lake Chad (M.I.C.) | Main Project Area | Pilot Project (Ngala) | Temporary Rain Pools |
|---|---|---|---|---|
| B. rohlfsi | + | + | + | |
| B. forskali | + | + | + | |
| B. globosus Lake Chad | + | | | |
| B. globosus Ngala | | | + | |
| B. senegalensis | | + | | + |
| Biomphalaria sudanica | + | | | |

M.I.C. = Main Intake Channel

their infectivity to members of each of these species groups has been recognised and extensively discussed (Brown, 1980; Wright and Southgate, 1981; Webbe, 1982). S. haematobium from different locations within its range shows definite specificity for those species groups of Bulinus occupying the same area (see Fig 1.2). Two main strains of the parasite are thought to exist, the first in the Mediterranean area of North Africa and the Middle East using intermediate hosts of the B. truncatus group, the second south of the Sahara where it develops in members of the B. africanus group. S. haematobium from the northern area cannot normally develop in B. africanus group species, while parasite from regions south of the Sahara usually fails to infect B. truncatus group snails. The situation in West Africa, where the distribution of both species groups overlaps, is more complex. There is evidence that both strains of parasite occur, and in Ghana there appears to have been a breakdown in the host restriction, with S. haematobium from some locations able to develop in members of both snail groups. This may reflect a mixing of the two S. haematobium strains within human hosts (Chu, Kpo and Klumpp, 1978) or result from hybridisation between the two forms, as suggested by Wright and Southgate (1976). This situation is further complicated by the involvement of members of the B. forskali group as intermediate hosts in some areas.

The bulinids reported from the S.C.I.P. area represent all the three main species groups involved in the transmission of S. haematobium in Africa, and all except B. forskali are known

FIGURE 1.2

Distribution of Schistosoma haematobium using different intermediate host groups throughout its range. (After Wright and Southgate, 1981)

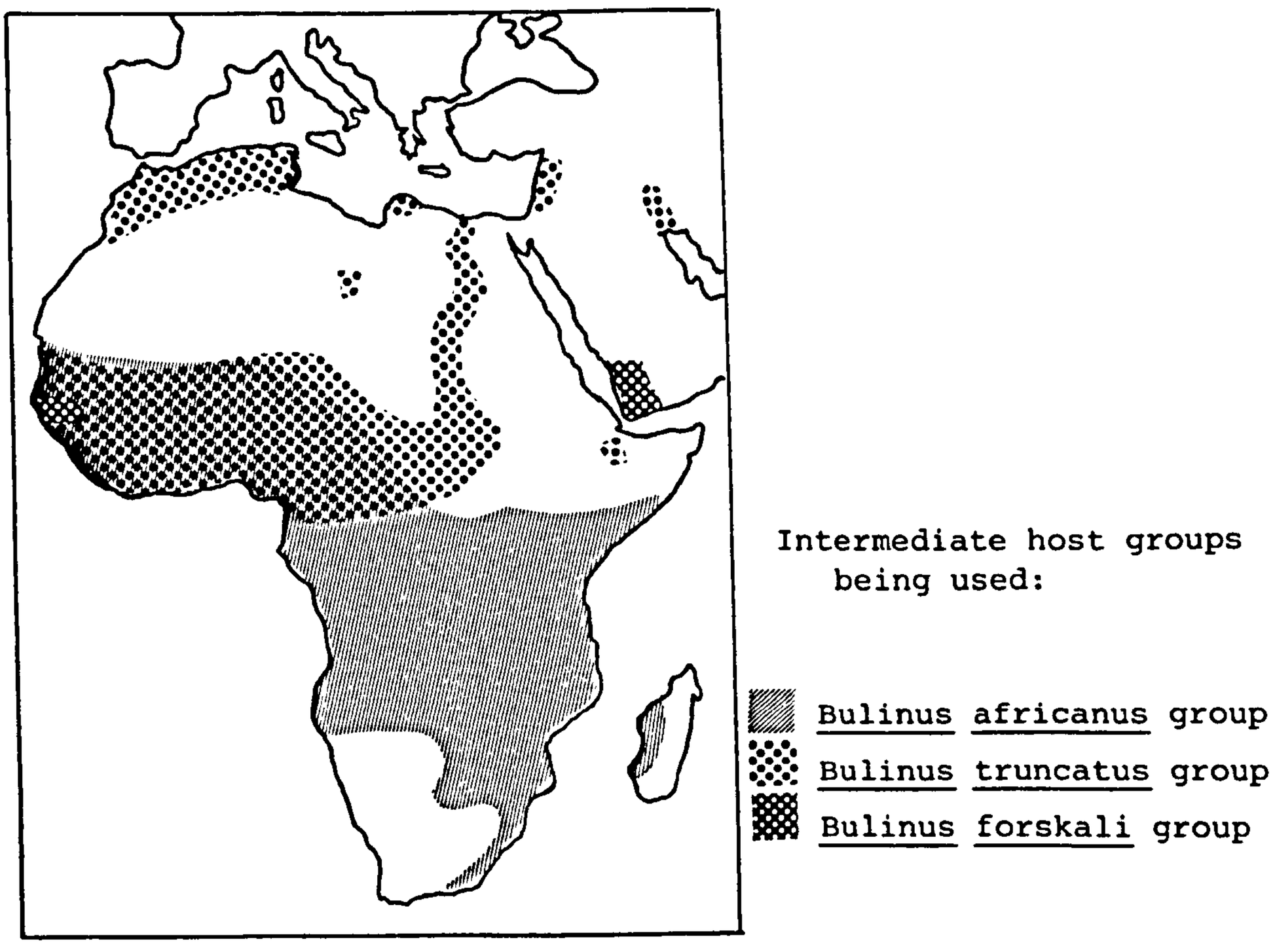


**TABLE 1.2**

**Summary of the Bulinus species reported from the S.C.I.P. area, their taxonomic status and countries where they are involved in the transmission of S. haematobium**

| Species Group | Representatives in S.C.I.P. | Areas of known transmission (From Brown, 1980) |
|---|---|---|
| B. africanus | B.globosus | Angola, Cameroun, Central African Republic, Ghana, Guinea, Kenya, Malawi, Mozambique, Nigeria, Zimbabwe, Sierra Leone, South Africa, Sudan (south), Tanzania, Uganda, Zaire, Zambia. |
| B. truncatus | B. rohlfsi | Angola, Cameroun, Ghana, Mauritania, Nigeria, Zaire (lower). |
| B. forskali | B. senegalensis | Gambia, Mauritania |
| | B. forskali | No proven records of natural infections |

to act as intermediate hosts in various parts of the continent (Table 1.2). As in Ghana (Chu et al., 1978), B. globosus is present as a potential host for africanus borne strains of the parasite, and B. rohlfsi for truncatus strains. However, the presence in the area of B. senegalensis, a B. forskali group species known to be capable of acting as a host for both strains (Wright, 1977), must also be considered in any study of the epidemiology of the disease in S.C.I.P.

Field collections have revealed natural S. haematobium infections in B. globosus from Ngala, and unidentified mammalian schistosome cercariae were shed from specimens of B. rohlfsi from a branch of the main intake channel of S.C.I.P. (Betterton, 1984 a). It therefore seemed probable that both africanus and truncatus strains of the parasite exist in the area.

The study described in the following chapters was designed to complement the field work being undertaken in S.C.I.P.. Its main aims were: to identify which bulinids present within the scheme are susceptible to S. haematobium from the local population; whether both africanus and truncatus strains of the parasite exist in the area; and to investigate various aspects of the intermediate host/parasite relationship that would provide information on the potential relative importance of each combination in the epidemiology of the disease within S.C.I.P..

Cultures of the molluscan hosts were set up at U.C.N.W. Bangor from material collected at a variety of sites within the

project area during a field trip in July/August 1980. During a second visit to the scheme in February/March 1981, a parasitological survey was carried out at the Government Secondary School at New Marte (Chapter 3). This provided background information on both the local human population and immigrants moving to the scheme from other areas of Borno State. It also provided a source of S. haematobium from carefully selected children known to have lived in the immediate area throughout their lives. This parasite material was taken back to Bangor and used in all subsequent laboratory investigations.

Once cultures of both hosts and parasite had become established in the laboratory (Chapter 2), controlled infection experiments were performed to determine the relative susceptibility of each bulinid species to infection with S.C.I.P. S. haematobium (Chapter 4). In addition, infected snails were monitored to obtain data on various aspects of the host/parasite relationship for each Bulinus species/parasite strain combination. Cercarial production, throughout the active stage of infections, was recorded (Chapter 5) and the effects of parasite infection on the growth and reproductive biology of its host assessed (Chapter 6).

Two further areas were investigated as a result of interesting observations made in Nigeria: the ability of B. senegalensis to carry viable infections through periods of aestivation, and the possible existence of two taxonomically distinct strains of B. globosus.

B. senegalensis was the only potential molluscan host present in the numerous temporary rain-filled pools around villages in the project area (Table 1.1) (Betterton et al., 1983; Betterton, 1984 a). As such it seems likely that it has played an important role in local transmission since before the irrigation project was constructed, and may still be the main source of infection for a large proportion of the human population. In order to repopulate these temporary habitats each rainy season, some portion of the snail population must survive a long period of aestivation each year. Any ability of individuals to carry schistosome infections through the dry season would have a marked effect on the duration of the relatively limited season when infection of the human population at these transmission sites takes place. Although recent observations in the Gambia by Goll and Wilkins (1984) supported Smithers' view that infections do not survive the dry season in B. senegalensis, a series of experiments was performed to investigate their ability to carry the parasite through periods of aestivation in the laboratory (Chapter 7).

During her field collections, Betterton (1984 a) noted interesting differences between the two populations of B. globosus from the main intake channel of the scheme (Lake Chad) and the Pilot Project (Ngala). The most notable of these was the complete absence of trematode infections in the Lake Chad population, whereas B. globosus from Ngala were frequently found infected with a variety of trematodes including S. haematobium. In addition, laboratory experiments (Chapter 4)

showed that snails from the Lake Chad population were refractory to infection with S. haematobium which developed readily in members of the Ngala population. These observations led to a comparative study of the B. globosus populations involving examination of certain morphological characteristics, and cross-breeding experiments using enzyme groups as genetic markers in an attempt to determine the taxonomical relationship of these two snail populations (Chapter 8).

## REFERENCES


Betterton, C, 1984 a. Ecological studies on the snail hosts of schistosomiasis in the South Chad Irrigation Project Area, Borno State, northern Nigeria. Journal of Arid Environments 7 : 43-57.

Betterton, C, 1984 b. Spatio-temporal distributional patterns of Bulinus rohlfsi (Clessin), Bulinus forskali (Ehrenberg) and Bulinus senegalensis Muller in newly irrigated areas in northern Nigeria. Journal of Molluscan Studies 50 : 137-152.

Betterton, C., Fryer, S.E. and Wright, C.A., 1983. Bulinus senegalensis (Mollusca: Planorbidae) in northern Nigeria. Annals of Tropical Medicine and Parasitology 77 : 143-149.

Brown, D.S., 1980. Freshwater Snails of Africa and their Medical Importance. Taylor and Francis Ltd., London. 487pp.

Chu, K.Y., Kpo, H.K. and Klumpp, R.K., 1978. Mixing of Schistosoma haematobium strains in Ghana. Bulletin of the World Health Organisation 56 : 601-608.

Goll, P.H. and Wilkins, H.A., 1984. Field studies on Bulinus senegalensis Muller and the transmission of Schistosoma haematobium infection in a Gambian community. Tropinmedizin und Parasitologie 35 : 29-36.

Iarotski, L.S. and Davis, A., 1981. The schistosomiasis problem in the world: results of a W.H.O. questionnaire survey. Bulletin of the World Health Organisation 59 : 115-127.

Menu, J.P., Dove, J.E., Noamesi, G.K. and Dazo, B.C., 1973. Health component in South Chad Irrigation Project - Feasibility study. Report on Missions Sept. 1972 - March 1973. World Health Organisation Unpublished document **AFR/PHA/117** 92pp.

Noamesi, G.K. and Morcos, G., 1974. Health component in South Chad Irrigation Project - Feasibility study. Follow-up studies on schistosomiasis and malaria. World Health Organisation Unpublished document **AFR/SCHIST/30** 26pp.

Odei, M.A., 1977. Health component in South Chad Irrigation Project - Feasibility study. Follow-up studies on the snail hosts of schistosomiasis. World Health Organisation Unpublished document **AFR/SCHIST/37** 73pp.

Smithers, S.R., 1956. On the ecology of schistosome vectors in the Gambia, with evidence of their role in transmission. Transactions of the Royal Society of Tropical Medicine and Hygiene **50** : 354-365.

Webbe, G., 1982. The intermediate hosts and host parasite relationships. In: Schistosomiasis: Epidemiology, Treatment and Control Ed. P. Jordan and G. Webbe. William Heinemann Medical Books Ltd., London. Chapter 2. pp16-49.

Wright, C.A., 1977. Co-evolution of bulinid snails and African schistosomes. In: Medicine in a Tropical Environment Ed. J.H.S. Gear, pp291-302. Cape Town: Balkema.

Wright, C.A. and Southgate, V.R., 1976. Hybridization of schistosomes and some of its implications. Symposia of the British Society for Parasitology 14 : 55-86.

Wright, C.A. and Southgate, V.R., 1981. Coevolution of digeneans and molluscs, with special reference to schistosomes and their intermediate hosts. In: The Evolving Biosphere Ed. P.L. Forey. British Museum (Natural History), Cambridge University Press. Chapter 15 pp191-205.

# CHAPTER 2

# ROUTINE PROCEDURES AND CULTURE TECHNIQUES

## INTRODUCTION

Various methods for maintaining both schistosomes and their molluscan intermediate hosts under laboratory conditions have been described, including those by Standen (1949), Claugher (1960), Hopf and Muller (1962), McClelland (1964), Wright (1972), Sodeman and Dowder (1973) Malek and Cheng (1974), Brown (1980) and Frandsen (1981). The techniques employed by many of these authors were reviewed by Webbe and James (1971) after a survey of several laboratories. Fraga de Azevado, Mattos dos Santos and Simoes (1974) described the problems which they had encountered in keeping Schistosoma haematobium in their laboratories; they and other workers consider that this species together with its bulinid hosts, is harder to maintain than S. mansoni and Biomphalaria species. Liang (1974) also made an extensive study of the effects of different culture methods on the perfomance of host snails of both genera.

When setting up cultures of Bulinus and S. haematobium at U.C.N.W. Bangor for the present study, it was necessary to devise a simple culture system requiring the minimum amount of labour since no technical assistance was available for routine maintenance.

The main requirement for the study was a reliable and continuous supply of young individuals of all snail species,

together with a steady supply of S. haematobium eggs for snail infection experiments.

Snail culture methods were based on those developed by the Experimental Taxonomy Unit at the British Museum (Natural History), using unaerated shallow trays for the main cultures, and the leaves of Sycamore (Acer pseudoplatanus) as the main snail diet.

The following methods of routine maintenance were used throughout the study. More detailed methods for individual experiments are included in the relevant chapters.

## MATERIALS AND METHODS

### A. Snail Cultures

i) Water Supply:

Until December 1982 copper-free mains water was used for all snail cultures. Mains water was brought through plastic pipes and held in a large aerated plastic storage tank to remove chlorine. This was mixed 10 parts to 1 with similar water from the holding tanks of African Clawed Toads (Xenopus laevis) filtered through a coarse gauze. This was used to introduce some organic material and micro-organisms which are provided in some laboratories by the use of guppies (Lebistes reticulatus) in reservoir tanks (e.g. Claugher, 1960; Wright, 1972).

Early problems with thin and pitted shells were experienced in all cultures. Since the quantity of inorganic salts in Bangor mains water was known to be extremely low, this

was increased by the addition of the Nolan-Carriker salt mixture described by Malek and Cheng (1974), viz:

50g Calcium carbonate

5g Magnesium carbonate

5g Sodium chloride

1g Potassium chloride

This was made up in 3 litres of distilled water to form a stock solution. The mixture was then added to all water for snail cultures at a rate of 4ml per litre. The resulting pH fell in the range of 5.5 to 6.

In mid December 1982, with very little warning, the Welsh Water Authority commenced a programme of mains relining. The cement compounds used changed the mains water pH from an existing value of around 5.5 to a value of 11 within 24 hours. To avoid such an abrupt change, and deleterious effects on cultures, an alternative water supply from a large, well established artificial pond was used. This pond, containing a wide variety of flora and fauna, is fed by rain and spring water. All pond water was double filtered, firstly through glass wool, followed by Whatman No.4 filter paper. As before, the stock salt solution was added at a rate of 4ml per litre, with a resultant pH of 6.5 to 7.

Despite assurances that these changes in mains water quality were temporary, after which it was planned to return to the original supply, several months later the pH was still extremely high. It was therefore decided to continue experimental work using the filtered pond water.

ii) Containers:

Three types of container were used in the routine maintenance of snails:

a) Cat litter trays (coloured plastic) 350x250x45mm covered with sheets of glass.

b) Clear polystyrene "Lunch Packs" with pushfit lids, 175x115x41mm (Stewart Plastics p.l.c. Industrial).

c) Clear polystyrene pots with snap on lids, 105mm diameter, 40mm deep (A. W. Gregory and Co. Ltd.).

All were soaked, prior to use, for a minimum of 24 hours in copper-free water to remove any volatiles.

iii) Culture Conditions:

All cultures of Bulinus were maintained in water-filled containers without the addition of gravel, plants or the provision of artificial aeration.

B. globosus (Lake Chad and Ngala) and B. rohlfsi were kept in a temperature controlled room at $26\pm1^{o}C$ by day, dropping to $24\pm1^{o}C$ at night. B. senegalensis was housed in glass fronted incubators at $30\pm1^{o}C$ by day, $29\pm1^{o}C$ night.

All cultures were maintained on a 12 hour light/dark cycle, with illumination provided by fluorescent lighting.

iv) Diet:

Snails were fed ad libitum on the leaves of sycamore (Acer pseudoplatanus). These were collected during spring and summer, then air dried and stored in lightproof polythene

sacks. Dry leaves were soaked for a minimum af 12 hours before feeding to snails.

The basic diet was supplemented with two commercial fish foods: Aquarian Tropical Fish Flakes (Thomas's, Halifax, England) and Magnet Pond Pellets (Magnet Pet Foods Ltd., Rishton, Blackburn). These were fed to snails alternately at weekly intervals at the rate of approximately 0.1g Pellets or 0.2g Flakes per 10 adult snails, with any uneaten remains being removed the day after feeding to prevent clouding of the water.

v) Handling and Cleaning:

The handling of snails was kept to a minimum to avoid undue stress and shell damage. Where manipulation of individual animals was necessary, broad bladed "Feather Light" forceps (Griffin) were employed.

Cleaning of containers was carried out by removing leaves and leaf skeletons with adhering snails and pouring the old water through a large sieve, thus retaining any snails that had not remained attached to the container. Except where very heavy growths of algae had accumulated, containers were rinsed gently in a small volume of fresh culture water before being refilled. In cases of heavy algal growth, snails were temporarily removed and containers cleaned using an abrasive cloth while being rinsed in copper-free water.

Great care was taken to prevent accidental transference of young snails and egg masses between culture vessels. Forceps and sieve were thoroughly wiped and dried between handling

individual trays.

vi) Routine Maintenance:

A routine procedure was developed to provide a regular supply of young snails of each bulinid species for experimental work.

All cultures were checked frequently to remove dead and dying snails, thus preventing deterioration in water quality. At the same time, more sycamore leaf was added where required, to provide a constant supply.

One of the two fish foods was added to each vessel at weekly intervals.

Breeding stocks of B. globosus (Lake Chad and Ngala) and B. rohlfsi were kept in the large trays filled with approximately 1500ml of water. The maximum stocking density was kept at 30 adult B. rohlfsi or 25 adult B. globosus (Lake Chad and Ngala) per tray.

To avoid disturbance and undue loss of newly hatched snails the water in these breeding trays was changed as infrequently as possible, either when snail faeces built up (approximately 6 weeks at highest densities) or if the water quality deteriorated.

Breeding cultures of B. senegalensis, maintained at the higher temperature, required more frequent changes of water, as water quality dropped more rapidly than at the lower temperature. To allow ease of handling, these snails were kept in the smaller "Lunch Packs", holding 500ml of water, with a

maximum of 15 of these much smaller adult snails in a single container. The water was then changed weekly.

To obtain large numbers of snails for experimental work, it was necessary to remove young from the breeding trays. Although these would develop in the main cultures, mortality rates during the first few weeks after hatching were very high, and the availability of young erratic.

At weekly intervals, old leaf skeletons from each breeding culture were carefully removed, together with attached young snails and adhering snail faeces. These were transferred to clean water in small round containers (one corresponding to each breeding culture), and left with a portion of fresh leaf for a week. Young snails, now large enough to handle easily, were then picked off the leaf skeletons and transferred to "Lunch Pack" containers used for snails of suitable size for experimental work. Up to 50 snails under 5mm in length and of mixed age were kept per container, being fed as the main cultures and cleaned weekly. Any unused snails over this size were then returned to breeding trays.

In order to avoid any possibility of selectively breeding snails resistant to infection with S. haematobium, the offspring of animals used in experiments were not returned to the main breeding cultures.

B **Parasite Cultures.**

i) Final Host:

*S. haematobium* adults were passaged through Golden Hamsters (*Mesocricetus auratus*) of either sex. All hamsters were individually caged and kept on a bedding of mixed-wood shavings. Hamsters were fed *ad libitum* on Labsure CRM pelleted diet.

ii) Collection of eggs:

Eggs of *S. haematobium* for experimental and routine infection of snails, were obtained from the liver and posterior portion of the gut (including faecal contents), of infected hamsters.

Hamsters with mature infections (100 days plus) were killed by cervical dislocation after light anaesthetization with chloroform. The liver and gut posterior to the caecum, were dissected out and macerated through a coarse sieve, being washed through with normal saline. The tissues with trapped eggs, and faecal material, were then washed in cold (3-4$^{o}$C) saline, sedimenting for 20 minutes between washes, until the supernatant was clear (3 or 4 washes). After a final wash in cold distilled water (3-4$^{o}$C) the sediment was transferred to petri dishes with warm distilled water (25-27$^{o}$C) under bright illumination. The first miracidia hatched within 10 minutes and could be collected under a binocular microscope using a Pasteur pipette.

iii) Infection of Snails:

Clear plastic vented tissue culture petri-plates (Flow-Labs, Irvine, Scotland) were used for the exposure of snails to miracidia, having been weathered in copper-free water for at least 24 hours before first use. Each lidded petri-plate consists of 25 compartments of $2cm^2$.

Counted miracidia, (normally 5 per snail), were transferred to each compartment in 5ml of water using a Pasteur pipette. All miracidia used were collected within one hour of hatching. Individual snails, normally 2-5mm in height, were then placed in each compartment, and left with the lid in place for a minimum of 5 hours.

Exposed snails were subsequently kept in "Lunch Packs", cleaned weekly and fed in the same way as main cultures. A maximum of 25 snails were maintained in a single container.

iv) Detection of Cercariae:

Snails were screened for cercarial production in tissue culture petri-plates. Individuals to be checked were placed in alternate compartments with 5ml of water at approximately 9a.m., and left under bright illumination. After a minimum of 8 hours, compartments were searched under a dissecting microscope, and any snails found to be producing cercariae were isolated individually in small round containers in 150ml of water. Cercariae could be transferred passively on the animals' shell or body, therefore all positive snails were rescreened within a few days.

v) Infection of the Final Host:

Cercariae for the infection of hamsters were obtained by placing infected snails in 150ml of fresh water at approximately 9a.m., leaving them under bright illumination. After up to 8 hours, cercariae were collected using a Pasteur pipette under a dissecting microscope. Wherever possible, cercariae from a minimum of 10 individual snails were used in the infection of each hamster. This was to ensure that a mixed sex infection became established.

Each hamster to be infected (approximately 6-10 weeks old) was placed in a large glass vessel containing sufficient warm water (30°C) to reach half way up its abdomen. It was then left for 15 to 20 minutes to void excretory products, before being temporarily removed. 250 to 300 cercariae were then placed in the same depth of clean water at 25-27°C, and the hamster allowed to paddle in the cercarial suspension for 30 to 45 minutes.

## DISCUSSION

The culture methods employed in this study for both bulinids and schistosome provided a reasonably reliable supply of material for experimental work. Some logistical problems were encountered, however, in simultaneously obtaining enough young snails of all species.

<u>B. globosus</u> Lake Chad proved the most difficult snail to keep in culture, with extremely high mortality rates amongst newly hatched snails in the breeding trays. Survival in this

case was improved by the daily removal of hatchlings and provision of polythene rafts as an egg laying substrate (Webbe and James, 1971). Rafts facilitated transference of egg masses to smaller containers before hatching without damage.

B. senegalensis was initially kept at the lower temperature, but did not perform well. Snails persistently crawled out of the water, few fed actively, and only very small numbers of egg masses were produced. Since this species is naturally restricted to the rainy season, when water temperatures are high (Betterton, Fryer and Wright, 1983; Betterton, 1984) culture temperatures were raised to 30°C. Fewer snails crawled out and the performance of cultures was greatly improved with increased fecundity and excellent survival of young snails.

Many laboratories employ more complicated culture methods, including the use of biologically balanced aquaria (e.g. Standen, 1949; Frandsen, 1981) or large glass aquaria with gravel or sand substrates and oxygenating plants (e.g. Claugher, 1960). Some workers also use artificial aeration (Webbe and James, 1971; Sodeman and Dowder, 1973), but this is rendered unnecessary by the high surface area to volume ratio of trays. As McClelland (1964) pointed out, shallow trays are also easier to handle and store than glass aquaria, and with the absence of gravel or sand, changing water is much simplified.

The diet of sycamore leaves, as used extensively by the British Museum (Natural History) (Brown, 1980), proved

satisfactory for all species. Although Webbe and James (1971) mention that many workers consider that very young snails require an alternative diet, newly hatched snails were regularly observed browsing on fresh leaves and old leaf skeletons. It is probable that they were feeding on the microflora and fauna growing on the decaying leaf material. Young snails were thus provided with a suitable food source by transferring them with old leaf skeletons. This also eliminated the problems of deteriorating water quality associated with the presence of unconsumed fish food. Young snails also often fed on the substance of fresh sycamore leaves within a fortnight of hatching. The method employed in cleaning out containers without the physical wiping of the tray sides allowed a build up of microflora and fauna as an additional food source.

Claugher (1960) considered snail faecal material as an important food source for newly hatched snails, and small quantities of this were always transferred with leaf skeletons and young snails.

Even newly hatched snails could be handled safely with the feather light forceps used, employing the broad flat blades as a spatula. The flexbility and large surface area of these forceps make them ideal, as it is virtually impossible to crush even very small snails.

The tissue culture petri-plates proved particularly suitable for both exposing snails to miracidia, and detecting shed cercariae. The individual compartments held 5ml of water

easily, allowing a larger volume per snail than the haemagglutination plates used by some laboratories (Webbe and James, 1971). They are also easier to handle than individual tubes or small beakers as used by others (e.g. Frandsen, 1981), and, in addition, each tray has a closely fitting lid.

It was found that very occasionally cercariae could pass from one compartment to the next if both were full of water and a snail crawled up on to the underside of the lid. It was for this reason that in all screening for cercarial production alternate compartments only were used.

Many workers use glass tubes for the isolation of snails to detect cercariae. Apart from being easier to handle, the petri-plates were easier to search under the microscope than tubes. The square sides to each compartment and relatively thin walls resulted in less diffraction of light around the edges than was encountered with round sided glass tubes. To test this observation, a comparison of the detectability of small numbers of cercariae in the two types of container was carried out. For the petri-plates, one worker placed from 0 to 5 cercariae per compartment in 4 plates (100 compartments). Each of these was then searched under a dissecting microscope and scored for the presence or absence of cercariae. In the case of 2X1 inch (50X25mm) glass tubes, the cercariae used were those produced by infected snails undergoing their weekly monitoring (see Chapter 5). Having removed the snails, tubes were searched and scored as positive or negative as above. The actual number of cercariae in each case was then checked

against the counts of trapped, stained cercariae, having passed the contents through filter paper and stained with 1% Ninhydrin (Chapter 5).

The number of false results is summarised in Table 2.1. Detection of small numbers of cercariae was considerably more sensitive in the compartments of petri-plates than in glass tubes. The three false results in glass tubes where cercariae were seen, but not detected on filter papers probably indicates the lack of sensitivity of the filtration technique, therefore the scores of cercariae for the test with tubes may actually be underestimates. $X^2$ and p values are included in Table 2.1 for each pair of results where the expected cell values for each part of the 2X2 contingency table are equal to or greater than 5. These show that significantly more false negative results were recorded in glass tubes than in compartments of petri-plates, at the 5% level, when less than 5 cercariae were present. The glass tubes continued to produce false negative results even when much larger numbers of cercariae were present. In one case a tube was scored negative when 17 cercariae were subsequently detected in the filtered sample.

The paddling method employed for the infection of hamsters does not give as high or consistent worm returns as other methods (Preston and James, 1972). Quantitative measurements of worm returns were not, however, required in this study and the results obtained were acceptable. In addition, the use of the paddling method avoided the need for anaesthetization of hamsters. Other workers also find this method to be adequate,

## TABLE 2.1

**Comparison of sensitivity of visual screening under a binocular microscope for the presence of small numbers of cercariae in petri-plates and glass tubes.**

| | PETRI-PLATES | | | GLASS TUBES | | | | |
|---|---|---|---|---|---|---|---|---|
| No. of cercariae | n | No. false | % Error | n | No. false | % Error | $X^2$ | p |
| 0 | 20 | 0 | 0 | 28 | 3 | 10.7 | 0.82 | N.S. |
| 1 | 39 | 7 | 17.9 | 18 | 16 | 88.9 | 22.89 | <0.001 |
| 2 | 24 | 1 | 4.2 | 24 | 19 | 79.2 | 24.77 | <0.001 |
| 3 | 13 | 0 | 0 | 21 | 10 | 47.6 | 6.63 | 0.01<p<0.02 |
| 4 & 5 | 4 | 0 | 0 | 15 | 13 | 86.7 | - | - |
| 6 to 10 | - | - | - | 26 | 17 | 65.4 | - | - |
| 11 to 15 | - | - | - | 12 | 4 | 33.3 | - | - |
| 16 to 20 | - | - | - | 3 | 1 | 33.3 | - | - |

N.S = not significant at the 5% level of confidence

and less time consuming than alternatives (Frandsen, 1981).

The culture methods developed thus proved suitable for the studies involved and the limited manpower available. In addition, they had some advantages over other methods described by previous workers.

## REFERENCES


Betterton, C., 1984. Spatio-temporal distribution patterns of Bulinus rohlfsi (Clessin), Bulinus forskali (Ehrenberg) and Bulinus senegalensis Muller in newly irrigated areas in northern Nigeria. Journal of Molluscan Studies 50 : 137-152.

Betterton, C., Fryer, S.E., and Wright, C.A., 1983. Bulinus senegalensis (Mollusca:Planorbidae) in northern Nigeria. Annals of Tropical Medicine and Parasitology 77 : 143-149.

Brown, D.S., 1980. Freshwater Snails of Africa and their Medical Importance. Taylor and Francis Ltd. London 487pp.

Claugher, D., 1960. The transport and laboratory culture of snail intermediate hosts of Schistosoma haematobium. Annals of Tropical Medicine and Parasitology 54 : 333-337.

Fraga de Azevado, J., Mattos dos Santos, M.A. and Simoes, M., 1974. Das dificuldades encontradas no mantuncao do Schistosoma haematobium, agente de bilharziose vesical, no nosso laboratorio. Anais do Instituto de Higiene and Med. trop. 2 : 357-361.

Frandsen, F., 1981. Cultivation of schistosomes for chemotherapeutic studies. Acta Pharmacologica et Toxicologia 49 (Supplement 5) : 118-122.

Hopf, H.S. and Muller, R.L., 1962. Laboratory breeding and testing of Australorbis glabratus for molluscicidal screening. Bulletin of the World Health Organisation 27 : 783-789.

Liang, Y.S., 1974. Cultivation of Bulinus (Physopsis) globosus (Morelet) and Biomphalaria pfeifferi pfeifferi (Krauss) snail hosts of schistosomiasis. Sterkiana 53 : 7-52 and 54 : 1-23.

Malek, E.A. and Cheng, T.C., 1974. Medical and Economic Malacology. Academic Press: New York and London. 398pp.

McClelland, W.F.J., 1964. A method of breeding Bulinus (Physopsis) nasutus in the laboratory. Annals of Tropical Medicine and Parasitology 58 : 265-269.

Preston, J.M. and James, C., 1972. Infection of hamsters with terminal-spined schistosomes. Journal of Helminthology 46 : 291-296.

Sodeman, W.A. and Dowder, M., 1973. Laboratory maintenance of Bulinus vectors of Schistosoma haematobium. American Journal of Tropical Medicine and Hygiene 22 : 678-681.

Standen, O.D., 1949. Experimental schistosomiasis. 1 - The culture of the snail vectors Planorbis boissyi and Bulinus truncatus. Annals of Tropical Medicine and Parasitology 43 : 13-22.

Webbe, G. and James, C., 1971. The importation and maintenance of schistosomes of human and veterinary importance. In Isolation and Maintenance of Parasites in vivo. Symposium of the British Society for Parasitology 9 : 77-107.

Wright, C.A., 1972. Land and Freshwater Mollusca. In The UFAW Handbook on the Care and Management of Laboratory Animals. Churchill Livingston: Edinburgh and London. Chapter 52 pp604-609.

# CHAPTER 3

# A SURVEY OF THE PREVALENCE OF SCHISTOSOME AND INTESTINAL HELMINTH INFECTIONS IN CHILDREN FROM NEW MARTE SECONDARY SCHOOL, BORNO STATE, NIGERIA

## INTRODUCTION

Both urinary and intestinal schistosomiasis are known to be endemic in Nigeria. The results of a World Health Organisation questionnaire survey (Iarotski and Davis, 1981) indicated that in 1977 some 15 million Nigerians (19.1% of the population) were exposed to infection with schistosomes. The country as a whole is considered to be "highly endemic" for Schistosoma haematobium (responsible for urinary schistosomiasis) and of "medium endemicity" for S. mansoni (causing intestinal schistosomiasis).

Cowper (1963 and 1973) reviewed the work carried out on schistosomiasis in Nigeria since early this century. He considered the northern region of the country to be the most heavily infected with S. haematobium, with infection rates probably reaching hyperendemic proportions in the north eastern (Chad Basin) area. The high incidence of haematuria, the main symptom of urinary schistosomiasis, in Bornu Province was commented upon by the German explorer Gustav Nachtigal in his travels in the region in 1881. Ramsay (1934) quoted the "mallams" of the area accepting that "every boy at some time in his early life passes blood in the urine, but that he grows out of it".

The potential for increase in prevalence and intensity of schistosome infections through major water developments in northern Nigeria, where the endemicity of these parasites is so well documented, has been recognised. The small irrigation scheme at Yau, in the extreme North West of Borno State, was the subject of a study by the Oxford University Expedition to Nigeria, 1971 (O.U.E.N., 1974). Evidence was obtained of an association between contact with the irrigation scheme and S. haematobium infection. The results were complicated by the presence of workers who had moved to the scheme from other areas, a very common situation with irrigation developments.

The South Chad Irrigation Project (S.C.I.P.) is a major irrigation development in Borno State. The O.U.E.N. team (1974) recognised the potential for increased schistosomiasis transmission in the S.C.I.P. Pilot Project at Ngala/Gamboru. The main scheme, planned to incorporate 66,000 hectares of irrigated land, is likely to have a profound effect on the epidemiology of this and other endemic, water related diseases.

Two World Health Organisation surveys carried out during the Feasibility Study for S.C.I.P. found overall S. haematobium prevalence rates of 27% (Menu, Dove, Noamesi, and Dazo, 1973) and 30.25% (Noamesi and Morcos, 1974). Isolated cases of S. mansoni infection were also found in the local population (Menu et al., 1973) and amongst workers in the Pilot Project (Noamesi and Morcos, 1974).

The present survey (1981) was carried out as part of a wider study on the epidemiology of schistosomiasis within

S.C.I.P. The Government Secondary School at New Marte, the administrative centre for the scheme, was selected as the location for a survey of schistosomiasis and other helminth parasites. An extensive questionnaire (see Appendix A) was included to obtain socio-economic information.

Being a residential school, follow-up studies would be easy to carry out. Since the school contained male pupils from the whole of Borno State, this allowed an examination of patterns of parasite infection, and the identification of potential importation of parasite species by people moving to S.C.I.P. from these regions.

It is generally considered that young males are the greatest source of schistosome infection in endemic areas. Pugh (1979 b) considered that males aged 5 to 20 years were responsible for over 77% of the transmission of S. haematobium in Malumfashi (Kaduna State). Thus, by examining the secondary school boys at New Marte, information would be obtained on the most important section of the population in the epidemiology of schistosomiasis.

## MATERIALS AND METHODS

The survey was carried out at the New Marte Secondary School during February and March 1981, with the assistance of Dr Christine Betterton and members of the Chad Basin Development Authority Diagnostic Unit.

Each pupil was interviewed for details of age, location of home village and certain aspects of home life, and the

responses recorded on the questionnaire. Where necessary, translation into the child's first language (Kanuri, Hausa, Shuwa or Arabic) was provided by multilingual members of the Diagnostic Unit. Each child was then requested to provide urine and stool samples in 120ml plastic cups with fitted lids and 50 ml plastic screw top containers respectively. All samples were collected between 1000 and 1200hrs, catching the early part of the period of daily peak in output of S. haematobium ova in the urine (Bell,1969; Pugh, 1979 a; Doehring, Feldmeier and Daffalla, 1983) and fitting in with the normal school routine. Subsequent examination of samples was then carried out in the laboratory.

The volume of the majority of urine samples was measured, and any macroscopic haematuria noted. After allowing urine samples to stand for a minimum of 20 minutes for any ova to settle, the upper portion was discarded, leaving approximately 10ml in the bottom of the container. This was then transferred to a centrifuge tube and centrifuged for 2 minutes at 1500 revolutions per minute. The bulk of the spun urine was discarded by inverting the tube, and the sediment resuspended in the small volume remaining by giving the bottom a sharp tap. The whole sediment of each sample was transferred to slides for examination under the compound microscope. Any occurrence of erythrocytes (microscopic haematuria) was noted, and any S. haematobium ova counted. The intensity of infection was then calculated and expressed as ova/10ml of urine from the original volume of the sample.

The appearance of faecal samples was noted, including the presence of blood and/or mucus. Each sample was then mixed thoroughly with 10% formalin and left for several days before further processing. Examination for helminth ova was performed by taking three thin smears from the bottom sediment of each sample for microscopical examination. Any helminth ova present were identified, but no attempt was made to count the number of eggs of each species.

Statistical analysis of results was performed using SPSS and SPSS-X statistical packages on the mainframe Dec-10 computer at U.C.N.W., Bangor. The 5% level of significance was used in all tests.

## RESULTS

A total of 303 boys were included in the survey, ranging in age from 8 to 18 years, although many seemed uncertain of their exact age.

All of the boys questioned provided urine samples, and 301 gave stool samples (99.3%).

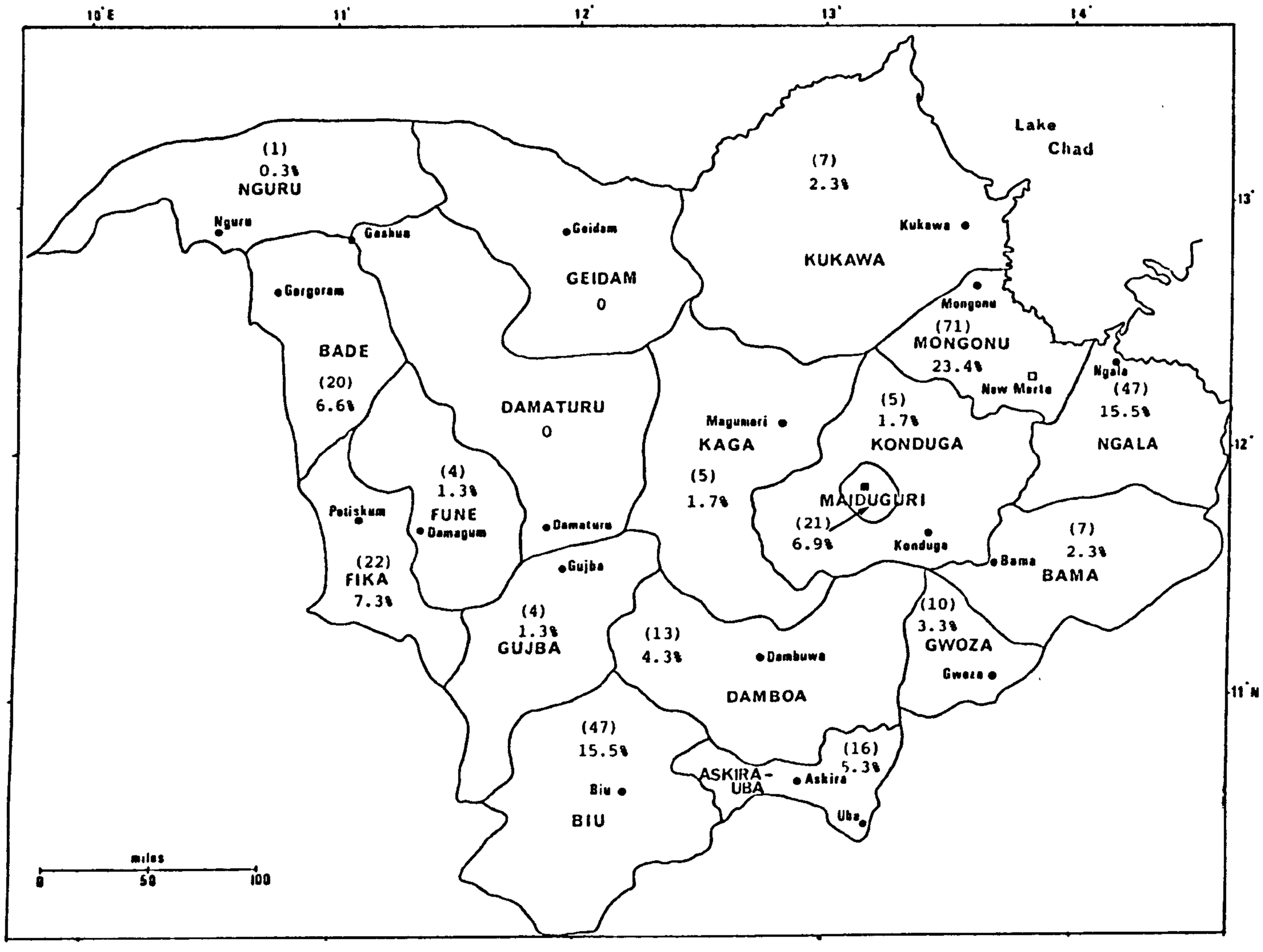
### Background Information

#### Geographical Distribution of Sample

The boys sampled were from villages in 16 of the 18 Local Government Areas of Borno State (Fig 3.1) Two individuals came from villages in other States of Nigeria, and one gave the name of a village whose location was unknown.

FIGURE 3.1

Distribution of New Marte schoolboys by Local Government Area within Borno State



Eighty-four boys gave villages in the S.C.I.P. area as their home residence, however 13 of these had lived elsewhere or visited other areas regularly. Throughout the analyses below, this group of 71 is presented separately, in addition to the results from the whole sample, as being representative of the local S.C.I.P. population.

Religion and Ethnic Groups

Two hundred and seventy (89.1%) of the boys were Muslim, including all those from the S.C.I.P. area.

Representatives of 24 different ethnic groups were included in the sample (Table 3.1) with the majority group of Borno State, the Kanuri, being the most numerous. There was also a large number of Bura in the sample, reflecting the relatively high proportion of boys from the Biu area.

Only three ethnic groups were represented in the S.C.I.P. sub-sample, Kanuri, Shuwa and Hausa, with Kanuri being in the majority.

Father's Occupation

Table 3.2 shows the occupation of the boys' fathers divided into four main categories. The majority were farmers. Market traders (8.9% of the group) included tatooists, blacksmiths, scribes, carpenters, tailors and shoemakers. Office jobs represented included policemen, prison officers, politicians, librarians, teachers, doctors and drivers. The S.C.I.P. sub-sample showed a similar distribution of

TABLE 3.1

Distribution of ethnic groups in the sample of schoolboys from New Marte Secondary School

| | Whole sample | | SCIP sub-sample | |
|---|---|---|---|---|
| | n | % | n | % |
| Kanuri | 144 | 47.5 | 60 | 84.5 |
| Bura | 37 | 12.2 | 0 | 0 |
| Hausa | 30 | 9.9 | 2 | 2.8 |
| Shuwa | 16 | 5.3 | 9 | 12.7 |
| Margi | 13 | 4.3 | 0 | 0 |
| Bode | 13 | 4.3 | 0 | 0 |
| Chibok | 7 | 2.3 | 0 | 0 |
| Kankura | 5 | 1.7 | 0 | 0 |
| Mandara | 4 | 1.3 | 0 | 0 |
| Ngusun | 4 | 1.3 | 0 | 0 |
| Bolewa | 4 | 1.3 | 0 | 0 |
| Babur | 4 | 1.3 | 0 | 0 |
| Kare Kare | 4 | 1.3 | 0 | 0 |
| Fulani | 4 | 1.3 | 0 | 0 |
| Muri | 2 | 0.7 | 0 | 0 |
| Gwoza | 2 | 0.7 | 0 | 0 |
| Idoma | 2 | 0.7 | 0 | 0 |
| Kotoko | 2 | 0.7 | 0 | 0 |
| Waha | 1 | 0.3 | 0 | 0 |
| Igala | 1 | 0.3 | 0 | 0 |
| Higi | 1 | 0.3 | 0 | 0 |
| Babole | 1 | 0.3 | 0 | 0 |
| Ibo | 1 | 0.3 | 0 | 0 |
| Yoruba | 1 | 0.3 | 0 | 0 |
| TOTAL | 303 | 100% | 71 | 100% |

**TABLE 3.2**

**Occupations of the fathers of the schoolboys from New Marte Secondary School**

| | Whole sample | | SCIP sub-sample | |
|---|---|---|---|---|
| | n | % | n | % |
| Farmer | 246 | 81.2 | 59 | 83.1 |
| Market Trader | 27 | 8.9 | 6 | 8.5 |
| Office Jobs | 22 | 7.3 | 5 | 7.0 |
| Labourer | 7 | 2.3 | 1 | 1.4 |
| Unknown | 1 | 0.3 | 0 | 0 |
| TOTAL | 303 | 100% | 71 | 100% |

**TABLE 3.3**

**Housing types of schoolboys from New Marte Secondary School**

| | Whole sample | | SCIP sub-sample | |
|---|---|---|---|---|
| | n | % | n | % |
| Adobe | 190 | 62.7 | 61 | 85.9 |
| Straw | 53 | 17.5 | 3 | 4.2 |
| Tin Shack | 5 | 1.7 | 1 | 1.4 |
| Block | 54 | 17.8 | 6 | 8.5 |
| Stone | 1 | 0.3 | 0 | 0 |
| TOTAL | 303 | 100% | 71 | 100% |

occupations.

Livestock was owned by 51.5% of the boys' families (50.7% in S.C.I.P.).

## Housing Types

Table 3.3 shows the type of housing inhabited by the boys in their home village. The majority lived in the adobe and straw dwellings typical of the region.

## Shoes and Mosquito Nets

Ninety-eight percent of the boys claimed to wear shoes at all times outdoors (100% in S.C.I.P.) and 75.2% claimed to use mosquito nets regularly when at home (85.9% in S.C.I.P.).

## Water Supplies and Sanitation

Only 13.5% (8.5% in S.C.I.P) claimed to have a tap either inside, or immediately outside, their house in their home village.

The responses to the questions on water supplies used for various domestic purposes in home villages were very confused, and it was obvious that a variety of natural sources were used in addition to any borehole or well in the village, where these were present, during the short rainy season. The responses were divided into three main categories (Table 3.4): those whose home village possessed a borehole or pump, a well, or natural water sources alone.

The proportion of the sample claiming to have a borehole

TABLE 3.4

Main water supplies in the home villages of boys at New Marte Secondary School

| | Whole sample | | SCIP sub-sample | |
|---|---|---|---|---|
| | n | % | n | % |
| Borehole/Pump | 193 | 63.7 | 69 | 97.2 |
| Well | 85 | 28.1 | 2 | 2.8 |
| Natural | 25 | 8.3 | 0 | 0 |
| TOTAL | 303 | 100% | 71 | 100% |

TABLE 3.5

Sanitation facilities at the homes of boys from New Marte Secondary School

| | Whole sample | | SCIP sub-sample | |
|---|---|---|---|---|
| | n | % | n | % |
| Water Closet | 22 | 7.3 | 4 | 5.6 |
| Pit Latrine | 242 | 79.9 | 65 | 91.5 |
| None | 39 | 12.9 | 2 | 2.8 |
| TOTAL | 303 | 100% | 71 | 100% |

in their village was very high, especially in the S.C.I.P. area. This was borne out by personal observations within the region, with boreholes present in almost all villages visited. Traditional wells made up a smaller proportion, with very few respondents relying on natural water sources alone (none in S.C.I.P.).

The boys were asked whether they swam, and if so where. Although over 90% claimed they swam or "played in water" some of the responses as to the location indicated confusion.

The majority of the group had access to some form of sanitation (Table 3.5) with pit latrines being very common, especially in the S.C.I.P. area.

## Helminth Infections

Only 59 (19.6%) of all the boys providing samples were without any detectable helminth infections. Table 3.6 gives the prevalence rate of each helminth species seen in the present survey, both for the whole group and the S.C.I.P. sub-sample.

Examination of urine specimens revealed 177 cases of *S. haematobium* (58.4%) with a similar proportion of the S.C.I.P. sample infected.

Ova of *S. mansoni* were detected in urine from two boys, with one of these having a mixed infection of the two species. Examination of a stool specimen from this child revealed no schistosome ova.

Examination of stool samples disclosed further cases of *S.*

**TABLE 3.6**

**Prevalence of helminth ova in urine and stool samples**

| Helminth species | Whole sample n | Whole sample Infected n | Whole sample Infected % | SCIP sub-sample n | SCIP sub-sample Infected n | SCIP sub-sample Infected % |
|---|---|---|---|---|---|---|
| Schistosoma haematobium | 303 | 177 | 58.4 | 71 | 43 | 60.6 |
| Schistosoma mansoni | 301 | 26 | 8.6 | 71 | 1 | 1.4 |
| Hookworm | 301 | 118 | 38.9 | 71 | 17 | 23.9 |
| Hymenolepis nana | 301 | 25 | 8.3 | 71 | 6 | 8.5 |
| Ascaris lumbricoides | 301 | 3 | 1.0 | 71 | 0 | 0 |
| Enterobius vermicularis | 301 | 2 | 0.7 | 71 | 0 | 0 |
| Trichuris trichiura | 301 | 1 | 0.3 | 71 | 0 | 0 |

mansoni, with a total of 26 individuals having detectable infections (8.6% of the whole sample). Ova of 5 further helminths were seen in specimens, with the commonest being hookworm, present in 118 samples (38.9%). No attempt was made to identify which species of hookworm these ova were from. Twenty-five cases of infection with the dwarf tapeworm Hymenolepis nana (8.3%) were found. Other helminth species detected were Ascaris lumbricoides (3 cases), Enterobius vermicularis (2 cases) and Trichuris trichiura (1 case).

Table 3.7 shows the distribution of these helminth species within individuals. Multiple infections were common with 28.9% of the boys showing ova of more than one species in their specimens.

**Infection with S. haematobium**

Fig.3.2 shows the infection rates of S. haematobium in boys from each of the local government areas of Borno State, as detected in this survey. Although sample sizes are very uneven, a simple cross-tabulation of infection rate by Local Government Area shows a significant interaction ($X^2$=29.9332; 17 d.f.; p=0.0268) thus indicating an uneven distribution of this parasite within the state.

Since local government boundaries provide a very artificial division of the State, the sample was sub-divided according to the rainfall distribution, dividing Borno State into 6 rain zones (based on inches of rainfall per year (Agboola, 1979)) (Fig.3.3). Individuals were only included if

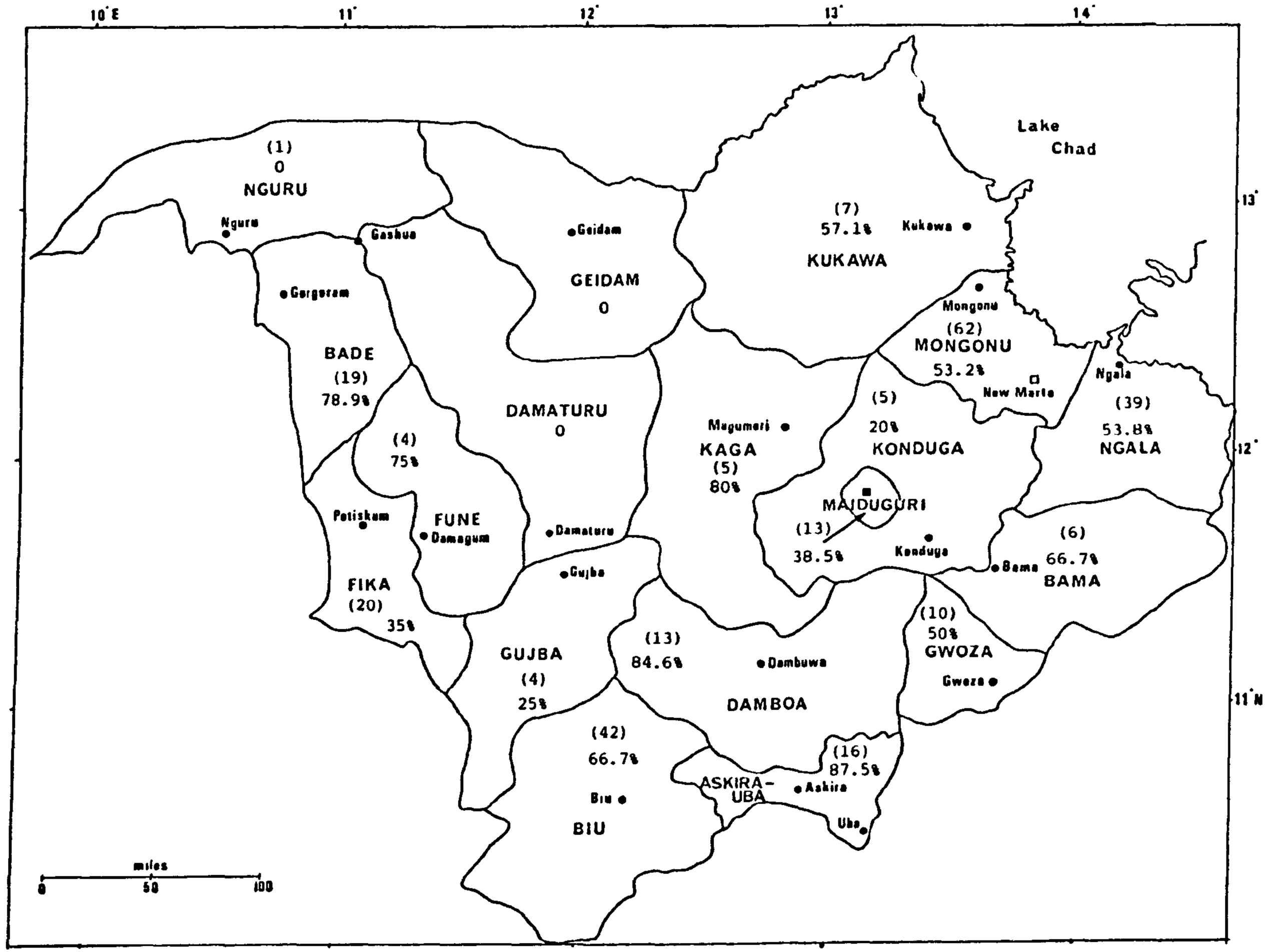
## TABLE 3.7

**Distribution of helminths in boys from New Marte Secondary School**

| | n | % |
|---|---|---|
| **No ova detected** | 59 | 19.6 |
| **Single species** | | |
| *S. haematobium* | 95 | 31.6 |
| *S. mansoni* | 8 | 2.7 |
| Hookworm | 44 | 14.6 |
| *H. nana* | 6 | 2.0 |
| *A. lumbricoides* | 1 | 0.3 |
| *E. vermicularis* | 1 | 0.3 |
| TOTAL | 155 | 51.5 |
| **Two species** | | |
| *S. haematobium* & Hookworm | 53 | 17.6 |
| *S. haematobium* & *H. nana* | 7 | 2.3 |
| *S. haematobium* & *S. mansoni* | 2 | 0.7 |
| *S. haematobium* & *T. trichiura* | 1 | 0.3 |
| *S. mansoni* & Hookworm | 4 | 1.3 |
| Hookworm & *H. nana* | 4 | 1.3 |
| TOTAL | 71 | 23.6 |
| **Three species** | | |
| *S. haematobium*, Hookworm & *S. mansoni* | 6 | 2.0 |
| *S. haematobium*, Hookworm & *H. nana* | 5 | 1.7 |
| *S. haematobium*, *S. mansoni* & *H. nana* | 2 | 0.7 |
| *S. haematobium*, *A. lumbricoides* & *S. mansoni* | 1 | 0.3 |
| *S. mansoni*, Hookworm & *H. nana* | 1 | 0.3 |
| TOTAL | 15 | 5.0 |
| **Four species** | | |
| *S. mansoni*, Hookworm, *A. lumbricoides* & *E. vermicularis* | 1 | 0.3 |

FIGURE 3.2

Prevalence of S. haematobium infections by Local Government Area of boys' home villages



they had lived within the same rain zone all their life, except for attending New Marte School. Cross-tabulation of the infection rates by rain zones indicated a significant interaction ($X^2$=22.7018; 5 d.f.; p=0.0004). Fig.3.4 shows that there is not a linear relationship between the rain zone and S. haematobium infection rates. Loglinear analysis incorporating the overall infection rate and distribution of the sample population between rain zones (for rain zones 1 to 6 where n>10) confirmed that the observed interaction was not linear. The interaction was, however, explained adequately by incorporating the quadratic component alone (likelihood ratio $X^2$=3.1218; 3 d.f.; p=0.372 i.e. the observed data do not differ significantly from the loglinear model). Infection rates were thus significantly lower in the areas of intermediate rainfall.

Cross-tabulation of S. haematobium infection and other responses to sections of the questionnaire revealed significant interactions with housing type (traditional and modern) (Table 3.8) and water supply in the child's home village (Table 3.9). The infection rates were significantly higher in the group of children living in traditional dwellings compared to those living in modern block houses ($X^2$=4.8760; 1 d.f.; p=0.0272). The infection rate was highest in the group of children with wells as their main water source in the home village and lowest where natural sources alone were available ($X^2$=16.4861; 2 d.f.; p=0.0003).

An examination of the interaction between rain zone and both housing type and village water supply, however, shows that

FIGURE 3.3

Prevalence of S. haematobium infections by rain zone of boys' home villages

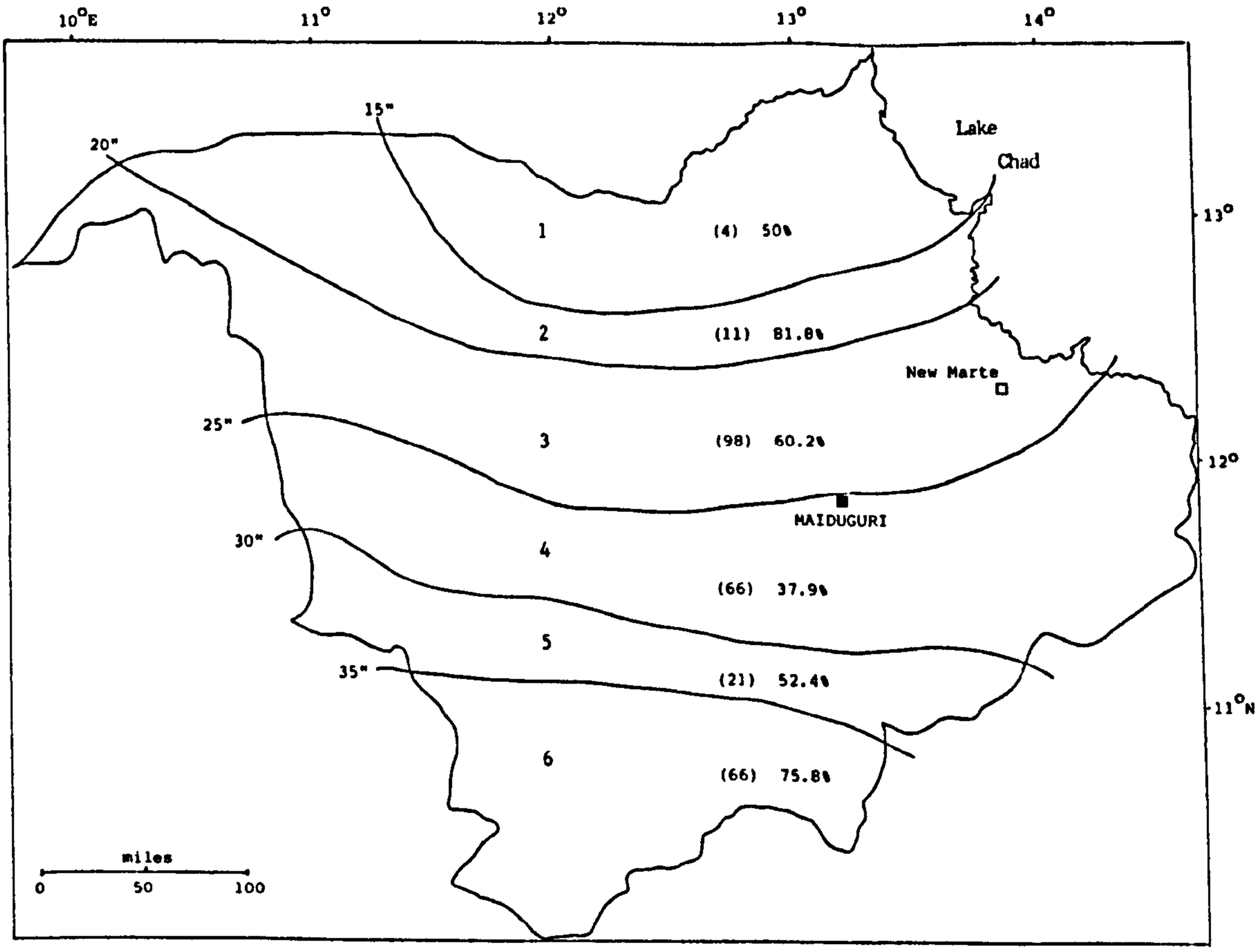



FIGURE 3.4

Relationship between S. haematobium prevalence and rain zone of boys' home villages

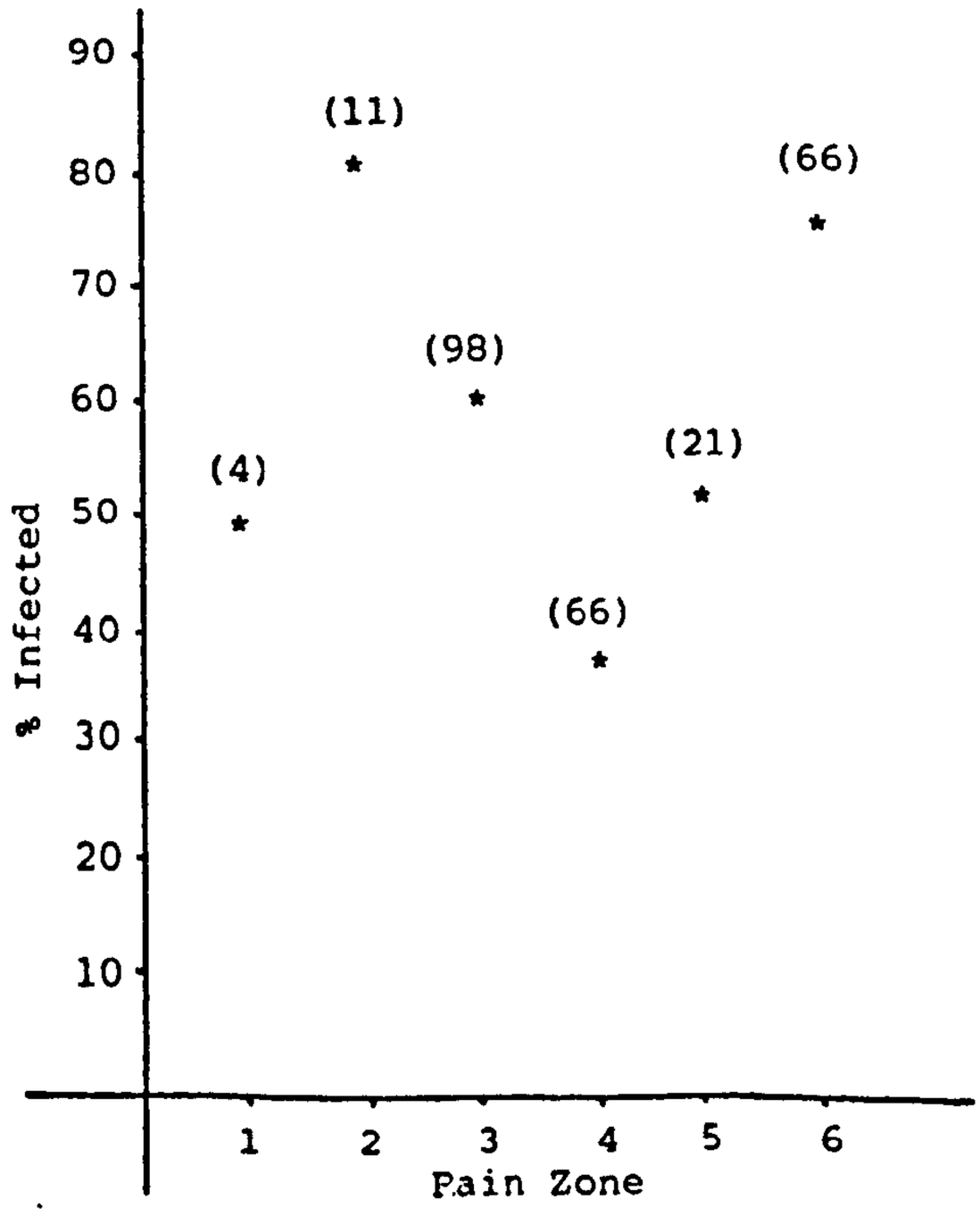


TABLE 3.8

**Prevalence of *S. haematobium* infections with housing type in boys' home villages**

| Housing type | n | n infected | % infected |
|---|---|---|---|
| Traditional | 243 | 150 | 61.7 |
| Modern | 60 | 27 | 45.0 |

$X^2$=4.8760 d.f.=1 p=0.0272

TABLE 3.9

**Prevalence of *S. haematobium* infections with main water supply in the boys' home villages**

| Water source | n | n infected | % infected |
|---|---|---|---|
| Borehole/pump | 193 | 101 | 52.3 |
| Well | 85 | 65 | 76.5 |
| Natural only | 25 | 11 | 44.0 |

$X^2$=16.4861 d.f.=2 p=0.0003

there are significant interactions in both cases (Table 3.10 and 3.11). Loglinear analysis incorporating rain zone, village water and S. haematobium infection shows that there is an additive relationship between these factors, indicating a significant interaction between village water and infection rates, and rain zone and infection rate independently, but no evidence of a dependence on the combination of both rain zone and village water (likelihood ratio $X^2$=10.3454; 8 d.f.; p=0.242. i.e. the observed results do not differ significantly from the loglinear model).

Further analysis incorporating housing types was not possible due to the lack of sufficient children living in modern houses in some rain zones.

Haematuria was closely related to the presence of S. haematobium ova in the urine (Table 3.12) ($X^2$=195.1071; 2 d.f.; p<0.0001). Ova were detected in all samples with macroscopic haematuria, and only 5 out of 35 samples with erythrocytes present on microscopical examination did not also contain ova.

Fig. 3.5 shows the distribution of egg counts (ova/10ml urine) in the 142 infected specimens where urine volumes were known. The intensity of infections was generally low, with a geometric mean of $13.04 \pm 6.36$. Counts ranged from 0.2 to 1663.3 ova/10ml.

As with the presence of ova in samples, the level of haematuria was closely related to the intensity of the infection. An analysis of variance on log transformed data showed a significant interaction both when uninfected cases (0

## TABLE 3.10

**Relationship between rain zone and housing type**

| Rain zone | n | Traditional | | Modern | |
|---|---|---|---|---|---|
| | | n | % | n | % |
| 1 | 4 | 2 | 50.0 | 2 | 50.0 |
| 2 | 11 | 10 | 90.9 | 1 | 9.1 |
| 3 | 98 | 89 | 90.8 | 9 | 9.2 |
| 4 | 66 | 44 | 66.7 | 22 | 33.3 |
| 5 | 21 | 19 | 90.5 | 2 | 9.5 |
| 6 | 66 | 56 | 84.8 | 10 | 15.2 |

$X^2$=20.9857 d.f.=5 p=0.0008

## TABLE 3.11

**Relationship between rain zone and main village water supply**

| Rain zone | n | Borehole | | Well | | Natural | |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % |
| 1 | 4 | 4 | 100 | 0 | 0 | 0 | 0 |
| 2 | 11 | 6 | 54.5 | 5 | 45.5 | 0 | 0 |
| 3 | 98 | 83 | 84.7 | 12 | 12.2 | 3 | 3.1 |
| 4 | 66 | 43 | 65.2 | 20 | 30.3 | 3 | 4.5 |
| 5 | 21 | 6 | 28.6 | 12 | 57.1 | 3 | 14.3 |
| 6 | 66 | 17 | 25.8 | 34 | 51.5 | 15 | 22.7 |

$X^2$=75.4185 d.f.=10 p<0.0001

## TABLE 3.12

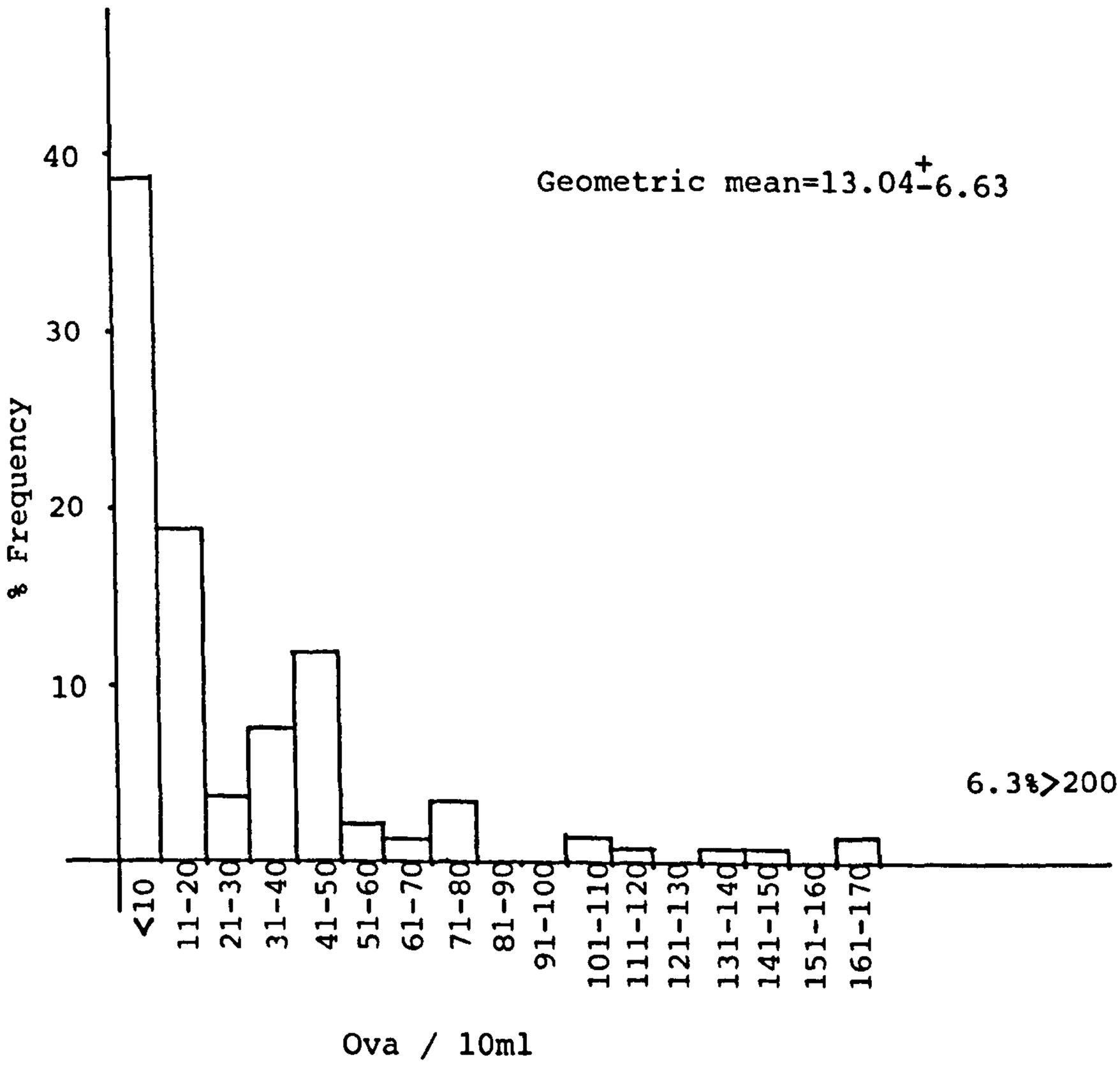
**Relationship between haematuria and <u>S. haematobium</u> infection**

| Haematuria | n | n infected | % infected |
|---|---|---|---|
| None | 147 | 26 | 17.7 |
| Microscopic | 130 | 125 | 96.2 |
| Macroscopic | 26 | 26 | 100 |

$X^2$=195.1071 d.f.=2 p<0.0001

FIGURE 3.5

Distribution of S. haematobium egg counts in infected samples



ova/10ml) were included (Table 3.13a) and with infected individuals alone (Table 3.13b).

Unlike the interactions between infection rates with S. haematobium and rain zone, village water and housing, no significant relationship was found between these parameters and the intensity of infections.

**Other Helminth Infections**

Cases of S. mansoni infection were in general restricted to those boys from areas of Borno State away from S.C.I.P., mostly in the Biu area in the south, and around Maiduguri (Fig. 3.6). There was however one infected child who claimed to have lived in a village in Mongonu local government area all his life.

Hookworm infections showed an interaction with the rain zone of the boy's home village (Fig.3.7), with prevalence increasing with rain zone in all but rain zone 1, where only a very small data set was available. Simple cross-tabulation showed that this interaction was significant ($X^2$=27.6352; 6 d.f.; p=0.0001).

Although blood and mucus were seen in some stool samples, there was no significant relationship between their presence and any of the helminth infections. This was also the case with the state of stools, which ranged from liquid to very well formed.

## TABLE 3.13

## Relationship between the intensity of *S. haematobium* infections and haematuria

### A: Including uninfected individuals

| Haematuria | n | Ln(eggs/10ml urine + 1) mean | Standard deviation |
|---|---|---|---|
| None | 118 | 0.1341 | 0.4322 |
| Microscopic | 107 | 2.6316 | 1.3613 |
| Macroscopic | 22 | 4.5162 | 1.4596 |

**Analysis of variance:**

| | Sum of squares | d.f. | Mean square |
|---|---|---|---|
| Between groups | 554.525 | 2 | 277.262 |
| Within groups | 263.015 | 244 | 1.078 |

F=257.217 p<0.0001

### B. Excluding uninfected individuals

| Haematuria | n | Ln(eggs/10ml urine) mean | Standard deviation |
|---|---|---|---|
| None | 18 | 0.0619 | 1.2346 |
| Microscopic | 102 | 2.5977 | 1.4595 |
| Macroscopic | 22 | 4.4828 | 1.5104 |

**Analysis of variance:**

| | Sum of squares | d.f. | Mean square |
|---|---|---|---|
| Between groups | 193.802 | 2 | 96.901 |
| Within groups | 288.975 | 139 | 2.079 |

F=46.610 p<0.0001

FIGURE 3.6

Prevalence of S. mansoni infection by Local Government Area of boys' home village

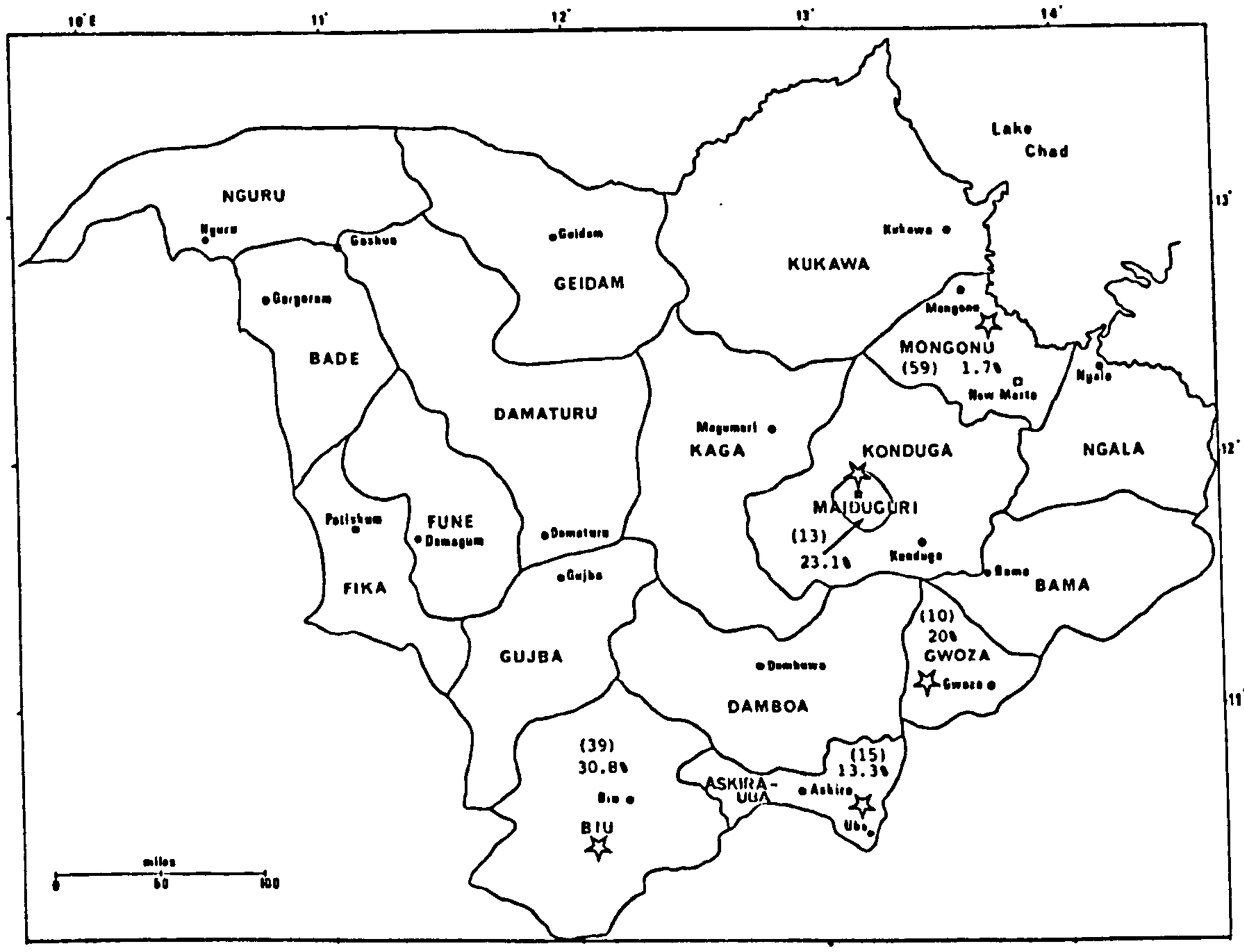
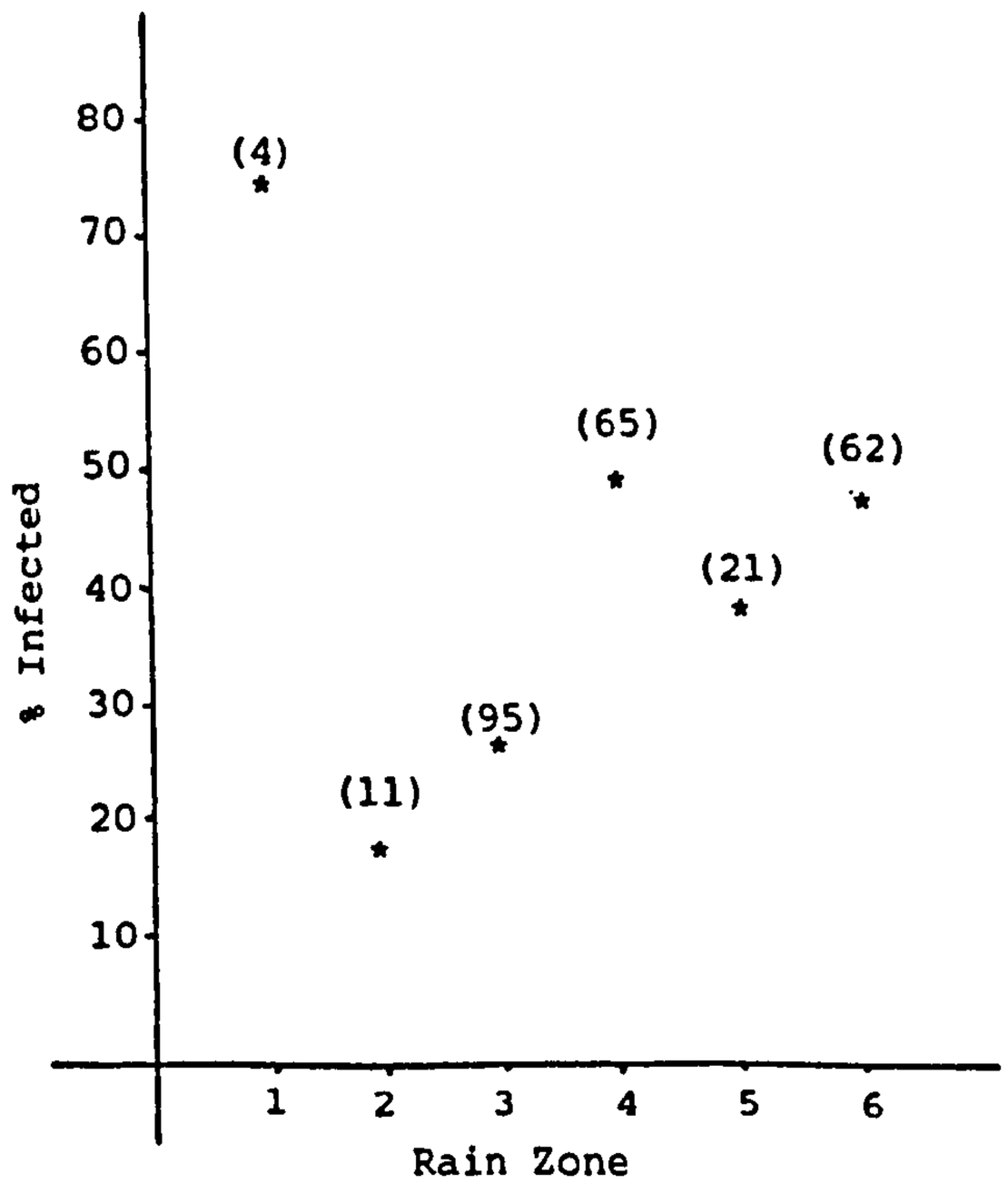



FIGURE 3.7

Relationship between hookworm infection and rain zone of boys' home villages



## DISCUSSION

The 303 boys sampled at New Marte Secondary School came from villages widely dispersed throughout Borno State. They included a large enough sample from within the area influenced by S.C.I.P. to enable patterns of helminth infection to be established, as well as providing information on the parasites present in the population moving to the area from other parts of Borno.

All the boys sampled were in the stated age range of 8 to 18 years. Since many seemed uncertain of their precise age, no attempt was made to break the sample down into smaller age groups.

Responses to the questionnaire provided valuable information on each boy's background. A few sections produced unexpected and almost certainly inaccurate replies. Although 98% of those questioned claimed to wear shoes at all times when outdoors, observations on the children playing in the school grounds indicated that it was very common for them to be barefoot. Similarly, although 75% claimed to sleep under mosquito nets at home, local sources indicated that their use was uncommon. It seems likely that the children were aware of the social stigma attached to their answers, and were not providing accurate information.

An attempt was made to obtain details of any past history of haematuria and other medical complaints. Once more, it seemed likely that responses may have been influenced by cultural attitudes. There was also some confusion over the

question, thus no attempt was made to analyse responses received. Ellis, Long and Friedland (1968) and Holzer, Saladin, Saladin, Dennis and Degremont (1983) encountered similar problems.

Although an attempt was made to obtain detailed information on the sources of water for a variety of domestic uses in both dry and wet seasons, responses were too confused for analysis. A much simpler breakdown into the main sources available in home villages was eventually used.

The concept of "swimming" was particularly difficult to convey to the boys, yet it became clear that the children were making extensive use of the Njine Branch Canal of S.C.I.P. where it runs close to the school grounds.

Throughout the analysis of helminth infection data in relation to the geographical origin of the boys, only the children who had lived in the same area all their life were included. Although it is theoretically possible that some infections had been acquired while the children were at the school, this seems very unlikely in the case of the two schistosome species. The school's water supply consisted of storage tanks filled by water tankers that collected it directly from a nearby borehole standpipe. There was thus very little chance of contamination with schistosome cercariae. The only other main source of water contact was at a section of the Njine Branch Canal and this had been found to be free from intermediate host species up to the time of the survey. The other potential sources of water contact, seasonal rainpools,

are only present during the short rainy season. Potential intermediate hosts of S. haematobium (Bulinus senegalensis) were only present in such pools from mid-July to late September (Betterton, Fryer and Wright, 1983), a period which coincides with the long vacation. It is thus likely that all schistosome infections had been acquired away from the school.

Transmission of the other helminth species encountered in the survey, none of which require an intermediate host, may have been occurring within the school.

All the helminth species detected have been reported previously in northern Nigeria (Ramsay,1934). Menu et al. (1973) and Noamesi and Morcos (1974) working in the S.C.I.P. area reported cases of hookworm, Ascaris, Taenia, Trichuris and Strongyloides. The only species present in any number in the present survey were hookworm and H. nana. No attempt was made to identify which species of hookworm was present since both Necator americanus and Ancylostoma duodenale coexist in the area and their eggs are difficult to separate. Although H. nana was not reported in either of the previous S.C.I.P. surveys, Ramsay (1934) detected it in 3.5% of samples from northern Nigeria, particularly commonly in children. More recently Oomen (1974) found 6.4% of Hausa schoolchildren at Malumfashi to be infected.

Hookworm was found in a much higher proportion of the present survey group (38.95% overall, 23.9% in S.C.I.P.) than had been previously reported by Menu et al. (1973) or Noamesi and Morcos (1974) even when allowances are made for the age of

the boys involved. Menu et al. (1973) found 13.8% of 10 to 14 year olds infected and Noamesi and Morcos (1974) found an overall prevalence of only 4.5%. Thirteen percent of Malumfashi schoolchildren aged 7 to 14 years had hookworm ova in samples examined by Oomen (1974). The only higher prevalence rate was that reported by Ramsay (1934) where 45% of males in northern Nigeria were found to be infected. Nwosu (1982) considered that the prevalence rate of hookworm throughout rural Nigeria was 12 - 21%, rising to 70 - 80% in urban areas. Although it seems likely that hookworm transmission was occurring within the tight residential community of New Marte Secondary School, the close association between prevalence and the rainfall in the boys' home villages indicates that the majority of cases were acquired away from the school.

The other helminth species, Ascaris, Enterobius and Trichuris were all present in only 1 to 3 of the boys sampled. Their presence does, however, provide opportunities for their transmission within this close community.

The prevalence rate of S. haematobium infections in local children at New Marte Secondary School (60.6%) was higher than that reported previously in S.C.I.P.. Boys in the age range of the present study are generally considered to be the most heavily infected section of a population (Jordan and Webbe, 1982), yet the proportion of boys infected was higher than in the sample of 10 - 14 year old males (38.8%) and 15 - 24 year old males (41.3%) examined by Menu et al. (1973). Noamesi and

Morcos (1974) do not present data from each sex, but found only 19.8% of 10 - 19 year olds to be infected.

The analysis of S. haematobium prevalence rates with the geographical origin of the boys' home village was restricted by the uneven distribution of the sample. The lack of details regarding the geographical features around each village has made the analysis very oversimplified. Division of the sample into areas of rainfall examines only one of the several geographical variables that might be interacting to affect the transmission rate of schistosomes. The pattern of S. haematobium prevalence with rainfall areas is, however, of interest, indicating that infection rates do not simply increase with rainfall. The pattern shown (Fig. 3.4) could be a result of a combination of increased snail habitats as rainfall increases with a greater concentration of human activity around water resources where rainfall levels are low. Thus transmission is high where there are many natural water bodies suitable as habitats for the snail intermediate hosts of the parasite, yet where low levels of rainfall make surface water less common, human use of these bodies is intense resulting in greater contamination and increased transmission.

The provision of "safe" piped water supplies is often associated with a decrease in the transmission of schistosomiasis (e.g. Farooq, Nielsen, Samaan, Mallah and Allam, 1966; O.U.E.N., 1974; Jordan, Woodstock, Urrau and Cook, 1975; De Wolfe Miller, Hussein, Mancy, Hilbert, Monto and Barakat, 1981; Schutte, Deventer and Lamprecht, 1981). In the

present study, although an association was found between the main water source in the children's home village and S. haematobium prevalence, those with natural water sources only had lower infection rates than those boys with either boreholes or wells available. Even when variations in water supply with the geographical location of the village and the subsequent interactions with S. haematobium prevalence are taken into consideration, there is no evidence for the provision of "safe" boreholes reducing parasite transmission. Although many artesian boreholes have been sunk in the State, particularly within S.C.I.P., many of these are inadequate for the population they serve (Menu et al.,1973). Boreholes are often poorly maintained, with many leaking constantly, thus producing overspill pools which are often colonised by intermediate hosts of schistosomes (Odei, 1977; Betterton et al., 1983). The inadequacy of these "safe" supplies leads to extensive use of natural pools when these are present during the rainy season. It is therefore probable that the majority of S. haematobium transmission is occurring in the rainy season, with "safe" water supplies failing to reduce infection rates.

Although the present sample distribution did not allow the apparent association between housing type and S. haematobium prevalence to be separated from the effects of the geographical location of the boys' home village, other studies have shown a significantly lower infection rate in people inhabiting modern dwellings (e.g. Farooq et al., 1966). Since housing type is a strong indicator of social and economic standards, other

variables may also play a role in the reduction in levels of infection in this section of the population.

The provision of pit latrines is often considered important in schistosomiasis control, yet in this survey there was no reduced prevalence of infection associated with the presence of a latrine. The high proportion of homes possessing a pit latrine in the S.C.I.P. area (91.5% of boys questioned) is probably the result of efforts to encourage their construction over recent years (Menu et al., 1973). Although pit latrines are common, personal observations indicate that they are not used regularly, particularly by the young boys of the household.

The intensities of S. haematobium infections in this survey were generally low, with 50% of positive specimens containing fewer than 16 ova/10ml urine, and only just over 10% with more than 100 ova/10ml. Direct comparison with Noamesi and Morcos's study (1974) in local villages is not possible due to the differences in the counting techniques used in both studies.

The strong association between haematuria and S. haematobium infection, with 85% of positive samples containing erythrocytes, has been reported in many studies (e.g. Ellis et al., 1968; O.U.E.N., 1974; Wilkins, Goll, Marshall and More, 1979; Schutte et al., 1981; Feldmeier, Doehring and Daffalla, 1982; Holzer et al.,1983; Mott, Dixon, Osei-Tutu and England, 1983; Tanner, Holzer, Marti, Saladin and Degremont, 1983; Zumstein, 1983).

Microscopic haematuria occurred in 7.8% of samples that contained no schistosome ova. Considering the low intensity of infections, and the well known fluctuations in daily output of ova in S. haematobium infections (Stimmel and Scott, 1956; Scott, 1957; McCullough and Bradley, 1973; Wilkins, 1977; Warren, Mahmoud, Muruka, Whittaker, Ouma and Arap Siongok, 1979) it is likely that examination of further samples would have revealed infections in at least some of the subjects. Such "false negative" results have been reported by other workers ( e.g. Feldmeier et al., 1982; Zumstein, 1983).

The absence of erythrocytes in samples was not a good indicator of the absence of S. haematobium infection, since 17.7% of specimens with no detectable haematuria contained schistosome ova.

Despite the low intensity of S. haematobium infections, there was a significant association between the degree of haematuria (absent, microscopic or macroscopic) and the number of ova/10ml urine. A similar relationship has been shown in studies both where haematuria has been quantified visually (Schutte et al., 1981) and when reagent strips have been used (Wilkins et al., 1979; Pugh, Bell and Gilles, 1980; Feldmeier et al., 1982; Mott et al., 1983; Zumstein, 1983).

Previous surveys in the area have detected cases of S. mansoni. Menu et al. (1973) found two infected individuals (of 610 sampled), one a fisherman thought to have travelled extensively around Lake Chad, and the other a woman permanently resident in a village within S.C.I.P. (Logomani). Noamesi and

Morcos (1974) found no S. mansoni infections in 179 people sampled from S.C.I.P. villages, but did detect ova in 5 of 41 specimens from workers in the Pilot Project at Ngala. No indication was given of the origin of these workers. The survey at Yau (O.U.E.N., 1974), also close to Lake Chad, detected infections in 19 of 396 people sampled, but only one of these was a permanent resident of the town. Similarly, although S. mansoni ova were found in specimens from 26 of the 301 boys sampled in the present survey, only one of these claimed permanent residence in the S.C.I.P. region. It therefore seems that although some transmission of this species occurs, it is extremely rare in this region of Borno State.

Cowper (1963 and 1973) commented on the widespread but localised distribution of S. mansoni in Nigeria. Infection rates were reported to be high in Biu and Maiduguri (Cowper, 1963), two of the areas from which many of the infected boys originated in this study.

A possible intermediate host of S. mansoni, Biomphalaria sudanica, has been found on the shores of Lake Chad (Menu et al., 1973; Odei, 1977) in this region. Betterton (1984) found this species both in the Lake itself and in the main intake channel of S.C.I.P.. The increased likelihood of contact with this intermediate host with improved access to Lake Chad via the Main Intake Channel, and its possible spread to other areas of the irrigation scheme, combined with the movement of infected individuals into the area from regions where prevalence of S. mansoni is high, could lead to an increase in

the transmission of this schistosome in the S.C.I.P. area.

## REFERENCES


Agboola, S.A., 1979. An Agricultural Atlas of Nigeria. Oxford University Press. 248pp.

Bell, D.R., 1969. Clinical trials and diagnostic methods in schistosomiasis. Annals of the New York Academy of Science **160** : 593-601.

Betterton, C., 1984. Ecological studies on the snail hosts of schistosomiasis in the South Chad Irrigation Project Area, Borno State, northern Nigeria. Journal of Arid Environments **7** : 43-57.

Betterton, C., Fryer, S.E. and Wright, C.A., 1983. Bulinus senegalensis (Mollusca:Planorbidae) in northern Nigeria. Annals of Tropical Medicine and Parasitology **77** : 143-149.

Cowper, S.G., 1963. Schistosomiasis in Nigeria. Annals of Tropical Medicine and Parasitology **57** : 307-322.

Cowper, S.G., 1973. Bilharziasis (schistosomiasis) in Nigeria. Tropical and Geographical Medicine **25** : 105-118.

DeWolfe Miller, F., Hussein, M., Mancy, K.H., Hilbert, M.S., Monto, A.S. and Barakat, R.M.R., 1981. An epidemiological study of Schistosoma haematobium and S. mansoni infection in thirty-five rural Egyptian villages. Tropical and Geographical Medicine **33** : 355-365.

Doehring, E., Feldmeier, H. and Daffalla, A.A., 1983. Day-to-day variation and circadian rhythm of egg excretion in urinary schistosomiasis in the Sudan. Annals of Tropical Medicine and Parasitology **77** : 587-594.

Ellis, C.R., Long, S. and Friedland, G., 1968. Prevalence of S. haematobium in the Okene Area of Nigeria, West African Medical Journal 17 : 21-24.

Farooq, M., Nielsen, J., Samaan, S.A., Mallah, M.B. and Allam, A.A., 1966. The epidemiology of Schistosoma heamatobium and S. mansoni infections in the Egypt-49 Project Area. 3. Prevalence of bilharziasis in relation to certain environmental factors. Bulletin of the World Health Organisation 35 : 319-330.

Feldmeier, H., Doehring, E. and Daffalla, A.A., 1982. Simultaneous use of a sensitive filtration technique and reagent strips in urinary schistosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 76 : 416-421.

Holzer, B., Saladin, K., Saladin, B., Dennis, E.P. and Degremont, A., 1983. The impact of schistosomiasis among rural populations in Liberia. Acta Tropica 40 : 239-259.

Iarotski, L.S. and Davis, A., 1981. The schistosomiasis problem in the World: results of a W.H.O. questionnaire survey. Bulletin of the World Health Organisation 59 : 115-127.

Jordan, P. and Webbe, G., 1982. Schistosomiasis: Epidemiology, Treatment and Control. William Heinemann Medical Books Ltd. London. 361pp.

Jordan, P., Woodstock, L., Urrau, G.O. and Cook, J.A., 1975. Control of Schistosoma mansoni transmission by the provision of domestic water supplies: Preliminary report on a study in St. Lucia. Bulletin of the World Health Organisation 52 : 9-20.

McCullough, F.S. and Bradley, D.J., 1973. Egg output stability of Schistosoma heamatobium. Part I: Variation and stability of Schistosoma haematobium egg counts. Transactions of the Royal Society of Tropical Medicine and Hygiene 67 : 475-490.

Menu, J.P., Dove, J.E., Noamesi, G.K. and Dazo, B.C., 1973. Health component in the South Chad Irrigation Project - Feasibility Study, report on missions (September 1972 - March 1973). W.H.O. Unpublished document AFR/PHA/117 116pp.

Mott, K.E., Dixon, H., Osei-Tutu, E. and England, E.C., 1983. Relation between intensity of Schistosoma haematobium infection and clinical haematuria and proteinuria. The Lancet 1 (8332) : 1005-1008.

Nachtigal, G., 1881. Sahara und Sudan: Ergebnisse Sechsjahriger Reisen in Afrika, Band 2 (1881), Berlin. Reprinted: Akademische Druck und Verlagsanstalt, Graz, Austria (1967):465.

Noamesi, G.K. and Morcos, G., 1974. Health component in the South Chad Irrigation Project - Feasibility Study. Follow-up studies on schistosomiasis and malaria. World Health Organisation Unpublished document AFR/SCHIST/30 26pp.

Nwosu, A.B.C., 1982. Intestinal helminthiasis in Nigeria: epidemiology, infection dynamics, and control strategies. Medecine d'Afrique Noire 29 : 587-591.

Odei, M.A., 1977. Health component in the South Chad Irrigation Project - Feasibility Study. Follow-up studies on the snail hosts of schistosomiasis. World Health Organisation Unpublished document AFR/SCHIST/37 73pp.

Oomen, J.M.V., 1974. Health of Hausa schoolchildren in Northern Nigeria. Tropical and Geographical Medicine 26 : 137-146.

O.U.E.N., 1974. Oxford University Expedition to Nigeria, 1971. Bulletin of the Oxford University Exploration Club 20, Special Series 1. : 1-95.

Pugh, R.N.H., 1979 a. Periodicity of output of Schistosoma heamatobium eggs in the urine. Annals of Tropical Medicine and Parasitology 73 : 89-90.

Pugh R.N.H., 1979 b. Malumfashi Endemic Diseases Research Project VII: The importance of young males fom the Malumfashi area, northern Nigeria, in the transmission of Schistosoma heamatobium infection. Annals of Tropical Medicine and Parasitology 73 : 189-190.

Pugh, R.N.H., Bell, D.R. and Gilles, H.M., 1980. Malumfashi Endemic Diseases Research Project XV: the potential medical importance of bilharzia in northern Nigeria: a suggested rapid, cheap and effective solution to control of Schistosoma haematobium infection. Annals of Tropical Medicine and Parasitology 74 : 597-613.

Ramsay, G.W. St.C., 1934. A study of schistosomiasis and certain other helminthic infections in Northern Nigeria. West African Medical Journal 8(2) : 2-10 and 8(3) : 2-7.

Schutte, C.H.J., van Deventer, J.M.G. and Lamprecht, Y., 1981. A cross-sectional study on the prevalence and intensity of infection with Schistosoma haematobium in students of Northern Kwazulu. American Journal of Tropical Medicine and Hygiene 30 : 364-372.

Scott, J.A., 1957. Egg counts as estimates of intensity of infection with S. haematobium. Texas Reports on Biology and Medicine 15 : 425-430.

Stimmel, C.M. and Scott, J.A., 1956. The regularity of egg output of S. haematobium. Texas Reports on Biology and Medicine 14 : 440-458.

Tanner, M., Holzer, B., Marti, H.P., Saladin, B. and Degremont, A.A., 1983. Frequency of haematuria and proteinuria among Schistosoma haematobium infected children of two communities from Liberia and Tanzania. Acta Tropica 40 : 231-237.

Warren, K.S., Mahmoud, A.A.F., Muruka, J.F., Whittaker, L.R., Ouma, J.H. and Arap Siongok, T.K., 1979. Schistosomiasis haematobia in Coast Province, Kenya. Relationship between egg output and morbidity. American Journal of Tropical Medicine and Hygiene 28 : 864-870.

Wilkins, H.A., 1977. Schistosoma haematobium in a Gambian community. I. The intensity and prevalence of infection. Annals of Tropical Medicine and Parasitology 71 : 53-58.

Wilkins, H.A., Goll, P.de C., Marshall, T.F. and More, P., 1979. The significance of proteinuria and haematuria in Schistosoma haematobium infection. Transactions of the Royal Society of Tropical Medicine and Hygiene 73 : 74-80.

Zumstein, A., 1983. A study of some factors influencing the epidemiology of urinary schistosomiasis in Ifakara (Kilombero District, Morogoro Region, Tanzania). Acta Tropica 40 : 187-204.

# CHAPTER 4

# SUSCEPTIBILITY OF EACH *BULINUS* SPECIES TO INFECTION WITH THE LOCAL STRAINS OF *S. HAEMATOBIUM*

## INTRODUCTION

It has long been recognised that variation exists in the compatibility of *Schistosoma haematobium* and *Bulinus* species from different geographical locations. This was mentioned as early as 1947 by Cowper and has been discussed by numerous authors since, including Gismann (1954), McCullough (1955), Webbe (1971), Berrie (1973) and Brown (1980). The general pattern of parasite strain and snail host distribution was discussed by Wright and Southgate (1981). There are two main strains of *S. haematobium*, the first in the Mediterranean area of North Africa and the Middle East using intermediate hosts of the *Bulinus truncatus* group, the second south of the Sahara, where the parasite develops in snails of the *B. africanus* group. *S. haematobium* from the northern area cannot normally develop in *B.africanus* group snails, while the parasite from regions south of the Sahara usually fails to infect snails of the *B. truncatus* group. The situation in West Africa, where the distribution of both species groups overlaps is more complex, with the presence of an apparent mosaic of the two parasite strains.

Leroux (1958) proposed a case for recognising two distinct

species of the parasite, with S. haematobium transmitted by B. truncatus group snails occurring in the northern areas, and reinstating 'S. capense' (Harley) to describe the southern species developing in B. africanus group snails. It is now generally accepted that S.haematobium exists as a single but complex species with several different geographical strains (Webbe and James, 1971; Berrie, 1973).

In some areas of West Africa (e.g. Ghana) where members of both the B. africanus and B. truncatus groups exist within the same region, evidence has shown that both strains occur within the human population and even in the same individual (Chu, Kpo and Klumpp, 1978). There is also strong evidence that hybridisation occurs between the two forms (Wright and Southgate, 1976).

Field studies in the South Chad Irrigation Project (S.C.I.P.) Borno State, Nigeria (Betterton, 1984a) have shown the presence of Bulinus species representing three of the main species groups:- B. rohlfsi (B.truncatus group), two populations of the B. africanus group species B. globosus (Ngala and Lake Chad) which show some morphological and behavioural differences (see Chapter 7) and B. forskali and B. senegalensis of the B. forskali group. B. rohlfsi is recognised as a natural host of S. haematobium in many areas (Brown, 1980) transmitting truncatus strains. B. globosus acts as intermediate host to africanus strains of the parasite in many regions throughout its very wide range, this species being present in most of Africa south of the Sahara (Brown, 1980).

It is these two snail species that have been extensively studied in areas of Ghana where two distinct strains of parasite occur (Paperna, 1968; Chu *et al.*, 1978) with some apparent mixing of strains. *B. senegalensis*, a 'relic' species of the *B. forskali* group is known to be able to act as host for both africanus and truncatus strains of *S. haematobium* (Wright, 1977). *B. forskali*, an extremely widespread species occurring over much of the African continent, has not been proved to transmit *S. haematobium* under natural conditions (Brown,1980) although occasional successful laboratory infections have been reported, some of these may have been in misidentified closely related forskali group species.

Naturally occurring schistosome infections were reported by Betterton (1984a) in two of these snail species. *B. rohlfsi* was found shedding mammalian schistosome cercariae, although no positive identification of species was possible. *B. globosus* (Ngala) were collected carrying positively identified *S. haematobium* infections.

The aim of the present investigation was to test the relative susceptibility of each of the *Bulinus* species collected from the area of the S.C.I.P. to *S. haematobium* obtained from the human population within the scheme, and to compare the compatibility of snail host/parasite strain combinations.

## MATERIALS AND METHODS

All snails used in the present study were from laboratory bred cultures set up from wild caught material in 1980 and 1981. The culture methods used were as described in Chapter 2.

The susceptibility study reported here covered all the bulinid snail species from the area with the exception of B. forskali which failed to become established in the laboratory.

S. haematobium eggs were obtained from pooled urine samples collected from individuals taking part in the school survey described in Chapter 3. Donors were selected from those children who were, as far as possible, known to have lived in the Project area all their lives with no time spent out of the region.

Wild caught specimens of B. rohlfsi and the two B. globosus populations, which had been screened and shown to be free from any mature trematode infections, were exposed to freshly hatched miracidia from the pooled urine samples in the field laboratory. These snails were then transported back to the Bangor laboratory within two weeks of exposure. Infection and mortality data from these initial exposures are not included in the results due to the problems of using wild caught snails, uncontrolled laboratory conditions, and the extra stresses involved in packing and transportation.

Cercariae from B. rohlfsi and B. globosus Ngala snails that survived and developed mature infections were then used to expose golden hamsters (Mesocricetus auratus) to the known strain of parasite ('rohlfsi' or 'globosus') using the paddling

technique described in Chapter 2. This was then repeated with cercariae produced by infected snails in subsequent passages. No mature infections developed in B. globosus from the Lake Chad population.

Eggs isolated from livers, guts and faeces of infected hamsters were used in experimental exposures of the whole range of Bulinus species, where possible material from each individual hamster was used to expose the whole range of species so that direct comparisons could be made. The methods employed were as described in Chapter 2, with snails 3-5mm in length being exposed individually to 5 miracidia at 25°C for 5 hours under bright illumination.

Snails were then maintained under the standard conditions described in Chapter 2, with B. globosus (Ngala and Lake Chad) and B. rohlfsi kept at 26±1°C and B. senegalensis at 30±1°C.

From approximately 20 days after initial exposure, snails were screened individually (using the standard technique in Chapter 2) for the production of cercariae at 2 - 3 day intervals. A successful infection was defined as one that released cercariae. Two values for the precercarial period were recorded for each infected snail, the minimum value being one day after the last screening where no cercariae were produced, and a maximum value for the day that cercariae were first observed. All snails dying within the experimental period were crushed and searched visually for the presence of sporocysts.

Infected snails were kept individually in plastic

containers with 150ml of water. The number of days of cercarial production was recorded for each snail. Since no cases of 'self cure' were observed, this corresponded to the length of time from first appearance of cercariae to the snail's death.

Control groups of snails were set up in parallel with some experimental exposures to compare mortality rates during the precercarial stage. Control snails were treated identically to experimental ones, each being isolated for 5 hours at the time of the experimental exposure and isolated in parallel with experimental snails during the screening period for cercarial production. As infected snails were transferred to individual containers on first detection of cercariae, a control of similar size was selected for identical treatment, with both being subjected to the same conditions for the remainder of their lives. In this way the longevity of infected and control snails could be compared by recording the survival time, i.e. number of days from the start of the experiment to the snail's death.

## RESULTS

### 1. Infection Rates.

Table 4.1 presents a summary of the results of infection experiments involving the four bulinid populations. *B. globosus* Ngala proved susceptible to the 'globosus' strain of *S. haematobium* but refractory to the 'rohlfsi' strain. *B. rohlfsi* only became infected when exposed to the 'rohlfsi'

TABLE 4.1

**Infection rates in the four species of *Bulinus* exposed to 'rohlfsi' and 'globosus' strains of *S. haematobium* over two passages.**

| P* | SNAIL SPECIES | 'GLOBOSUS' STRAIN OF PARASITE | | | 'ROHLFSI' STRAIN OF PARASITE | | |
|---|---|---|---|---|---|---|---|
| | | Exposed | Survived | Infected | Exposed | Survived | Infected |
| I | *B. globosus*(Ng) | 50 | 45 | 27(60%) | 30 | 30 | 0 |
| | *B. rohlfsi* | 33 | 31 | 0 | 35 | 14 | 0 |
| | *B. senegalensis* | 75 | 69 | 18(26%) | 23 | 20 | 8(40%) |
| | *B. globosus*(LC) | 10 | 10 | 0 | 15 | 11 | 0 |
| II | *B. globosus* (Ng) | 404 | 299 | 97(32%) | 82 | 78 | 0 |
| | *B. rohlfsi* | 360 | 326 | 0 | 295 | 234 | 29(12%) |
| | *B. senegalensis* | 528 | 433 | 43(10%) | 146 | 137 | 29(21%) |
| | *B. globosus*(LC) | 145 | 126 | 0 | 0 | - | - |
| POOLED | *B. globosus* (Ng) | 454 | 344 | 124(36%) | 112 | 108 | 0 |
| | *B. rohlfsi* | 393 | 357 | 0 | 330 | 248 | 29(11%) |
| | *B. senegalensis* | 603 | 502 | 61(12%) | 169 | 157 | 37(23%) |
| | *B. globosus* (LC) | 155 | 136 | 0 | 15 | 11 | 0 |

P* = Passage
Ng = Ngala LC = Lake Chad

strain of the parasite while both 'globosus' and 'rohlfsi' strains developed successfully in B. senegalensis. B. globosus Lake Chad was refractory to both strains.

Examination of snails that died during the course of experiments failed to detect any developing sporocysts. Unfortunately, many dead snails had been consumed by live ones in the container before being retrieved so that it was not possible to examine every specimen.

In the first passage no B. rohlfsi became infected, even with the 'rohlfsi' strain of parasite. This was almost certainly due to the small numbers of snails exposed, and the high mortality experienced during the precercarial period. Some of the snails dying during this period may have been infected.

There was an apparent decrease in the infection rate of both parasite strains in the second passage, with this reduction being significant for the 'globosus' strain of S. haematobium in both B. globosus Ngala ($X^2$=11.72, p<0.001) and B. senegalensis ($X^2$=13.08, p<0.001). Although a reduction was seen in the case of B. senegalensis exposed to the 'rohlfsi' strain, this was not significant at the 5% level ($X^2$=2.47, p>0.1). Throughout the statistical analysis, results from each passage are therefore presented separately in addition to the pooled data.

A generalised linear model with Poisson error distribution was used to examine the three factor contingency table to test for interactions between infection rates, snail species,

parasite strain and their three way interaction (Table 4.2). This shows that at the 5% level there is a significant difference in infection rates between snail species and also a three way interaction between infection rate, parasite strain and snail species. There is, however, no difference in the infection rate between parasite strains alone, except in the very limited first passage experiments.

Each of these significant interactions was then examined more closely by analysing th 2X2 contingency tables for each component (using 1% level of significance).

The interaction between snail species and parasite infectivity indicated by the model is shown clearly in Table 4.3. This shows the percentage of all snails of each species that became infected when exposed to parasite miracidia, regardless of the strain of S. haematobium involved. Calculating $X^2$ values for each pair of snail species there is a significant difference in infection rates at the 1% level for all combinations except B. globosus Ngala and B. senegalensis in the first passage ($X^2$=0.57 $0.3<p<0.5$). In all cases B. globosus Ngala showed the highest infection rate and B.senegalensis showed higher infection rates than B. rohlfsi.

The linear model also shows a highly significant three way interaction between infection rate, parasite strain and snail species. This is clearly seen where a given snail species is susceptible to one parasite strain, but refractory to infection by the other. Table 4.4 shows that there are also significant differences in infection rates between compatible snail

TABLE 4.2

$X^2$ values from generalised linear model of three factor contingency table between parasite strain, snail species and infection rate.

| | First passage | Second passage | Pooled data |
|---|---|---|---|
| Parasite strain: Infection rate | $X^2$=8.84 .001<p<.01 | $X^2$=0.03 N.S. | $X^2$=1.86 N.S. |
| Snail species: Infection rate | $X^2$=33.36 p<.001 | $X^2$=84.26 p<.001 | $X^2$=108.44 p<.001 |
| Three way interaction | $X^2$=27.78 p<.001 | $X^2$=114.38 p<.001 | $X^2$=146.50 p<.001 |

N.S.= not significant at the 5% level of confidence

TABLE 4.3

Overall infection rates for each Bulinus species exposed to the two strains of S.haematobium under investigation.

| | First passage | Second passage | Pooled data |
|---|---|---|---|
| B. globosus(Ng) | 36% | 25.5% | 27.5% |
| B. rohlfsi | 0 | 5.0% | 5.0% |
| B. senegalensis | 29% | 12.5% | 15.0% |

Ng = Ngala

**TABLE 4.4**

**$X^2$ and p values for each pair of infection rate values in susceptible snail species / parasite strain combinations.**

| | First passage | | Second passage | | Pooled data | |
|---|---|---|---|---|---|---|
| *B. globosus* (Ng) 'g' v *B. rohlfsi* 'r' | 13.16 | p<0.001 | 28.13 | p<0.001 | 43.33 | p<0.001 |
| *B. globosus* (Ng)'g' v *B.senegalensis* 'g' | 11.73 | p<0.001 | 56.50 | p<0.001 | 66.82 | p<0.001 |
| *B. globosus* (Ng) 'g' v *B. senegalensis* 'r' | 1.50 | N.S. | 5.27 | N.S. | 7.14 | 0.001<p p<0.01 |
| *B. senegalensis* 'r' v *B. rohlfsi* 'r' | 5.27 | N.S. | 4.40 | N.S. | 9.08 | 0.001<p p<0.01 |
| *B.senegalensis* 'r' v *B.senegalensis* 'g' | 0.86 | N.S. | 12.67 | p<0.001 | 11.43 | p<0.001 |
| *B. senegalensis* 'g' v *B. rohlfsi* 'r' | 3.25 | N.S. | 1.48 | N.S. | 0.003 | N.S. |

N.S. = Not significant at the 1% level of confidence.
'g' = 'globosus' strain of *S. haematobium*
'r' = 'rohlfsi' strain of *S. haematobium*

species/ parasite strain combinations. B.globosus Ngala exposed to the 'globosus' strain of S. haematobium shows significantly higher infection rates at the 1% level than B. rohlfsi exposed to the 'rohlfsi' strain and B. senegalensis exposed to the 'globosus' strain. It also shows significantly higher infection rates than B. senegalensis exposed to 'rohlfsi' strain if combined data are examined.

The 'rohlfsi' strain of parasite shows higher infection rates in B. senegalensis than in B. rohlfsi, although this is only significant at the 1% level in second and combined passage results. This is also the case when comparing the two parasite strains in B. senegalensis, with the 'rohlfsi' strain showing higher infection rates than the 'globosus' strain.

**2. Mortality Rates in the Precercarial Period:**

The mortality figures up to 28 days from the start of each experiment are summarised in Table 4.5, which includes the results of control groups.

Significantly higher mortality rates occurred in the second passage in the case of both B. globosus Ngala ($X^2$=5.36, 0.01<p<0.05) and B. senegalensis ($X^2$=4.01, 0.01<p<0.05) exposed to the 'globosus' strain of S. haematobium. B. rohlfsi exposed to the 'rohlfsi' strain of parasite showed a significantly higher mortality rate in the first passage than in the second ($X^2$=23.84, p<0.001). Other combinations showed no significant difference in mortality rates between passage. The statistical analysis was therefore carried out on individual passages as well as combined results. Since control experiments were run

in parallel to second passage, these are only compared directly with the second passage data except in the initial linear model.

Table 4.6 shows the results of the generalised linear model with Poisson error distribution. This shows a significant interaction at the 5% level between snail species and mortality rate and a three way interaction of mortality rate, snail species and parasite strain (which includes no parasite, or control). There was no significant difference in mortality rates between parasite strains (and control) at the 5% level, except in the very limited first passage data.

These interactions were then further investigated by analysing the 2x2 contingency tables of each individual combination.

The interaction between snail species and mortality can be seen clearly by comparing the control mortalities of each species (see Table 4.5). Although there is no significant difference between the mortality rates up to 28 days between B. globosus Ngala and B. senegalensis ($X^2$=0.55, 0.3<p<0.5). B. rohlfsi shows significantly higher rates than both B. senegalensis ($X^2$=10.23, 0.001<p<0.01) and B. globosus Ngala ($X^2$=9.62, 0.001<p<0.01).

The linear model also showed a highly significant three way interaction between mortality rates, parasite strain and snail species. Analysing these interactions more closely, control mortalities were first compared to the mortalities after exposure to each parasite strain (see Table 4.5). In

## TABLE 4.5

**Mortality of exposed and control snails over the first 28 days of experiments.**

| PASSAGE | | 'GLOBOSUS' STRAIN | | | | 'ROHLFSI' STRAIN | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | DEAD | LIVE | %MORT. | N | DEAD | LIVE | %MORT. |
| I | B. globosus(Ng) | 50 | 5 | 45 | 10 | 30 | 0 | 30 | 0 |
| | B. rohlfsi | 33 | 2 | 31 | 6 | 35 | 21 | 14 | 60 |
| | B. senegalensis | 75 | 6 | 69 | 8 | 23 | 3 | 20 | 13 |
| | B. globosus(LC) | 10 | 0 | 10 | 0 | 15 | 4 | 11 | 26.5 |
| II | B. globosus(Ng) | 404 | 105 | 299 | 26 | 82 | 4 | 78 | 5 |
| | B. rohlfsi | 360 | 34 | 326 | 9.5 | 295 | 61 | 234 | 20.5 |
| | B. senegalensis | 528 | 95 | 433 | 18 | 146 | 9 | 137 | 6 |
| | B. globosus(LC) | 145 | 19 | 126 | 13 | 0 | - | - | - |
| POOLED | B. globosus(Ng) | 454 | 110 | 344 | 24 | 112 | 4 | 108 | 3.5 |
| | B. rohlfsi | 393 | 36 | 357 | 9 | 330 | 82 | 248 | 25 |
| | B. senegalensis | 603 | 101 | 502 | 17 | 169 | 12 | 157 | 7 |
| | B. globosus(LC) | 155 | 19 | 136 | 12.5 | 15 | 4 | 11 | 26.5 |

| Uninfected Controls | N | DEAD | LIVE | %MORT |
|---|---|---|---|---|
| B. globosus(Ng) | 125 | 13 | 112 | 10.5 |
| B. rohlfsi | 125 | 33 | 92 | 26.5 |
| B. senegalensis | 175 | 20 | 155 | 11.5 |
| B. globosus(LC) | 20 | 0 | 20 | 0 |

Ng = Ngala
L.C. = Lake Chad

## TABLE 4.6

**$X^2$ values from generalised linear model of three factor contingency table between parasite strain, snail species and mortality rate.**

| | First passage | Second passage | Pooled data |
|---|---|---|---|
| **Parasite strain: Mortality rate** | $X^2$=11.05 .001<p<.01 | $X^2$=4.36 N.S. | $X^2$=0.98 N.S. |
| **Snail species: Mortality rate** | $X^2$=36.08 p<.001 | $X^2$=7.29 .02<p<.05 | $X^2$=7.07 .02<p<.05 |
| **Three way interaction** | $X^2$=21.77 p<.001 | $X^2$=70.86 p<.001 | $X^2$=89.64 p<.001 |

N.S.= not significant at the 5% level of confidence

only one case (B. globosus Ngala exposed to the 'globosus' strain of S. haematobium) is there a significantly higher mortality rate than in controls ($X^2$=12.50, p<0.001). Where B. rohlfsi are exposed to the 'globosus' strain, the mortality is actually significantly lower than that of controls ($X^2$=22.94, p<0.001). In B. senegalensis exposed to each strain of parasite, and in all snail species exposed to 'rohlfsi' S. haematobium there is no significant difference between experimental and control mortalities ($X^2$<3.20, p<0.05).

Comparing the mortality rates of each snail species exposed to the two strains of parasite, for both B. globosus Ngala and B. rohlfsi (Table 4.5), mortality rates are significantly higher when exposed to their compatible strain than when exposed to the strain to which they are refractory, (except B. globosus Ngala first passage), (Table 4.7). In the case of B. senegalensis, where both strains are infective, in second and combined passage results exposure to the 'globosus' strain produces significantly higher mortality than the 'rohlfsi'strain.

Comparison of mortality rates in different snail species is only possible where there is either no significant difference between control mortalities, or the difference in mortality rates is in the opposite direction to the controls. Comparing all three species' mortality rates when exposed to the 'globosus' strain (Table 4.8) in all second and combined passage results, there is a significant difference between all the species at the 1% level, with B. globosus Ngala showing the

**TABLE 4.7**

**$X^2$ values comparing mortality rates of each snail species exposed to the two parasite strains.**

| | First passage | Second passage | Pooled data |
|---|---|---|---|
| B. globosus (Ngala) | $X^2$=1.72<br>N.S. | $X^2$=16.27<br>p<.001 | $X^2$=22.57<br>p<.001 |
| B. rohlfsi | $X^2$=19.73<br>p<.001 | $X^2$=15.61<br>p<.001 | $X^2$=31.19<br>p<.001 |
| B. senegalensis | $X^2$=0.10<br>N.S. | $X^2$=11.37<br>p<.001 | $X^2$=9.08<br>.001<p<.01 |

N.S.= not significant at the 1% level of confidence.

**TABLE 4.8**

**$X^2$ and p values comparing mortality rates to 28 days of each *Bulinus* species exposed to the 'globosus' strain of *S. haematobium***

| | First passage | | Second passage | | Pooled data | |
|---|---|---|---|---|---|---|
| B.globosus(Ng) v B.senegalensis | 4.15 | N.S. | 8.22 | 0.001>p<br>p<0.01 | 8.61 | 0.001<p<br>p<0.01 |
| B.globosus(Ng) v B.rohlfsi | 0.05 | N.S. | 33.91 | p<0.001 | 32.48 | p<0.001 |
| B.rohlfsi v B.senegalensis | 0.0019 | N.S. | 11.92 | p<0.001 | 10.92 | p<0.001 |

N.S. = not significant at the 1% level of confidence

highest mortality, and B. senegalensis the lowest. Comparison of the 'rohlfsi' strain exposures is only possible between B. globosus Ngala and B. senegalensis where there is no significant difference in the observed mortality rates ($X^2<2.1$ in all passages).

**3. Infected Snail Parameters**

Summaries of precercarial periods and days of cercarial production are shown in Table 4.9 and 4.10. Some results were obtained from snails exposed to third and fourth passage material. As no significant differences were observed between passages, all the data have been pooled.

Some unavoidable problems were experienced in the statistical analysis of this data. Firstly, in the combination of three snail species and two parasite strains only one species became infected with both strains. As this resulted in a two by three contingency table with two empty cells, it is impossible to test for any interaction between parasite strain and snail species. The analysis has therefore been carried out assuming that there is no such interaction. In addition, for most of the parameters there is a very poor homogeneity of variances. Where the variances increase with means, a $Log_{10}$ transformation was carried out before the analysis, but in most cases no suitable transformation could be made. The statistical analyses were still performed, although some caution must be observed in their interpretation.

A multivariate analysis applied to the precercarial periods showed that there was no significant difference

## TABLE 4.9

**Maximum and minimum precercarial periods of *S. haematobium* infections in bulinids.**

**A: MINIMUM PRECERCARIAL PERIOD**

| Species | Parasite strain | n | Mean (days) | S.D. | Range Min. | Range Max. |
|---|---|---|---|---|---|---|
| *B. globosus* Ng. | 'g' | 107 | 33.29 | 7.91 | 25 | 65 |
| *B. rohlfsi* | 'r' | 41 | 33.66 | 5.91 | 26 | 57 |
| *B. senegalensis* | 'g' | 64 | 38.61 | 11.64 | 21 | 67 |
| *B. senegalensis* | 'r' | 29 | 41.65 | 10.15 | 23 | 60 |

**B: MAXIMUM PRECERCARIAL PERIOD**

| Species | Parasite strain | n | Mean (days) | S.D. | Range Min. | Range Max. |
|---|---|---|---|---|---|---|
| *B. globosus* Ng. | 'g' | 131 | 33.89 | 9.41 | 25 | 76 |
| *B. rohlfsi* | 'r' | 43 | 33.07 | 6.47 | 27 | 74 |
| *B. senegalensis* | 'g' | 67 | 40.63 | 13.94 | 23 | 74 |
| *B. senegalensis* | 'r' | 32 | 41.94 | 10.96 | 25 | 63 |

'g' = globosus 'r' = rohlfsi

## TABLE 4.10

**Duration of cercarial production from bulinids infected with *S. haematobium***

| Species | Parasite strain | n | Mean (days) | S.D. | Range Min. | Range Max. |
|---|---|---|---|---|---|---|
| *B. globosus* Ng. | 'g' | 85 | 168.63 | 127.21 | 6 | 463 |
| *B. rohlfsi* | 'r' | 43 | 41.42 | 22.36 | 1 | 84 |
| *B. senegalensis* | 'g' | 66 | 56.59 | 29.12 | 14 | 129 |
| *B. senegalensis* | 'r' | 32 | 48.53 | 20.42 | 7 | 87 |

'g' = globosus 'r' = rohlfsi

observed between parasite strains for either the minimum (F=2.26, d.f.=1, p=0.134) or maximum (F=0.34, d.f.=1, p=0.562) values. There was, however, a highly significant difference between the snail species (Min: F=13.52, d.f.=2, p<.0001; Max: F=13.05, d.f.=2, p<.0001). A one way analysis of variance (Scheffe procedure) using mean values for each snail species corrected for the absence of difference between the two parasite strains, showed that B. senegalensis had a significantly higher minimum and maximum precercarial period than either of the other snail species at the 5% level of significance.

A similar multivariate analysis performed on the data for the period of cercarial production again indicated no significant difference in the values between parasite strains (F=0.21, d.f.=1, p-0.644) but a highly significant difference between snail species (F=35.81, d.f.=2, p<.0001). A one way analysis of variance (Scheffe procedure) on corrected means shows that B. globosus Ngala has a significantly higher mean period of cercarial production than either of the other snail species at the 5% level. A further multivariate analysis was then carried out omitting the B. globosus Ngala results, thus reducing the difference in variances, although this was still significant at the 5% level (Cochran's C=0.48, p=approx. 0.013). In this case there were no significant differences between either parasite strains (F=1.44 d.f.=1, p=0.232) or snail species (F=2.17 d.f=1, p=0.13) at the 5% level, thus confirming the result of the original analysis.

## TABLE 4.11

**Survival times of infected and control snails from the start of experiments**

| *Bulinus* species | | n | Mean (days) | S.D. | Student's t-test t | p |
|---|---|---|---|---|---|---|
| *B. globosus* Ng | Control | 20 | 342.30 | 109.21 | 4.59 | <0.0001 |
| | Infected | 85 | 201.92 | 126.05 | | |
| *B. rohlfsi* | Control | 20 | 69.55 | 13.92 | -1.36 | N.S. |
| | Infected | 43 | 76.49 | 20.78 | | |
| | Infected 'g' | 66 | 96.73 | 33.59 | 0.42* | N.S. |
| *B. senegalensis* | Control | 20 | 99.25 | 19.44 | 1.26 | N.S. |
| | Infected 'r' | 32 | 90.47 | 27.00 | | |

Ng = Ngala
'g'= globosus 'r' = rohlfsi
* Based on pooled variance estimate.
N.S. Not significant at the 5% level of confidence.

Table 4.11 shows the mean survival times of infected and control snails from the start of experiments. Although there was a significant reduction in the longevity of infected B. globosus Ngala compared to controls, this was not the case in either B. rohlfsi or B. senegalensis.

## DISCUSSION

Numerous laboratory studies have been carried out testing the relative susceptibility of Bulinus species to different strains of S. haematobium (e.g. Leroux, 1958; Cridland, 1955 and 1957; McCullough, 1959; Paperna, 1968; Webbe and James, 1971 and 1972; Lo, 1972; Chu et al., 1978; Frandsen, 1979 a and b). With the exception of McCullough (1959) and Chu et al. (1978) these studies involved intermediate host species and parasite strains from widely different geographical locations. In the present study the Bulinus species studied cover three of the main species groups, yet are all from the same geographical region as the S. haematobium involved.

The results of this study show the presence of at least two strains of S. haematobium in the human population within the South Chad Irrigation Project. This is similar to the findings in other areas of West Africa where members of both the B. africanus and B. truncatus species groups are present (e.g. McCullough, 1957; Paperna, 1968; Chu et al., 1978). In S.C.I.P. B. rohlfsi is potentially capable of transmitting the 'truncatus' strain of the parasite and B. globosus Ngala the 'africanus' strain, as has been found in Ghana (Chu et

al., 1978; McCullough, 1957 and 1959; Paperna, 1968). In S.C.I.P. however, a third species is also implicated in the transmission of S. haematobium, the forskali group snail B. senegalensis which is capable of carrying both parasite strains under laboratory conditions. If this is also the case in the natural situation, this species may play an important role in maintaining both parasite strains when the alternative host species are absent, either due to the season or lack of suitable habitats.

It is interesting to note the lack of any successful infections of B. globosus Lake Chad in this study, although unfortunately only small numbers of snails were available due to the poor performance of this snail in culture. Betterton (1984 a) noted that no trematode infections were ever observed in wild caught specimens of this population despite the high incidence of such infections in coexisting B. rohlfsi snails. This population of B. globosus poses several taxonomic problems, since although it shows some morphological and behavioural differences from the Ngala population, as well as this marked difference in susceptibility to one of the tested strains of S. haematobium, in laboratory experiments the two populations can successfully hybridize (Chapter 8).

The complete lack of cross infections of the 'truncatus' strain of parasite in B. globosus and 'africanus' strain in B. rohlfsi has not been observed in all other studies. Webbe and James (1971) and Frandsen (1979 a) both found some cross-infectivity, but they were using parasites from different

geographical areas to the snail hosts. In the case of Paperna's 1968 study it is likely that there was a mixing of the two parasite strains within the patients from whom miracidia were obtained (Chu et al., 1978) as may also have been the case with McCullough's 1959 work.

The failure to infect B. rohlfsi with the 'rohlfsi' strain of parasite in the first passage was probably due to the small numbers of snails used and the high mortalities experienced. There is no significant difference between the observed infection rates in the first and second passages ($X^2$=0.95, 0.3<p<0.5).

The results obtained for infection rates and mortality over the precercarial period, the precercarial period itself, duration of cercarial production and survival all show no significant differences between the two parasite strains involved. However, in all cases there are significant differences between the snail species, and also between each combination of snail species and parasite strain.

Comparing infection rates, regardless of parasite strain, B. globosus Ngala consistently shows the highest levels of infection and B. rohlfsi the lowest. Even looking at each individual parasite strain, B. globosus Ngala shows higher infection rates than B. senegalensis when both are exposed to the 'globosus' strain, and B. senegalensis shows a higher infection rate than B. rohlfsi when they are exposed to the 'rohlfsi' parasite.

The exposure of snails to S. haematobium miracidia had

very little adverse effect on the mortality rates of snails, compared to identically treated controls, over the precercarial period. In only one case (B. globosus Ngala exposed to 'globosus' strain) was there a significant increase in mortality over controls. Notably this combination produced the highest infection rates. In marked contrast, B. rohlfsi exposed to the same parasite strain showed a significant reduction in precercarial period mortality compared to controls, in this case the snail was refractory to infection with this parasite strain. Chu et al. (1978), working with the same snail species (B. rohlfsi and B. globosus) from Ghana, and with B. truncatus (Chu, Sabbaghian and Massoud, 1966) found no increase in mortality over the precercarial period in snails exposed to S. haematobium compared to controls.

A more striking effect on precercarial period mortality was seen when comparing each snail species exposed to the two parasite strains. In the case of both B. rohlfsi and B. globosus Ngala significantly higher mortalities occurred when snails were exposed to the parasite strain to which they were susceptible, compared to the strain to which they were refractory. In the case of B. senegalensis, susceptible to both parasite strains, this difference in mortality was not so marked, although in both second and combined passage results exposure to 'globosus' parasite produced significantly higher mortalities than exposure to the 'rohlfsi' strain.

In the limited comparison of precercarial period mortalities of each species exposed to the 'globosus' strain of

S. haematobium the same hierarchy applies as was observed in the infection rates: B. globosus Ngala highest and B. rohlfsi the lowest. Unfortunately the inherently higher mortality rates of B. rohlfsi controls compared to the other species do not allow a direct comparison between each species exposed to the 'rohlfsi' strain, although it can be concluded that there is no significant difference between B. globosus Ngala and B. senegalensis.

In both B. rohlfsi and B. senegalensis, schistosome infection had no significant effect on host longevity. However, the average survival time of infected B. globosus Ngala was significantly shorter than controls.

B. senegalensis shows a significantly higher mean precercarial period than the other snail species, with no significant difference between the two parasite strains within this snail. This is the case despite the maintenance of this species at higher temperatures, with an expected increase in the speed of development of the parasite (i.e. precercarial period would decrease) (Pfluger, Roushdy and Emam, 1984). A very small group of B. senegalensis maintained at a lower temperature ($26\pm1^{o}C$) showed an even higher mean precercarial period ( Min 43.61, Max 45.79, both strains combined).

The main difference between this snail species and the others can be seen when comparing the frequency distributions of the precercarial periods (Fig. 4.1 and 4.2). In both B. globosus Ngala and B. rohlfsi 90% of infected snails had produced cercariae by 42 days from exposure. With B.

FIGURE 4.1

Distribution of minimum precercarial periods of S. haematobium in each bulinid

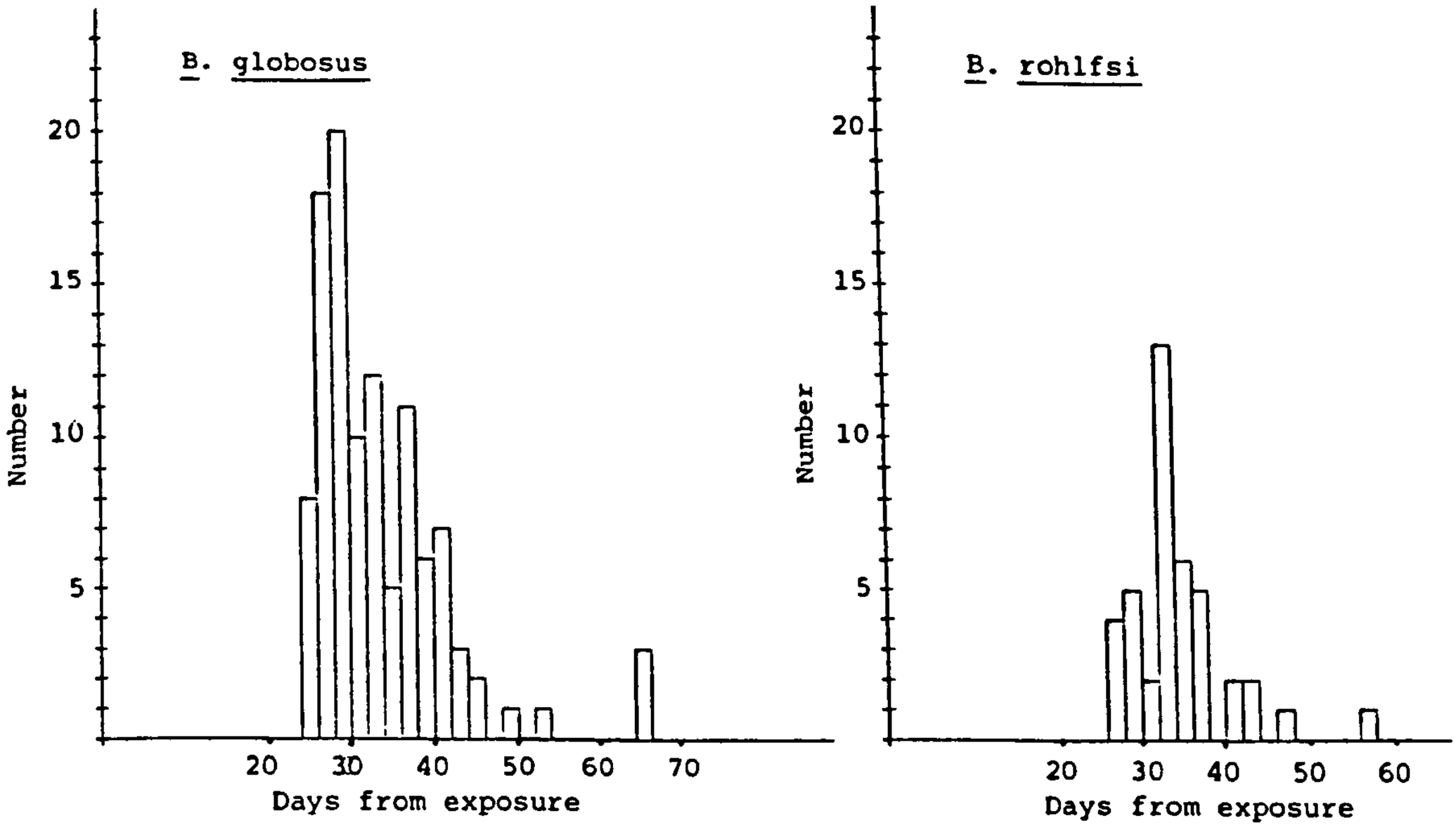
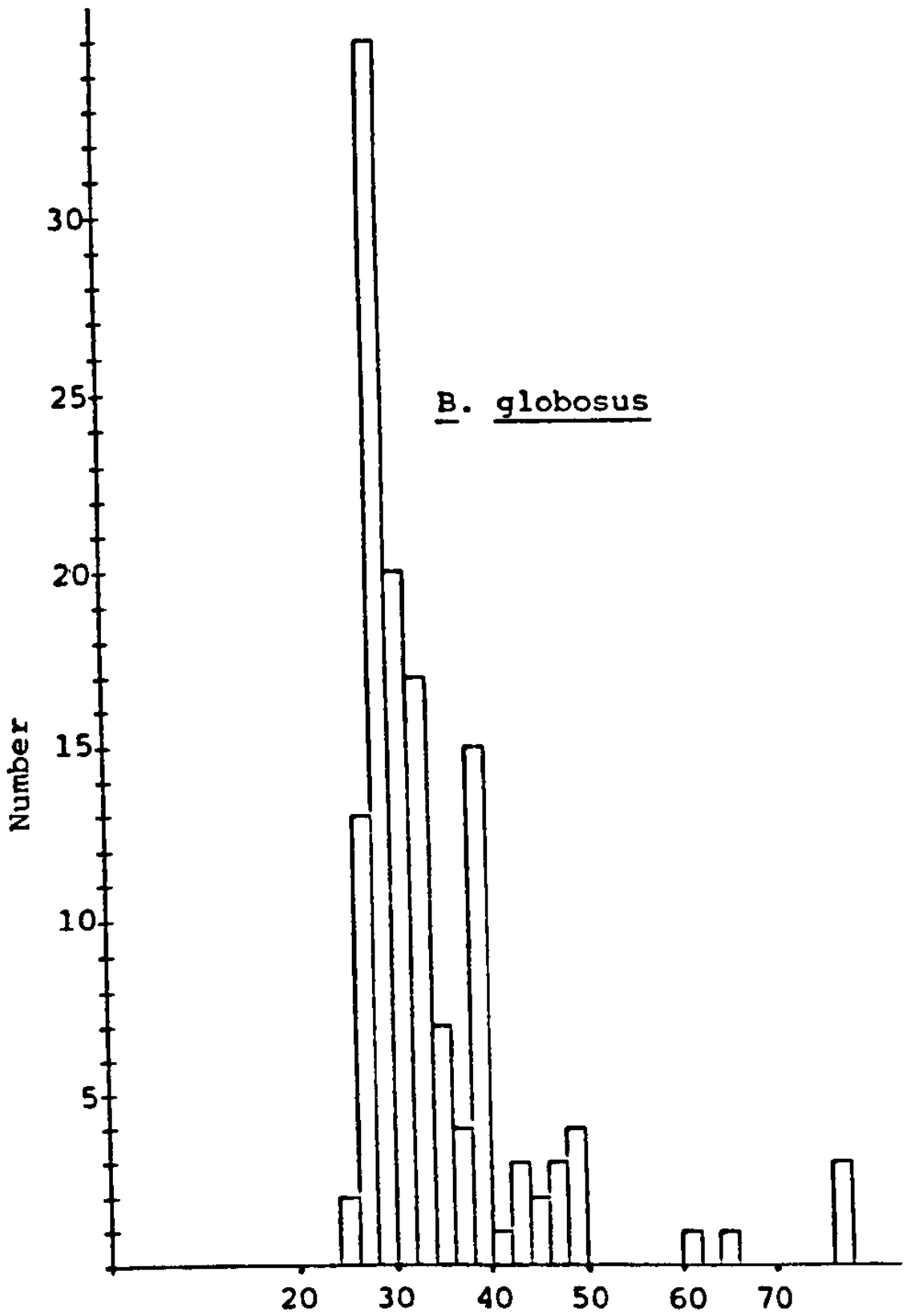
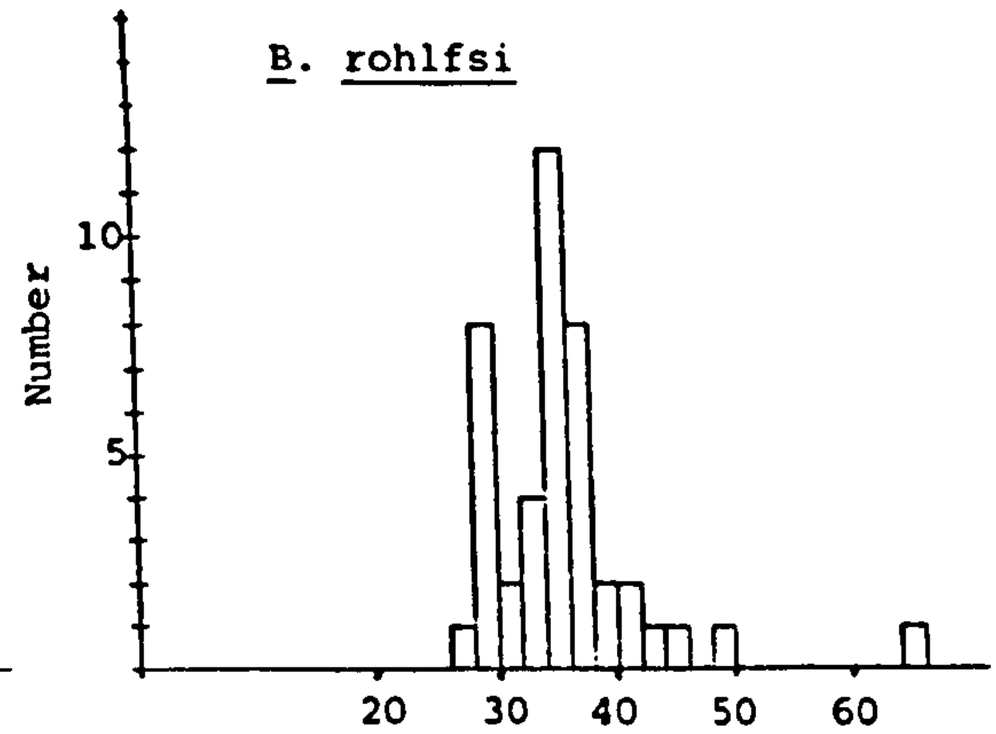
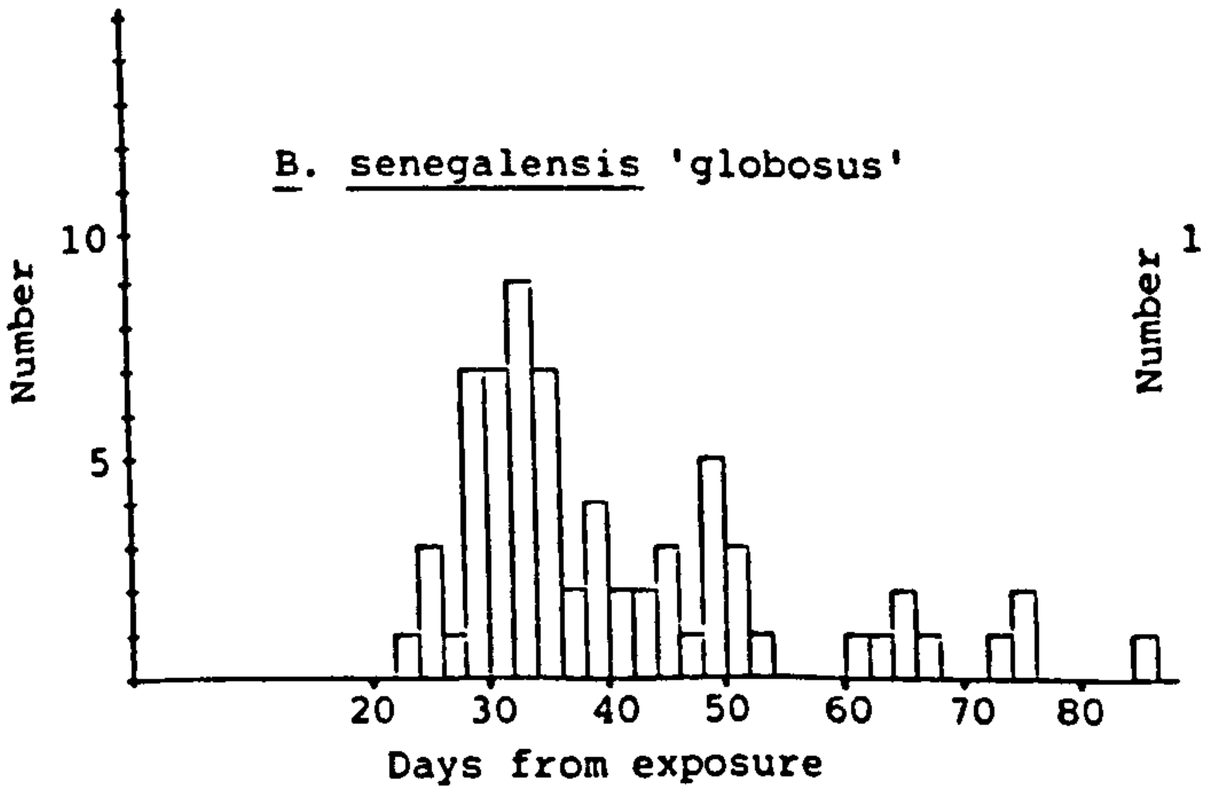


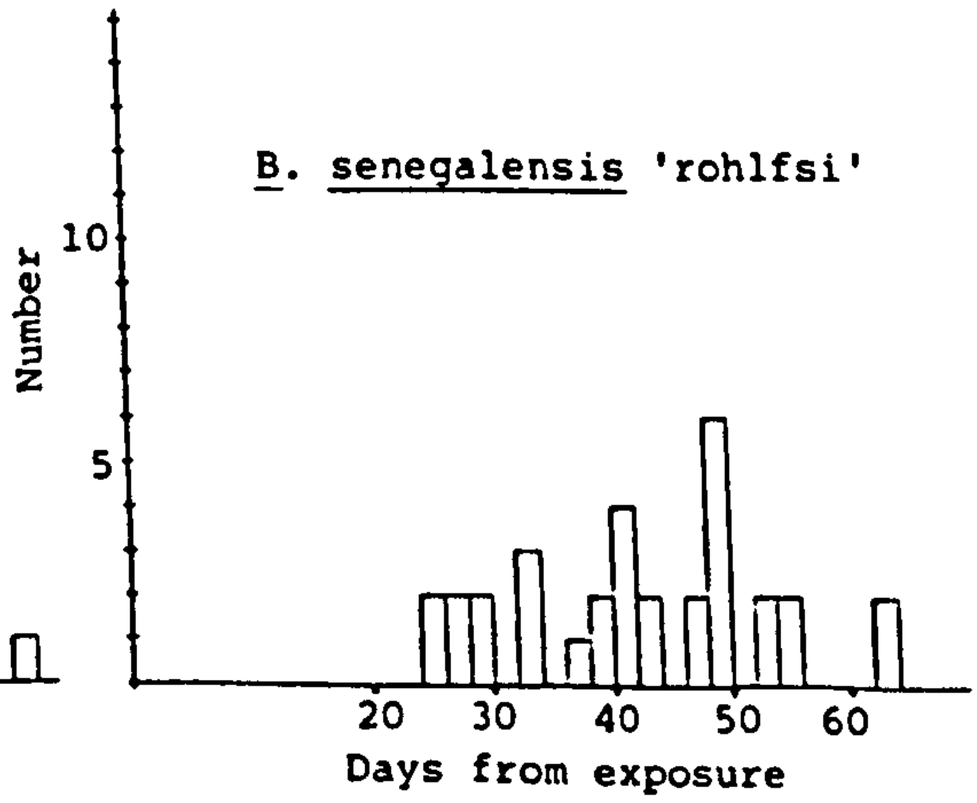
FIGURE 4.2

Distribution of maximum precercarial periods of S. haematobium in each bulinid



senegalensis this 90% level is not reached until 61 days post exposure.

This wide spread of precercarial periods, reflecting the development of the parasite, can be explained by the behaviour of B. senegalensis in the laboratory. Individual snails show a tendency to persistent crawling out of the water and cessation of feeding for periods of up to several weeks, after which they resume normal feeding and rapid growth. This probably reflects their behaviour in their natural habitats, consisting of highly seasonal rain-filled pools, where a proportion of snails aestivate each year to survive until the next rains (Betterton, Fryer and Wright, 1983). This 'voluntary' aestivation in the laboratory could result in a temporary halt of the parasite's development while the host's metabolic rate has dropped to a very low level, with renewed development as the snail resumes normal feeding. This would result in a very wide range of precercarial periods since there is no apparent synchronisation of this aestivatory behaviour under laboratory conditions. Small scale laboratory experiments have shown that B. senegalensis is capable of carrying immature S. haematobium infections through periods of enforced aestivation (see Chapter 7).

No significant differences were observed in the precercarial periods of B. globosus Ngala and B. rohlfsi in this study. Chu et al., (1978) observed a significantly longer precercarial period in B. globosus infected with some 'africanus' strains of S. haematobium compared to those in B.

rohlfsi infected with 'truncatus' strains in Ghana.

The duration of cercarial production in infected snails again showed no difference between parasite strains. However, B. globosus Ngala produced cercariae for significantly longer periods than the other two species. This is almost certainly a reflection of the natural longevity of the three snail species, since the same is true when comparing the survival times of uninfected control snails. No cases of 'self cure', i.e. permanent cessation of cercarial production during the snail's lifetime, were ever observed (see Chapter 5).

The results of this study have shown that there is a significant three way interaction between infection rates, snail species and parasite strains, and that this is followed closely by the observed mortality rates during the precercarial period. Throughout, although there are no significant differences between parasite strains, B. globosus Ngala shows both the highest overall infection and precercarial mortality rates and B. rohlfsi the lowest. B. globosus Ngala was also the only species to show reduced longevity when infected. The two parasite strains show little or no difference in either their precercarial development time, or the duration of cercarial production. Although there are significant differences in the parasite's performance within a given species, these result from the behaviour of the host or simply its natural longevity.

This infection study, using a very limited sample of S. haematobium, has shown the presence of at least two distinct

strains of the parasite in the area of the South Chad Irrigation Project with three species of Bulinus implicated in the transmission of urinary schistosomiasis. These intermediate host species cover the whole range of aquatic habitats and their differences in seasonal abundance (Betterton, 1984 a and b) provide the potential for transmission throughout the year.

The small sample size of S. haematobium used in the study means that the possibility that other strains of the parasite also occur cannot be ruled out. Neither can the possibility be ignored of mixing of strains (Chu et al., 1978), or as has been suggested for Ghana (Wright and Knowles, 1972; Wright and Southgate, 1976) the existence of natural hybrids.

**REFERENCES**


Berrie, A.D., 1973. Snails, schistosomes and systematics: some problems concerning the genus Bulinus. In The Systematics Association, special volume No. 5. Taxonomy and Ecology Ed. V.H. Haywood. pp.173-188.

Betterton, C., 1984 a. Ecological studies on the snail hosts of Schistosomiasis in the South Chad Irrigation Project Area, Borno State, northern Nigeria. Journal of Arid Environments 7 : 43-57.

Betterton, C., 1984 b. Spatio-temporal distributional patterns of Bulinus rohlfsi (Clessin), Bulinus forskali (Ehrenberg) and Bulinus senegalensis Muller in newly irrigated areas in northern Nigeria. Journal of Molluscan Studies 50 : 137-152.

Betterton, C., Fryer, S.E., and Wright, C.A., 1983. Bulinus senegalensis (Mollusca:Planorbidae) in northern Nigeria. Annals of Tropical Medicine and Parasitology 77 : 143-149.

Brown, D.S., 1980. Freshwater Snails of Africa and their Medical Importance. Taylor and Francis Ltd. London 487pp.

Chu, K.Y., Kpo, H.K., and Klumpp, R.K., 1978. Mixing of Schistosoma haematobium strains in Ghana. Bulletin of the World Health Organisation 56 : 601-608.

Chu, K.Y., Sabbaghian, H., and Massoud, J., 1966. Host-parasite relationship of Bulinus truncatus and Schistosoma haematobium in Iran. 2: Effect of exposure dosage of miracidia on the biology of the snail host and the development of the parasites. Bulletin of the World Health Organisation 34 : 121-130.

Cowper, S.G., 1947. Observations on the life-cycle of Schistosoma mansoni in the laboratory with a discussion on the snail vectors of S. mansoni and S. haematobium. Annals of Tropical Medicine and Parasitology 41 : 173-177.

Cridland, C.C., 1955. The experimental infection of several species of African freshwater snails with Schistosoma mansoni and S. haematobium. Journal of Tropical Medicine and Hygiene 58 : 1-11.

Cridland, C.C., 1957. Further experimental infection of several species of East African freshwater snails with Schistosoma mansoni and S. haematobium. Journal of Tropical Medicine and Hygiene 60 : 18-23.

Frandsen, F., 1979 a. Studies on the relationships between Schistosoma and their intermediate hosts. 1. The genus Bulinus and Schistosoma haematobium from Egypt. Journal of Helminthology 53 : 15-29.

Frandsen, F., 1979 b. Studies on the relationships between Schistosoma and their intermediate hosts. II. The genus Bulinus and Schistosoma haematobium from Sudan, Zaire and Zambia. Journal of Helminthology 53 : 205-212.

Gismann, A., 1954. Notes on various molluscan genera of the family Planorbidae involved in the transmission of Bilharziasis in Africa and the near East. Journal of the Egyptian Medical Association 37 : 1163-1184.

Leroux, P.L., 1958. The validity of Schistosoma capense (Harley,1864) amended as a species. Transactions of the Royal Society of Tropical Medicine and Hygiene 52 : 12-14.

Lo, C.T., 1972. Compatibility and host-parasite relationships between species of the genus Bulinus (Basommatophora: Planorbidae) and an Egyptian strain of Schistosoma haematobium (Trematoda:Digenea). Malacologia 11 : 225-280.

McCullough, F.S., 1955. Apparent resistance of a snail to infection in the Gold Coast. Nature 176 : 181-182.

McCullough, F.S., 1957. A preliminary note on the degree of compatibility between Schistosoma haematobium and its bulinid vectors. West African Medical Journal 6 : 98-100.

McCullough, F.S., 1959. The susceptibility and resistance of Bulinus (Physopsis) globosus and Bulinus (Bulinus) truncatus rohlfsi to two strains of Schistosoma haematobium in Ghana. Bulletin of the World Health Organisation 20 : 75-85.

Paperna, I., 1968. Susceptibility of Bulinus (Physopsis) globosus and Bulinus truncatus rohlfsi from different locations in Ghana to different local strains of Schistosoma haematobium. Annals of Tropical Medicine and Parasitology 62 : 13-26.

Pfluger, W., Roushdy, M.Z., and El Emam, M., 1984. The prepatent period and cercarial production of Schistosoma haematobium in Bulinus truncatus (Egyptian field strain) at different constant temperatures. Zeitschrift fur Parasitenkunde 70 : 95-103.

Webbe, G., 1971. The significance of infra-specific variations of hosts and parasites in the epidemiology of helminths of medical importance. Journal of Helminthology 45 : 325-327.

Webbe, G. and James, C., 1971. Infra-specific variations of Schistosoma haematobium. Journal of Helminthology. 45 : 403-413.

Webbe, G. and James, C., 1972. Host-parasite relationships of Bulinus globosus and Bulinus truncatus with strains of Schistosoma haematobium. Journal of Helminthology 46 : 185-199.

Wright, C.A., 1977. Coevolution of Bulinid snails and African Schistosomes. In Medicine in a Tropical Environment. Ed. J.H.S. Gear. pp291-302.

Wright, C.A., and Knowles, R.J., 1972. Studies on Schistosoma haematobium in the laboratory: Strains from Iran, Mauritius and Ghana. Transactions of the Royal Society of Tropical Medicine and Hygiene. 66 : 108-118.

Wright, C.A., and Southgate, V.R., 1976. Hybridisation of schistosomes and some of its implications. In Symposia of the British Society for Parasitology 14 : 55-86.

Wright, C.A., and Southgate, V.R., 1981. Coevolution of digeneans and molluscs, with special references to the schistosomes and their intermediate hosts. In The Evolving Biosphere Ed. P.L. Forey. British Museum (Natural History), Cambridge University Press. Chapter 15 pp191-205.

# CHAPTER 5

# CERCARIAL OUTPUT FROM BULINIDS INFECTED WITH LOCAL SCHISTOSOMA HAEMATOBIUM

## INTRODUCTION

The production of cercariae is of great importance in all digenetic trematode life-cycles. In schistosomes, where infection of the final host is by direct penetration of the cercarial stage, cercarial productivity is one of the major factors in the transmission dynamics of the parasite.

The cercarial productivity of a snail host - schistosome parasite combination is considered to be of importance when comparing the relative compatibility of different combinations, and is extensively discussed by Frandsen (1979c). In general highly compatible combinations, where there is considerable adaptation between the schistosome and its molluscan host, produce larger numbers of cercariae than those where there is poor compatibility. Factors other than compatibility may affect the number of cercariae produced. The number of miracidia penetrating the mollusc may be important (Chu, Sabbaghian and Massoud, 1966; Webbe and James, 1972) although this is possibly only of significance when comparing monomiracidial infections with those involving two or more miracidia, and then only in the early stages of infection (Theron, 1982).

The size of the snail host is probably also important in determining the number of cercariae produced, with larger

snails shedding more than smaller ones (McClelland, 1965; Berrie, 1970; Lo, 1972).

The cercarial output from schistosome-infected molluscs also varies with the age of the infection. Exhaustive and highly detailed studies on S. mansoni in Biomphalaria glabrata (Theron, 1981 a and b) where cercarial production was monitored continuously, have shown the presence of regular peaks and troughs in the levels of daily cercarial output. These waves reflect the cycles of cercarial production from primary daughter sporocysts, combined with their periodic replication to produce further generations of daughter sporocysts. The existence of replicating daughter sporocysts has also been shown in S. haematobium (Kechemir and Theron, 1980), but no studies have yet been carried out in sufficient detail to examine the patterns of cercarial output from Bulinus species throughout the period of infection.

As part of the overall study of host-parasite relationships between Bulinus species and S. haematobium strains from the area of the South Chad Irrigation Project, Nigeria, a number of laboratory reared and infected snails were monitored for daily cercarial production at weekly intervals throughout the duration of their infections.

## MATERIALS AND METHODS

Details of the experimental and maintenance procedures used in this study are described in Chapter 2.

Individual snails monitored had been exposed to 5 miracidia for a period of 5 hours at 25°C in 5ml of water as part of the susceptibility studies described in Chapter 4. From the first detection of cercariae, snails were housed individually in 150ml of water until their death.

One day's cercarial output from each infected snail was sampled for each week of the infection from first detection of cercariae, cercariae being collected from 9am to 5pm on the sample day. Cercarial production between 5pm and 9am is known to be negligible in this species as has been confirmed in these cultures (Raymond, 1984). Sample collections were made by isolating each snail in a 50x25mm flat-bottomed glass tube in 15ml of distilled water, having first rinsed the snail in distilled water and wiped its shell with soft tissue paper to avoid transferring any adhering cercariae. On removal from the tubes at the end of the sample period, snails were again rinsed with distilled water to wash any adhering cercariae into the sample tube.

Cercariae in the sample suspension were then counted by passing the contents of the tube through 7cm Whatman No.1 filter papers in a Buchner funnel under partial vacuum, with all cercariae being washed out of the tube by several rinses of distilled water. Cercariae trapped on the filter paper were then stained by spraying with 0.5% Ninhydrin in Butan-1-ol

(Ninhydrin spray for developing chromatograms, BDH Chemicals Ltd.) and placing the filter papers in a drying oven to develop the stain. Cercariae, stained purple, were then counted under a dissecting microscope with the aid of an overlay grid. Only the heads of cercariae were counted, since tails often became detached during the sampling process. Statistical analysis of the data was carried out as detailed in the results section.

## RESULTS

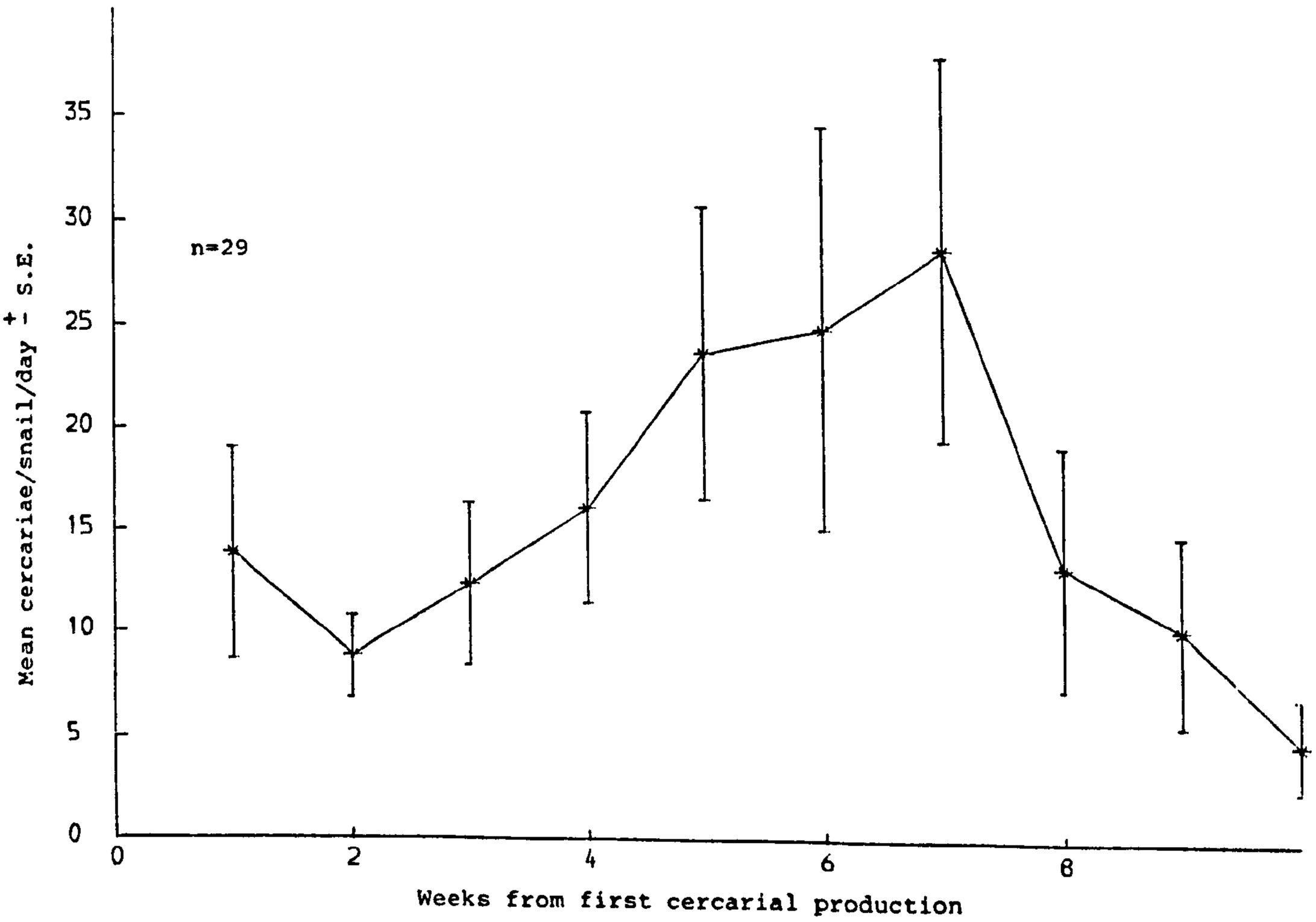
The weekly counts of daily cercarial production from each individual snail are represented graphically in Appendix C. Overall results for each combination are presented in Figures 5.1 to 5.3. A more detailed breakdown of this data is given in Appendix B. In all combinations there was a large variation in daily cercarial counts between individual snails, and also between weekly samples from the same individual. However, some general patterns of changes in cercarial output with time for each host/parasite pair are apparent.

As observed with larger numbers of infected snails (Chapter 4) B. globosus Ngala showed significantly longer durations of infection than any of the other snail species, reflecting the long life-span of this bulinid. This species infected with the 'globosus' strain of parasite also showed the highest levels of daily cercarial production, with over 1,000 cercariae being produced from individual snails on some sample days.

All combinations of host species/parasite strain showed

FIGURE 5.1

Cercarial output at weekly intervals from B. rohlfsi infected with the 'rohlfsi' strain of S. haematobium



some variation of daily cercarial production with age of the infection. This can be seen most clearly in the case of B. rohlfsi infected with the 'rohlfsi' strain of S. haematobium (Fig. 5.1). Levels of daily cercarial output increased gradually over the first seven weeks of the infection after initial shedding, after which they declined. A similar, but slightly less smooth pattern is shown with both parasite strains in B. senegalensis (Fig. 5.2).

Levels of daily cercarial output in B. rohlfsi infected with the 'rohlfsi' strain of the parasite were relatively low throughout the infection with even the peak mean value 7 weeks from initial production under 30 cercariae/snail/day. The maximum number of cercariae produced from a single infected B. rohlfsi in a sampled day was 151.

B. senegalensis showed similar levels of daily cercarial production when infected with each of the two parasite strains, in both cases these values were slightly higher than those seen from B. rohlfsi. The highest mean values were 144, 9 weeks from first production in the case of the 'rohlfsi' strain, and 91, 6 weeks from initial production from the 'globosus' strain. (The value of 130 on the 10th week may be distorted since it is based on results from only three surviving snails). Maximum daily production from individual B. senegalensis was 623 in the case of the 'rohlfsi' strain infections, and 783 from the 'globosus' strain.

The pattern of cercarial production from B. globosus Ngala infected with the 'globosus' strain of S. haematobium (Fig.

FIGURE 5.2

Cercarial output at weekly intervals from B. senegalensis infected with A: 'globosus' and B: 'rohlfsi' strains of S. haematobium

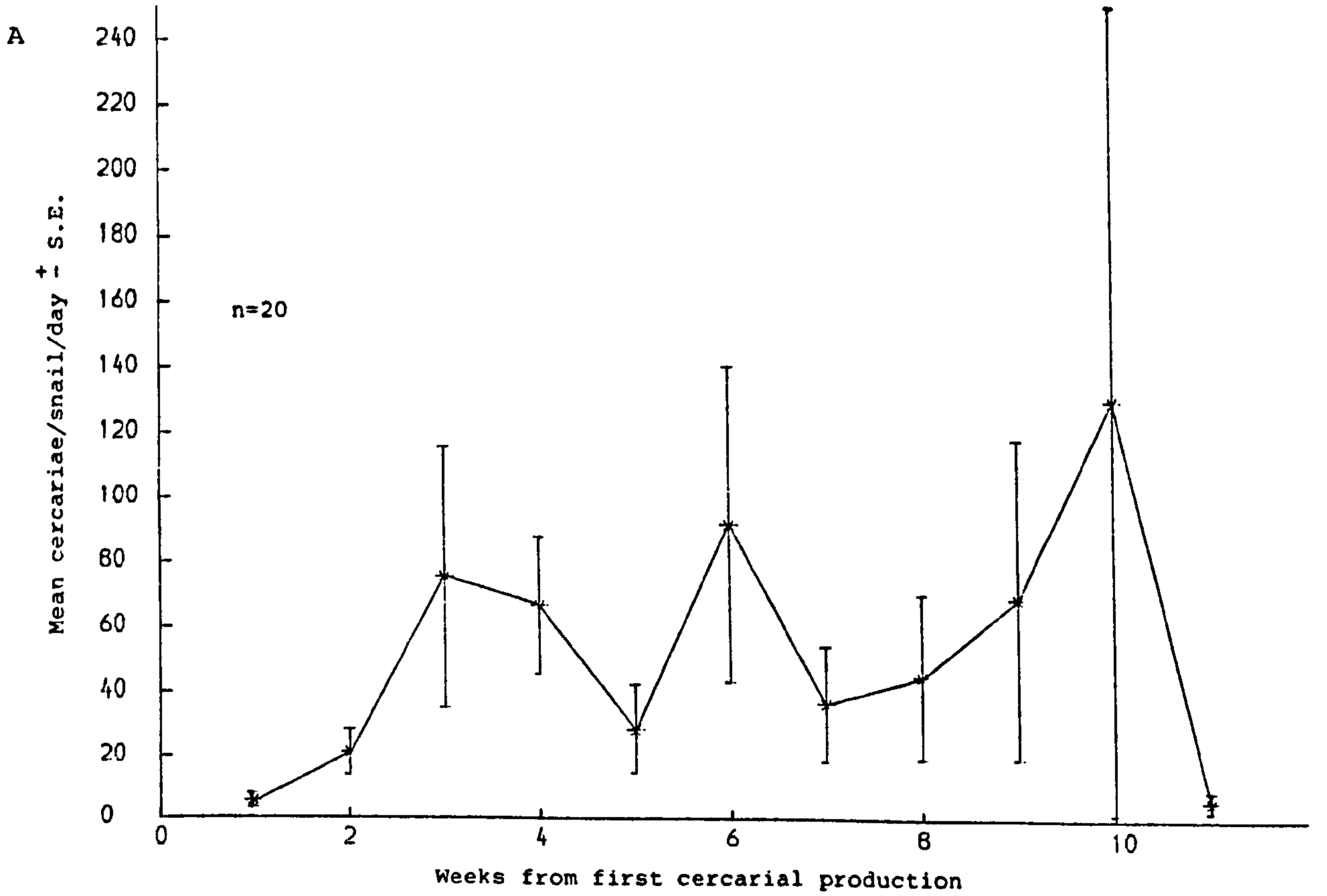


FIGURE 5.3

Cercarial output at weekly intervals from B. globosus infected with 'globosus' strain of S. haematobium

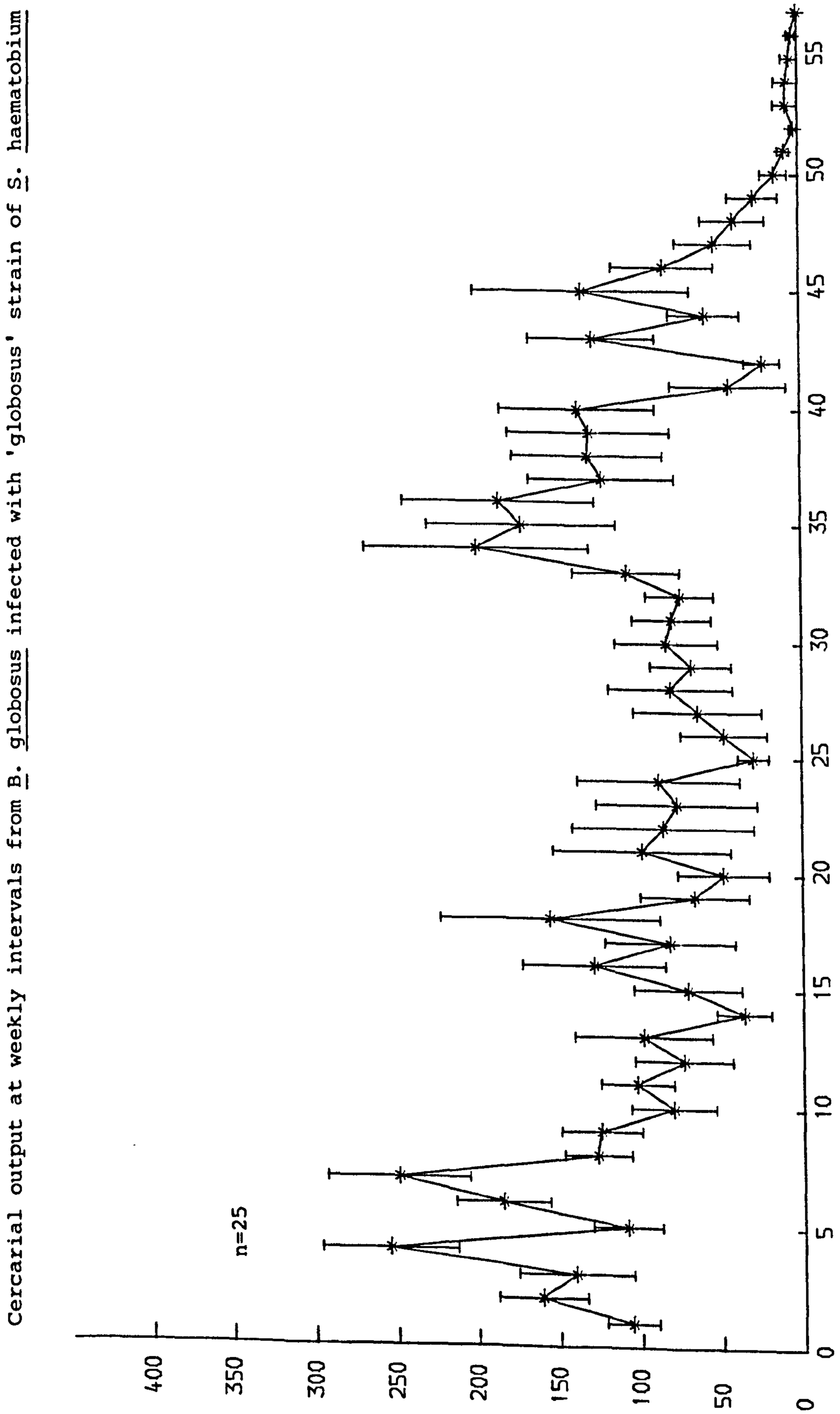


FIGURE 5.4

Cercarial output at weekly intervals from those B. glabosus infected with S. haematobium showing a single peak in cercarial production

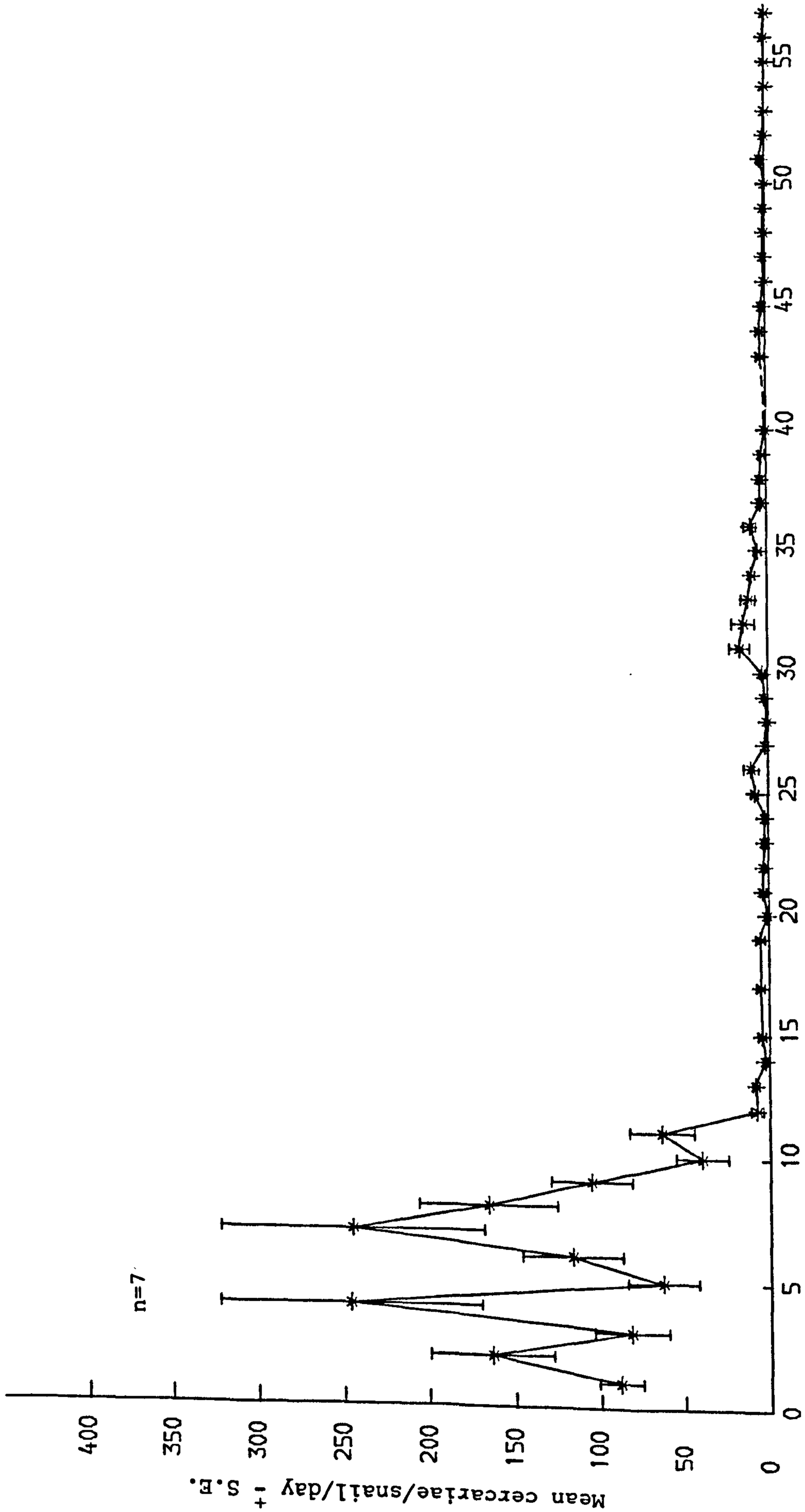
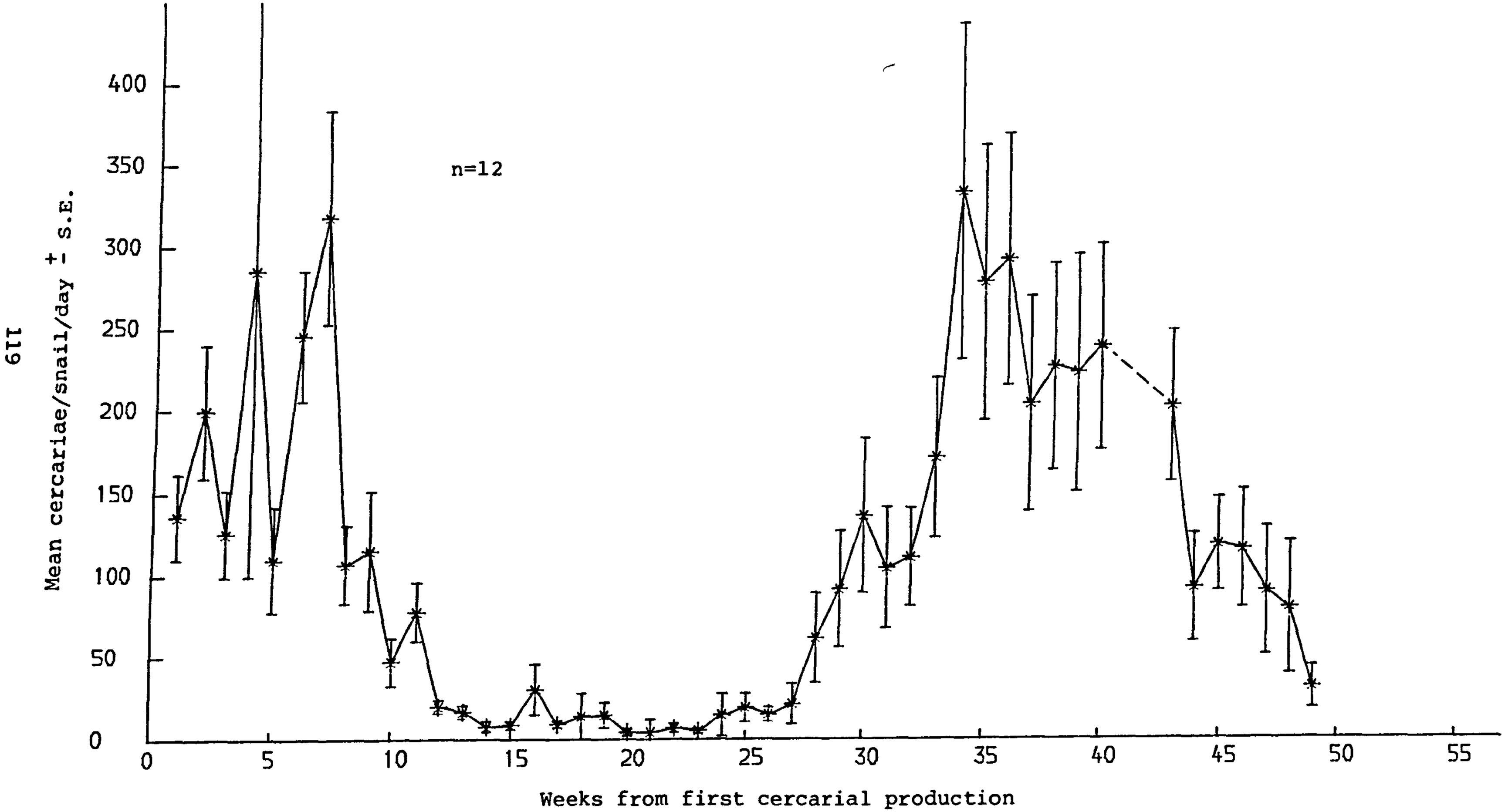


FIGURE 5.5

Cercarial output at weekly intervals from those B. globosus infected with S. haematobium showing two peaks in cercarial production



5.3) is not nearly as clear as those seen in the other combinations. This is complicated by the extremely long duration of the infection, and considerable variation between individual snails. Examining the daily cercarial output patterns from individual snails (Appendix C) two quite distinct patterns can be seen: some individuals show a single period of high output early in the productive phase of infection after which only low levels occur; other snails show the same early peak followed by a second peak late in the infection, with a long period when very few cercariae are released each day.

Of 25 individually infected B. globosus Ngala monitored, three did not survive long enough to be able to ascertain which shedding pattern was being shown, one snail produced very small numbers of cercariae throughout its infection, and two always produced high counts. Of the remaining snails seven showed a single peak in production, and 12 produced two peaks of high output. The combined results from the "single peak" group are shown in Figure 5.4 with the "double peak" results in Figure 5.5. A more detailed breakdown of this data is given in Appendix B.

Levels of daily cercarial production from B. globosus Ngala taken over the whole infection period are considerably higher than those shown by the shorter lived combinations, with a mean daily cercarial production of almost 100 cercariae per snail. Direct comparison of the overall mean daily cercarial counts of each combination would be misleading because of the differences in the durations of the infections. Table 5.1

TABLE 5.1

Maximum daily production of S.haematobium cercariae from each Bulinus species/parasite strain combination*

| Bulinus species | n | mean | S.D. | Mean rank (R) |
|---|---|---|---|---|
| B.globosus (Ngala) 'g' | 25 | 401.28 | 119.55 | 76.72 |
| B. rohlfsi 'r' | 29 | 41.83 | 41.58 | 31.47 |
| B. senegalensis 'r' | 20 | 95.05 | 136.22 | 41.17 |
| B. senegalensis 'g' | 20 | 120.85 | 190.79 | 40.55 |

* over the first 12 weeks of shedding
'r'= infected with 'rohlfsi' strain
'g'= infected with 'globosus' strain

compares the highest daily cercarial counts during the first 12 weeks of cercarial shedding. Since the standard deviations of each set of results were so variable, a Kruskal Wallis one-way analysis of variance by ranks was performed on the highest sample count obtained from each individual snail over the first 12 weeks of production, thus eliminating the results from the older infections in B. globosus Ngala. At the 5% level of significance, B. globosus Ngala infected with the 'globosus' strain of S. haematobium showed a significantly higher peak in daily cercarial output than any other combination in the study. There were no significant differences at the 5% level between the other host-parasite combinations.

## DISCUSSION

Frandsen (1979c) stressed the importance of standardized techniques in comparative studies of the compatibiliy of schistosomes and their intermediate hosts. In the present study, although care must be taken when comparing results with those of other workers, a direct comparison can be made of the two parasite strains under investigation in two different bulinid hosts, B. globosus Ngala and B. rohlfsi, since both were treated identically. In addition a comparison can be made of the two strains in the same molluscan host, B. senegalensis. The same can be done for each parasite strain in different host species, since experimental procedures were identical apart from the slightly higher maintenance temperature of B. senegalensis (see Chapter 2) which may have altered cercarial

production levels slightly (Pfluger, Roushdy and El-Emam, 1984).

The large variation observed in the levels of cercarial production between different individual snails of the same species infected with the same parasite strain has also been reported by many other workers (e.g. Gordon, Davey and Peaston, 1934; Lo, 1972; Webbe and James, 1972; Pfluger et al., 1984). Some of this variation may be due in part to the infrequency of sampling. Theron (1981 a) emphasised the problems of using a single day's test to deduce the weekly production of cercariae, since continuous daily monitoring of output shows very large day to day variations within individual snails. This problem has been experienced and recognised by many workers (e.g. Gordon et al., 1934; McClelland, 1967; Pitchford, Meyling, Meyling and DuToit, 1969; Frandsen, 1979a). However the logistical problems involved in continuous sampling of such a large number of snails are considerable. In addition, stress caused by handling the snails has an important effect on mortality and may also affect cercarial output.

Although the fine rhythms of cercarial production associated with sporocyst replication and regeneration shown by Theron (1981 a and b), in S. mansoni infected Biomphalaria glabrata were not seen in this discontinuous study, some interesting patterns were observed and gross differences between the levels of production of each host-parasite combination could be distinguished.

The phenomenon of 'self cure' as reported in other studies

on S. haematobium infections (e.g. Webbe and James, 1972; Pfluger et. al., 1984) was not conclusively shown in this investigation. In several cases no cercariae were detected on successive sample days, although later in the infection the same snail might produce high counts. The one B. globosus Ngala showing no cercariae on sample days for 12 successive weeks before its death may have been producing small numbers of cercariae on non-sampled days. The occurrence of long periods of extremely low cercarial production, as seen in the middle period of some infected B. globosus Ngala and in the case of those showing a "single peak" pattern, continuing for the rest of the infection, may lead to a false identification of 'self cure'. This could happen if monitoring is not continued until the snail's death, or if only rough visual checks are being performed which lack sensitivity when small numbers of cercariae are involved (see Chapter 2).

The long duration of infection in B. globosus Ngala is a reflection of the natural longevity of this species in laboratory cultures compared to the other bulinids under investigation (Chapter 4). The average duration of cercarial production for this species with the 'globosus' strain in the monitored group was very high (306 days, see Table 5.2), despite the handling involved in sampling. The other combinations all produced cercariae for shorter periods, B. rohlfsi having a mean duration of infection of 50 days and B. senegalensis infected with 'globosus' and 'rohlfsi' strains of parasite, 41 and 54 days respectively. In a wider

TABLE 5.2

Duration of cercarial production and mean daily cercarial output of each infected bulinid monitored, with correlation coefficients for each host/parasite combination

| B. globosus (Ng) 'globosus' | | B. rohlfsi 'rohlfsi' | | B. senegalensis 'globosus' | | B. senegalensis 'rohlfsi' | |
|---|---|---|---|---|---|---|---|
| Cer/day | Days | Cer/day | Days | Cer/day | Days | Cer/day | Days |
| 332 | 463 | 21 | 84 | 161 | 56 | 18 | 34 |
| 188 | 215 | 13 | 81 | 65 | 14 | 88 | 56 |
| 78 | 44 | 2 | 62 | 65 | 19 | 47 | 68 |
| 230 | 177 | 2 | 55 | 76 | 28 | 41 | 32 |
| 18 | 320 | 1 | 27 | 225 | 32 | 181 | 69 |
| 75 | 222 | 6 | 28 | 108 | 73 | 116 | 34 |
| 187 | 362 | 9 | 34 | 21 | 47 | 21 | 47 |
| 53 | 387 | 20 | 40 | 28 | 36 | 6 | 53 |
| 178 | 320 | 43 | 54 | 2 | 73 | 4 | 73 |
| 28 | 419 | 33 | 51 | 3 | 45 | 16 | 64 |
| 54 | 233 | 9 | 58 | 36 | 50 | 2 | 28 |
| 72 | 361 | 13 | 58 | 23 | 35 | 46 | 57 |
| 142 | 299 | 20 | 56 | 2 | 36 | 21 | 51 |
| 78 | 362 | 4 | 64 | 6 | 34 | 7 | 42 |
| 136 | 342 | 2 | 48 | 5 | 46 | 12 | 57 |
| 57 | 215 | 27 | 70 | 4 | 37 | 2 | 81 |
| 112 | 320 | 18 | 78 | 2 | 29 | 3 | 24 |
| 22 | 396 | 12 | 58 | 7 | 34 | 6 | 86 |
| 75 | 236 | 6 | 40 | 8 | 83 | 14 | 52 |
| 21 | 387 | 32 | 62 | 2 | 22 | 52 | 71 |
| 3 | 424 | 27 | 65 | | | | |
| 119 | 285 | 1 | 27 | | | | |
| 53 | 273 | 3 | 18 | | | | |
| 50 | 294 | 27 | 40 | | | | |
| | | 4 | 38 | | | | |
| | | 4 | 31 | | | | |
| | | 3 | 40 | | | | |
| | | 4 | 23 | | | | |
| | | 40 | 55 | | | | |
| Mean | 306±92 | | 50±17 | | 41±18 | | 54±17 |
| Corr (r) | 0.0192 | | +0.4316 | | -0.0133 | | +0.0692 |

investigation including unmonitored snails there were no significant differences in the longevities of these combinations whereas B. globosus Ngala lived significantly longer than any of them (Chapter 4).

Webbe and James (1972) working with B. truncatus from Iran and a Nigerian B. globosus, with strains of S. haematobium from the same countries, reported that snails with high cercarial shedding capacity were not as long-lived as those producing fewer cercariae. As with Frandsen's study (1979 a), the results from the present investigation do not agree with this finding. Table 5.2 shows the mean daily cercarial output from each snail together with its duration of infection, with the correlation coefficients (r) for each combination of host species-parasite strain. Only in the case of B. rohlfsi infected with the 'rohlfsi' strain is there evidence of a relationship between these parameters ($r=0.4316$, $p<0.02$). Multiple linear regression analysis confirms a positive relationship between mean daily cercarial output and duration of infection for B. rohlfsi infected with the 'rohlfsi' strain of S. haematobium ($p=0.02$). This finding is directly opposite to that of Webbe and James (1972).

The period of active cercarial production from B. globosus Ngala experienced in this study is longer than that reported by other workers for this species from other areas of Africa (summarised in Table 5.3), possibly reflecting the culture conditions or a low level of pathogenicity of this parasite strain. The comparative longevity of uninfected snails

## TABLE 5.3

### Summary of infection parameter details from other studies involving *B. globosus* and *S. haematobium*

| *S.haematobium* origin. | *B.globosus* origin | Cercariae/ snail/day | | Duration of production (days) | | | Source |
|---|---|---|---|---|---|---|---|
| | | Mean* | Max. | Mean* | Max. | | |
| Egypt | Rhodesia | - | 25 | 10 | 10 | 1 | Frandsen 1979(a) |
| Zaire | Rhodesia | - | 100 | 14 | 56 | 1 | Frandsen 1979(b) |
| Zambia | Kenya | - | 130 | 52 | 62 | 1 | Frandsen 1979(b) |
| | Rhodesia | - | 100 | 14 | 23 | 1 | |
| Sierra Leone | Sierra Leone | 50to 400 | - | 80to 90 | 120 | 2 | Gordon *et al* 1934 |
| Egypt | S. Rhodesia | 196 | - | - | - | 3 | Lo 1972 |
| | S. Africa | - | - | 15 | 56 | 3 | |
| Transvaal . | Transvaal | - | - | - | 45 | 4 | Pitchford & Visser 1965 |
| Kenya | Kenya | 45 | - | - | - | | Teesdale 1962 |
| Nigeria | Nigeria | 145 | - | 66 | - | 5 | Webbe & James |
| Tanzania | Tanzania | 359 | - | 56 | - | 5 | 1971 |
| Nigeria | Nigeria | | | | | | |
| | 1 miracidium | 337 | 10000 | 100-150 | 243 | 5 | Webbe & James |
| | 3 miracidia | 200 | - | - | - | 5 | 1972 |
| | 7 miracidia | 147 | - | - | - | 5 | |
| S. Africa | S. Africa | 950 | - | - | - | | Wright & Bennet 1967 |

* where true mean not available, 'average' or 'normal' figure quoted
1 sampled one day/week
2 75 minute sample, one day each week
3 not sampled over whole infection
4 outdoor aquaria
5 sampled one day/week 0930-1630h

(Chapter 4) is not reported by other workers.

Comparisons of the levels of daily cercarial output from the host-parasite combinations in this study with those from other laboratories may not be justified due to variations in techniques. Those observed from B. globosus Ngala do, however, fall in with the higher results reported from other studies (Table 5.3) e.g. Gordon et al., 1934; Webbe and James, 1971 and 1972. The only published study of cercarial production from S. haematobium infection of B. rohlfsi was that from Lo (1972) who obtained higher mean daily cercarial outputs with an Egyptian strain of the parasite in molluscs from Ghana, although these snails were only monitored over part of their infection. No published reports of cercarial production from B. senegalensis were found.

The gross patterns of change in cercarial production with the age of the schistosome infection for the three short-lived combinations in this study agree broadly with those reported previously (e.g. Gordon et al., 1934; Chu et al., 1966; Lo, 1972; Webbe and James, 1972; Pfluger et al., 1984) all working in controlled laboratory conditions, and Pitchford, et al. (1969), whose experiments were carried out under field conditions. In all cases daily cercarial production rose to a peak within a few weeks of the onset of shedding, after which it declined until the snail's death. The position of the peak, and the regularity of the decline are rather variable. No clear peak in output was observed by Frandsen (1979 a). Patterns of production of cercariae from other schistosomes

show similar patterns (reviewed by Berrie, 1970).

No other laboratory study of S. haematobium cercarial production has covered infections of the duration observed in B. globosus Ngala. This may explain why a second peak in cercarial output shown in a proportion of snails has not previously been reported. McClelland (1967) working with natural infections of S. haematobium in B. nasutus, in Tanzania, where the age of the infection was therefore unknown, did report a more frequent occurrence of high daily outputs of cercariae (over 2,000) from individuals towards the end of the infection season than in preceding months. He was unable to explain this phenomenon. It is possible, based on the present findings, that he was collecting snails with very old infections showing a similar second period of high productivity to B. globosus Ngala.

The long period of low cercarial production seen in most B. globosus Ngala in this investigation has no obvious physiological explanation. The waves of daughter sporocyst replication observed and linked with cercarial output patterns by Theron (1981 (a and b)) in Biomphalaria glabrata infected with S. mansoni occurred over much shorter periods (5 - 6 weeks between peaks) than that seen in the B. globosus Ngala showing two periods of high productivity. Kechmir and Theron (1980) observed replicating S. haematobium daughter sporocysts in B. truncatus 65 to 150 days post infection i.e. very early in the productive phase of this combination where first cercariae were only seen 55 days from initial exposure to

miracidia. Similar patterns of sporocyst replication may be occurring in B. globosus Ngala with the corresponding waves of cercarial production being undetected by the infrequent sampling. It is possible that the long period of low cercarial output is an extended phase of daughter sporocyst replication where, in a proportion of snails, renewed cercarial production from the fresh sporocysts produces a second spell of high output. In other snails either no new sporocysts are produced, or they fail to produce large numbers of cercariae. This hypothesis could only be investigated by an extensive histological study in parallel with continuous cercarial monitoring.

Frandsen (1979 a and c) and Theron (1981a) discuss the use of cercarial productivity in indicating the degree of adaptation between host-parasite combinations. Both point out the need for the standardization of techniques to enable comparison between studies in different laboratories. In practice, standardizing experimental conditions when dealing with cultures that are not easy to maintain presents problems. In addition, the obvious need for continuous monitoring of cercarial production to obtain true figures of the parasite's productivity (Theron, 1981a) combined with the attendant difficulties of processing such material and the stress on the snails caused by increased handling (Frandsen, 1979c) make the necessary exhaustive quantitative studies impracticable.

Frandsen (1978, 1979a) proposed a new index: the total cercarial production per 100 exposed snails (TCP/100) as a

measure of the compatibility of a host-parasite combination. This takes into account many aspects of the relationship: susceptibility of the host population to infection with the parasite strain; mortality of snails over the prepatent period; the cercarial productivity of the infection and its duration. Table 5.4 shows the TCP/100 indices calculated for the host-parasite combinations in the present study, using data from larger experimental groups for prepatent mortality, infection rates and survival times, (see Chapter 4) and the results from this study for cercarial productivity. Of the host/parasite combinations that produced successful infections, B. globosus Ngala infected with the 'globosus' strain of parasite is the most compatible and B. rohlfsi infected with the 'rohlfsi' strain the least.

Frandsen's TCP/100 index, although a useful expression of compatibility, is not ideal. Exhaustive studies would be necessary to obtain the required data. Theron (1981a) points out that the duration of the productive period of an infection is a function of the life-span of the snail and culture conditions; this is clearly shown in the present study with B. globosus Ngala being naturally long-lived compared to the other two species under investigation.

Theron (1981a) proposed expressing the index of production of a host-parasite combination by the cercarial production per day and per snail (c/d/s), calculated from a group of snails over a sufficiently long period and always specifying: number of miracidia; water temperature of prepatent and shedding

**TABLE 5.4**

**Indices of Total Cercarial Production per 100 Exposed Snails.**

| Host species/ parasite strain | Mean daily cercarial output | Mean duration of infection | % exposed snails infected | TCP/100 index | Class |
|---|---|---|---|---|---|
| B.globosus (Ng) | | | | | |
| 'globosus' | 100 | 169 | 27 | 456,300 | V |
| 'rohlfsi' | 0 | 0 | 0 | 0 | 0 |
| B. rohlfsi | | | | | |
| 'rohlfsi' | 16 | 41 | 9 | 5,904 | I |
| 'globosus' | 0 | 0 | 0 | 0 | 0 |
| B.senegalensis | | | | | |
| 'globosus' | 45 | 57 | 10 | 25,650 | II |
| 'rohlfsi' | 38 | 49 | 22 | 40,964 | II |

**Modified form of Frandsen's TCP/100** (Frandsen, 1979 c)

TCP/100 = Mean daily cercarial production X Mean duration of infection* X % exposed snails infected*

* these values obtained from larger study described in Chapter 4. Compatibility classes range from 0 (incompatible or refractory) to VI (highest degree of compatibility).

period; luminous intensity and photoperiod; number of daily emissions considered on average. Although having the advantage of requiring less extensive studies to obtain the data, it does not incorporate sufficient information to give a measurement of the degree of adaptation between host and parasite.

Both Frandsen's TCP/100 index and Theron's c/d/s fail to take into account the importance of the size of the host, a factor that has been shown to be of significance in schistosome infections (reviewed in Berrie, 1970) and may have been a factor in the present study (Chapter 6).

The measurement of the degree of compatibility between host and parasite is extremely complicated, both because of the number of interactions that should be investigated to obtain a full picture of the relationship, and the problems involved in standardizing techniques. In attempting to assess the epidemiological importance of a host-parasite combination, the essential measurement required is the potential output of the infective stage, in this case cercariae. For this purpose Frandsen's TCP/100 is highly suitable.

In the present study, it is essentially the relative importance of each Bulinus species in the transmission of S. haematobium that is under investigation. It would seem that B. globosus Ngala is likely to provide the largest source of infection, with B. senegalensis also being of significance due to its susceptibility to both parasite strains investigated. However, this laboratory study was carried out under controlled conditions and can only be assumed to give an indication of the

situation in the field. The relative abundance, habitat preferences and population dynamics of each species will also have a bearing on the role of each in transmission of the parasite.

## REFERENCES


Berrie, A.D., 1970. Snail problems in African schistosomiasis. Advances in Parasitology 8 : 43-96.

Chu, K.Y., Sabbaghian, H. and Massoud, J., 1966. Host-parasite relationships of Bulinus truncatus and Schistosoma haematobium in Iran. Bulletin of the World Health Organisation 34 : 121-130.

Frandsen, F., 1978. An assessment of the compatibility between Schistosoma sp. and their intermediate hosts using the cercarial production from a population of snails. Fourth International Congress of Parasitology, Warsaw C, : 105-106.

Frandsen, F., 1979 a. Studies on the relationship between Schistosoma and their intermediate hosts. I. The genus Bulinus and Schistosoma haematobium from Egypt. Journal of Helminthology 53 : 15-29.

Frandsen, F., 1979 b. Studies on the relationship between Schistosoma and their intermediate hosts. II. The genus Bulinus and Schistosoma haematobium from Sudan, Zaire and Zambia. Journal of Helminthology 53 : 205-212.

Frandsen, F., 1979 c. Discussion of the relationship between Schistosoma and their intermediate hosts, assessment of the degree of host-parasite compatibility and evaluation of schistosome taxonomy. Zeitschrift fur Parasitenkunde 58 : 275-296.

Gordon, R.M., Davey, T.H. and Peaston, H., 1934. The transmission of human bilharziasis in Sierra Leone, with an account of the life-cycle of the schistosomes concerned, S. mansoni and S. haematobium. Annals of Tropical Medicine and Parasitology 28 : 323-418.

Kechemir, N., and Theron, A., 1980. Existence of replicating sporocysts in the development cycle of Schistosoma haematobium. Journal of Parasitology 66 : 1068-1070.

Lo, C-T., 1972. Compatibility and host-parasite relationships between species of the genus Bulinus (Basommatophora: Planorbidae) and an Egyptian strain of Schistosoma haematobium (Trematoda: Digenea). Malacologia 11 : 225-280.

McClelland, W.F.J., 1965. Production of cercariae by Schistosoma mansoni and S. haematobium and methods for estimating the numbers of cercariae in suspension. Bulletin of the World Health Organisation 33 : 270-276.

McClelland, W.F.J., 1967. Production of Schistosoma haematobium and Schistosoma mansoni cercariae in Tanzania. Experimental Parasitology 20 : 205-218.

Pfluger, W., Roushdy, M.Z., and El-Emam, M., 1984. The prepatent period and cercarial production of Schistosoma haematobium in Bulinus truncatus (Egyptian field strains) at different constant temperatures. Zeitschrift fur Parasitenkunde 70 : 95-103.

Pitchford, R.J., and Visser, P.S., 1965. Some further observations on schistosome transmission in the Eastern Transvaal. Bulletin of the World Health Organisation 32 : 83-104.

Pitchford, R.J., Meyling, A.H., Meyling, J. and DuToit, J.F., 1969. Cercarial shedding patterns of various schistosome species under outdoor conditions in the Transvaal. Annals of Tropical Medicine and Parasitology 63 : 359-371.

Raymond, K., 1985. An investigation of the production of cercariae of Schistosoma haematobium from Bulinus globosus, and the effect of light on emission. M.Sc. Thesis, University of Wales.

Teesdale, C., 1962. Ecological observations on the molluscs of significance in the transmission of bilharziasis in Kenya. Bulletin of the World Health Organisation 27 : 759-782.

Theron, A., 1981 a. Dynamics of larval populations of Schistosoma mansoni in Biomphalaria glabrata. I. Rhythmic production of the cercariae in monomiracidial infections. Annals of Tropical Medicine and Parasitology 75 : 71-77.

Theron, A., 1981 b. Dynamics of larval populations of Schistosoma mansoni in Biomphalaria glabrata. II. Chronobiology of the intramolluscan larval development during the shedding period. Annals of Tropical Medicine and Parasitology 75 : 547-554.

Theron, A., 1982. Ecology of schistosome cercarian transmission: production, emergence, dispersion and infectivity of Schistosoma mansoni cercariae. In Parasites - Their World and Ours Ed. Mettrick, D.F. and Desser, S.S. Elsevier Biomedical Press 289-292.

Webbe, G. and James, C., 1971. Infra-specific variations of Schistosoma haematobium. Journal of Helminthology 45 : 403-103.

Webbe, G. and James, C., 1972. Host-parasite relationships of Bulinus globosus and B. truncatus with strains of Schistosoma haematobium. Journal of Helminthology 46 : 185-199.

Wright, C.A. and Bennet, M.S., 1967. Studies on Schistosoma haematobium in the laboratory: I. A strain from Durban, Natal, South Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene 61 : 221-227.

# CHAPTER 6

# THE EFFECT OF INFECTION WITH *SCHISTOSOMA HAEMATOBIUM* ON GROWTH AND REPRODUCTION IN *BULINUS* SPECIES

## INTRODUCTION

Many trematode infections have a marked effect on the biology of their molluscan host (Wright, 1966), particularly affecting growth and reproductive activity. Gigantism is often associated with trematode infections in both prosobranchs and pulmonates (Wright, 1971). In the majority of reports, infection of *Biomphalaria* species with *Schistosoma mansoni* produces a temporary acceleration of host growth (Pan, 1963, 1965; Sturrock, 1966; Sturrock and Sturrock, 1970; Meuleman, 1972). Where very young snails are infected, however, retardation in growth has been observed (Sturrock and Sturrock, 1970; Meier and Meier-Brook, 1981).

Studies involving *S. haematobium* infections in *Bulinus* species have shown less consistent effects on host growth. Sturrock (1967) showed an acceleration of growth of infected *B. nasutus productus* and Mohamed and Ishak (1982) reported giant growth of *B. truncatus*. Other studies involving a variety of bulinids have shown no changes in growth between infected and uninfected snails (Chu, Sabbaghian and Massoud, 1966; Lo, 1972).

Reductions in fecundity of molluscan hosts of schistosomes are also common (Berrie, 1970) with the extent of the

inhibitory effects apparently linked to the stage of reproductive maturity of the host when exposed to infection. Infection of immature Biomphalaria spp. with S. mansoni generally halts all subsequent oviposition (Sturrock and Sturrock, 1970; Meier and Meier-Brook, 1981) whereas exposure to miracidia after the snail has reached reproductive maturity usually inhibits egg laying, with only a few individuals showing a total suppression (Sturrock and Sturrock, 1970; Meuleman, 1972; Meier and Meier-Brook, 1981). Sturrock (1966) showed the reverse effect, with mature snails ceasing oviposition completely, whereas immature animals demonstrated a diminished and delayed egg laying rate.

Some studies have shown that egg laying recovers to a certain extent in the latter stages of an infection (Sturrock, 1966; Sturrock and Sturrock, 1970; Etges and Gresso, 1965) although, except in cases of snail 'self cure' (Sturrock, 1966) levels of oviposition never reached those of uninfected snails.

Inhibition of egg production has also been demonstrated in Bulinus species infected with S. haematobium (Najarian, 1961; Lo, 1972; Chu et al., 1966; Sturrock, 1967; Mohamed and Ishak, 1982) though without total stoppage of oviposition even when snails were exposed to very large numbers of miracidia (Chu et al., 1966).

In many studies of both S. mansoni and S. haematobium infections a reduction in the number of embryos contained in egg masses laid by the host snails has been observed (Etges and Gresso, 1965; Chu et al., 1966; Sturrock, 1966; Meuleman, 1972;

Lo, 1972; Meier and Meier-Brook, 1981). In the case of S. haematobium in B. truncatus this reduction in egg mass size was more marked than the decrease in oviposition (Najarian, 1961).

Since schistosome sporocysts do not cause direct damage to the molluscan host's ovotestis, the inhibition of host reproduction is thought to be indirect. Extensive studies into the possible mechanisms involved have been carried out using the avian schistosome Trichobilharzia ocellata in Lymnaea stagnalis as a model (Sluiters, Brussaard-Wust and Meuleman, 1980; Sluiters, 1981; Sluiters, Roubos and Joosse, 1984; Sluiters and Gerearts, 1984 a and b; Sluiters and Dogterom, 1984) since much is already known on the endocrine control of growth and reproduction in this snail species. Results support the hypothesis that the parasite in this case is intervening in the endocrine control of reproduction in the host at the level of the reproductive organs.

Meuleman (1972) suggested that the variability of results obtained in studies of host growth and reproduction even in the same host-parasite combination may be due to differences in experimental protocols applied, or to the use of different strains of parasite. As a part of a wider study of the host-parasite relationships of Bulinus species and S. haematobium, strains of the parasite from the area of the South Chad Irrigation Project, Borno State, Nigeria have been used to study the effects of parasite infection on host growth and reproduction. Three species of Bulinus known to be susceptible to infection with the two strains of S haematobium

(Chapter 4) have been used in this study allowing a comparison to be made of four host-parasite combinations under similar experimental conditions. These combinations are described in the Materials and Methods section below.

## MATERIALS AND METHODS

All three Bulinus species that proved susceptible to infection with the S. haematobium strains under investigation were studied, giving four host species-parasite combinations:

B. globosus Ngala infected with 'globosus' strain

B. rohlfsi infected with 'rohlfsi' strain

B. senegalensis infected with 'globosus' strain

B. senegalensis infected with 'rohlfsi' strain

Constraints in culturing sufficient suitable snail host and parasite material led to exposure experiments being spread over a long period. As the first priority was to establish large culture stocks of both parasite and snail hosts, experiments including parallel groups of unexposed control snails were only possible during the later stages of the study period.

Conditions were kept as constant as possible over the whole period of experimentation. As discussed in Chapter 2, it became necessary to change the water supply for snail cultures without warning in December 1982. Since all data for the uninfected controls were obtained after this date, where sufficient data are available comparisons between uninfected and infected snails have been restricted to data obtained after

the water change.

Details of the infection procedures and maintenance conditions for experimental snails are described in Chapters 2 and 4. In summary, infected snails had initially been exposed individually to five miracidia in 5ml of water at 25°C under bright illumination. Controls were isolated similarly, without the addition of miracidia. Over the precercarial period exposed and control snails were maintained in small groups. Detailed monitoring of growth and egglaying was not performed at this stage since it was impossible to predict in which of the exposed snails S. haematobium infections had become established. Snails were only classed as infected once shed cercariae had been detected, at which stage they were isolated in 100ml containers. As each infected snail was isolated, an individual from the parallel control group was similarly transferred. Monitoring of growth and egg production was then started.

At all stages infected and control snails were treated identically, with containers arranged randomly on culture room shelves.

Growth was assessed by weekly measurement of shell height. Snails were first wiped with a soft tissue to remove algae etc., and then measured on a 0.5mm graduated ruler.

Reproduction was assessed at weekly intervals by counting all egg masses and contained embryos deposited on the sides of containers, and on leaf skeletons. Egg-masses were allowed to remain on the sides of the container, but marked to prevent

them from being counted twice.

The reproductive output of each individual snail monitored was expressed by calculating the egg mass production and total embryos produced per day for the study period. The mean number of embryos contained in each egg mass laid was also calculated. Blank egg masses, where no embryos were contained in the gelatinous matrix were included in the total egg mass count. On a few occasions egg masses had been wholly or partially eaten by the snail before counting, although the outline of the mass could be seen clearly. In these cases an estimate was made of the number of embryos that were present using the calculated mean eggs per mass for that individual.

Monitoring of growth and reproduction continued until each snail died.

Statistical analyses were performed using SPSS statistical package on a mainframe Dec-10 computer.

## RESULTS

### 1. Snail Growth

At the time of exposure of S. haematobium miracidia, all snails were in the same size range (3 to 5mm shell height). By the start of the monitored period (when infected snails first produced cercariae) there was no significant difference between the shell heights of infected and control snails of each species.

Infection of B. globosus Ngala with the 'globosus' strain of S. haematobium resulted in an inhibition of growth over the

early stage of the active infection (Fig. 6.1). An analysis of variance of components of infected and control growth curves over the first 6 weeks shows a significant difference in both the linear component (F=9.5108; d.f.=1,36; p=0.004) and the quadratic component (F=6.5067; d.f.=1,36; p=0.015), indicating a significant reduction in the growth rate of infected snails over this period. Later in the infection the growth rates were similar, although infected snails never attained the same mean size as the controls.

In contrast, infection of B. rohlfsi with the 'rohlfsi' strain of the parasite produced an acceleration in growth compared with the controls (Fig 6.2), with a significant difference in the linear component of the two growth curves over the first 6 weeks of cercarial production (F=6.0246; d.f.=1,14; p=0.028). In this shorter lived species, analysis beyond this point was not possible due to the limited number of snails surviving.

Infection with the two strains of S. haematobium ('globosus' and 'rohlfsi') had no significant effect on the growth of B. senegalensis (Fig. 6.3) measured in this experiment. In this case there are insufficient data available for snails infected with 'rohlfsi' parasite after the change in water supply (Chapter 2), therefore data for 'globosus' strain infected snails prior to the change are also included. There is no significant effect of either parasite strain, or of water supply on the observed growth of snails.

FIGURE 6.1

Growth of B. globosus infected with the 'globosus' strain of S. haematobium and uninfected controls

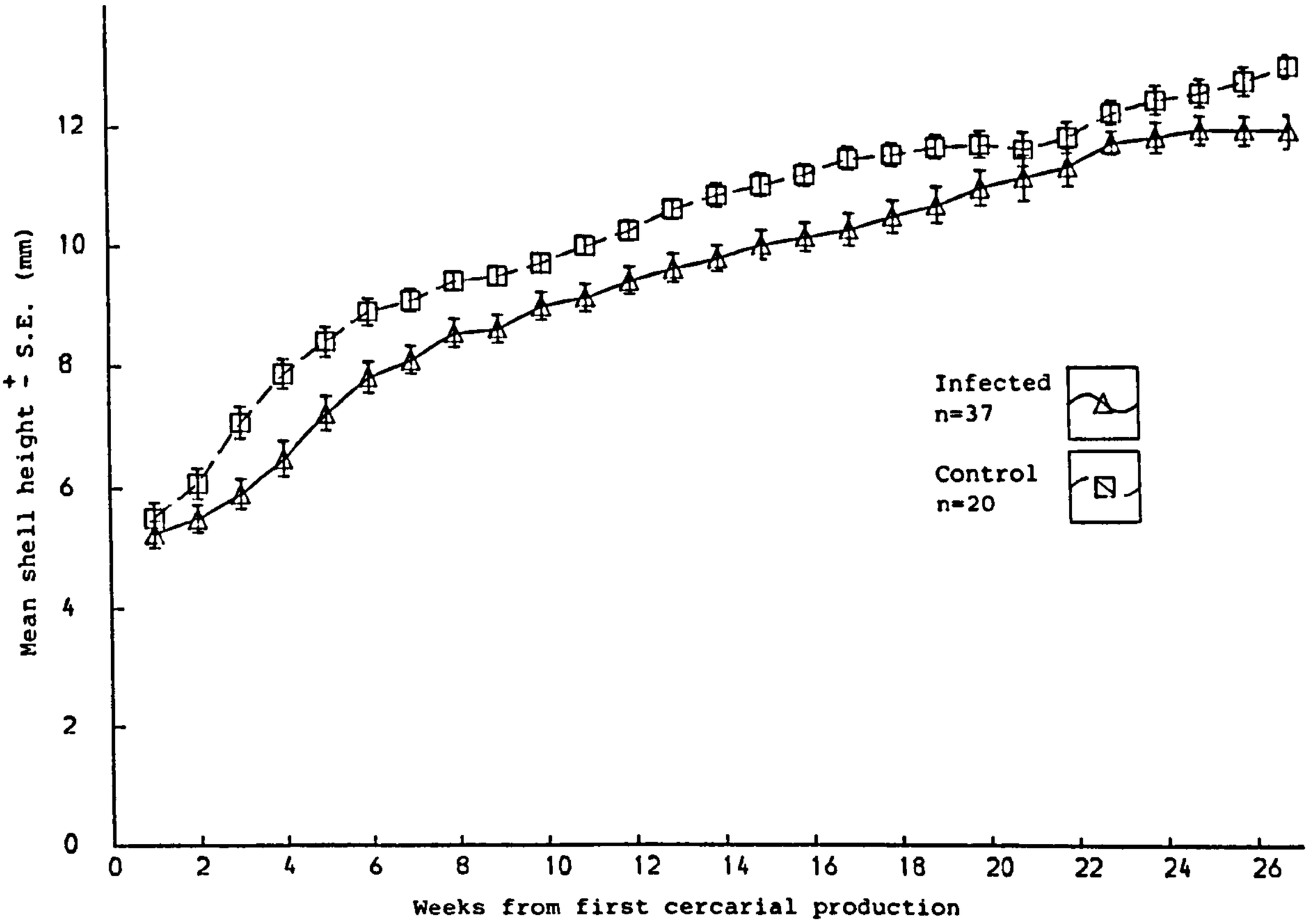



FIGURE 6.2

Growth of B. rohlfsi infected with the 'rohlfsi' strain of S. haematobium and uninfected controls

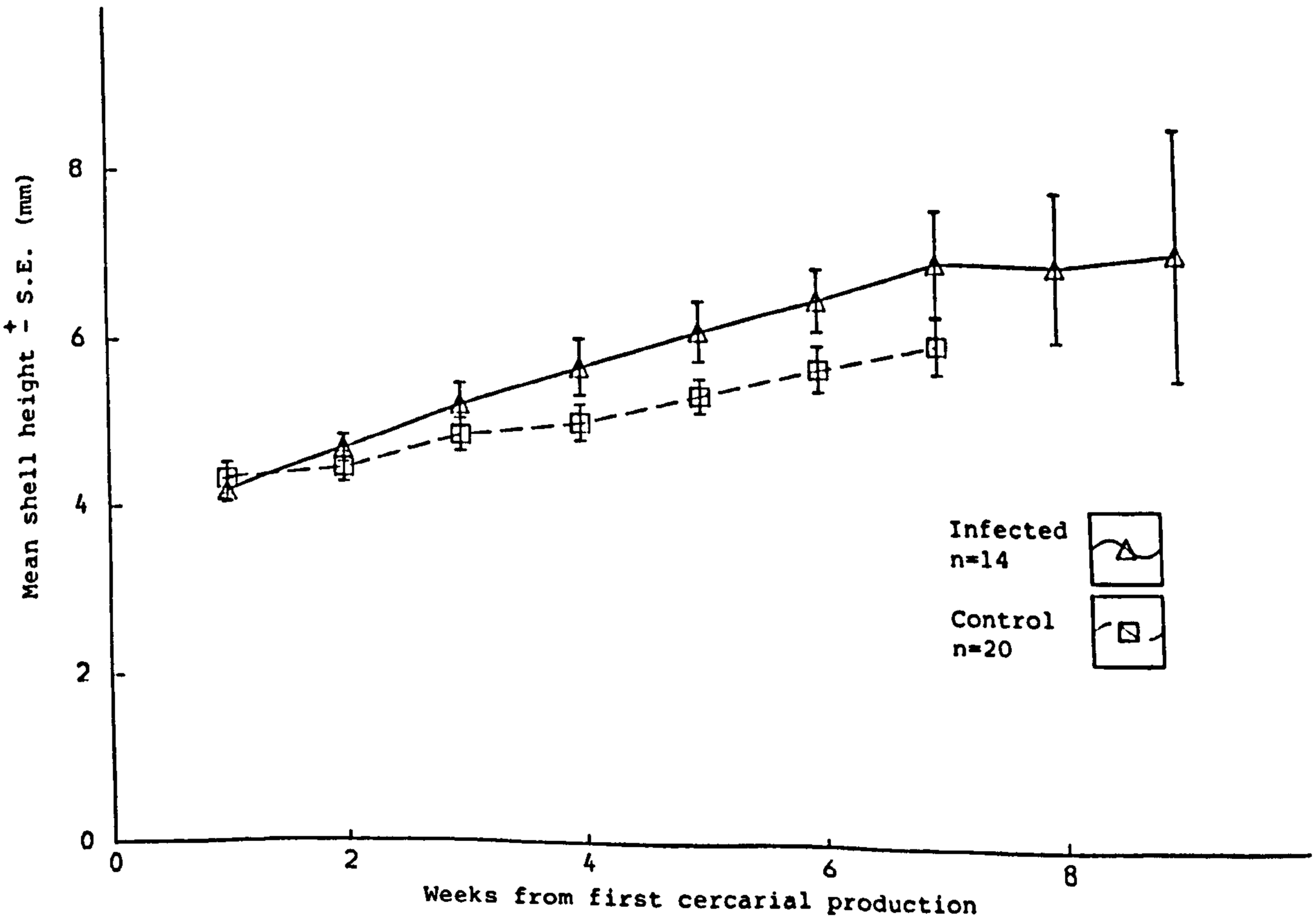
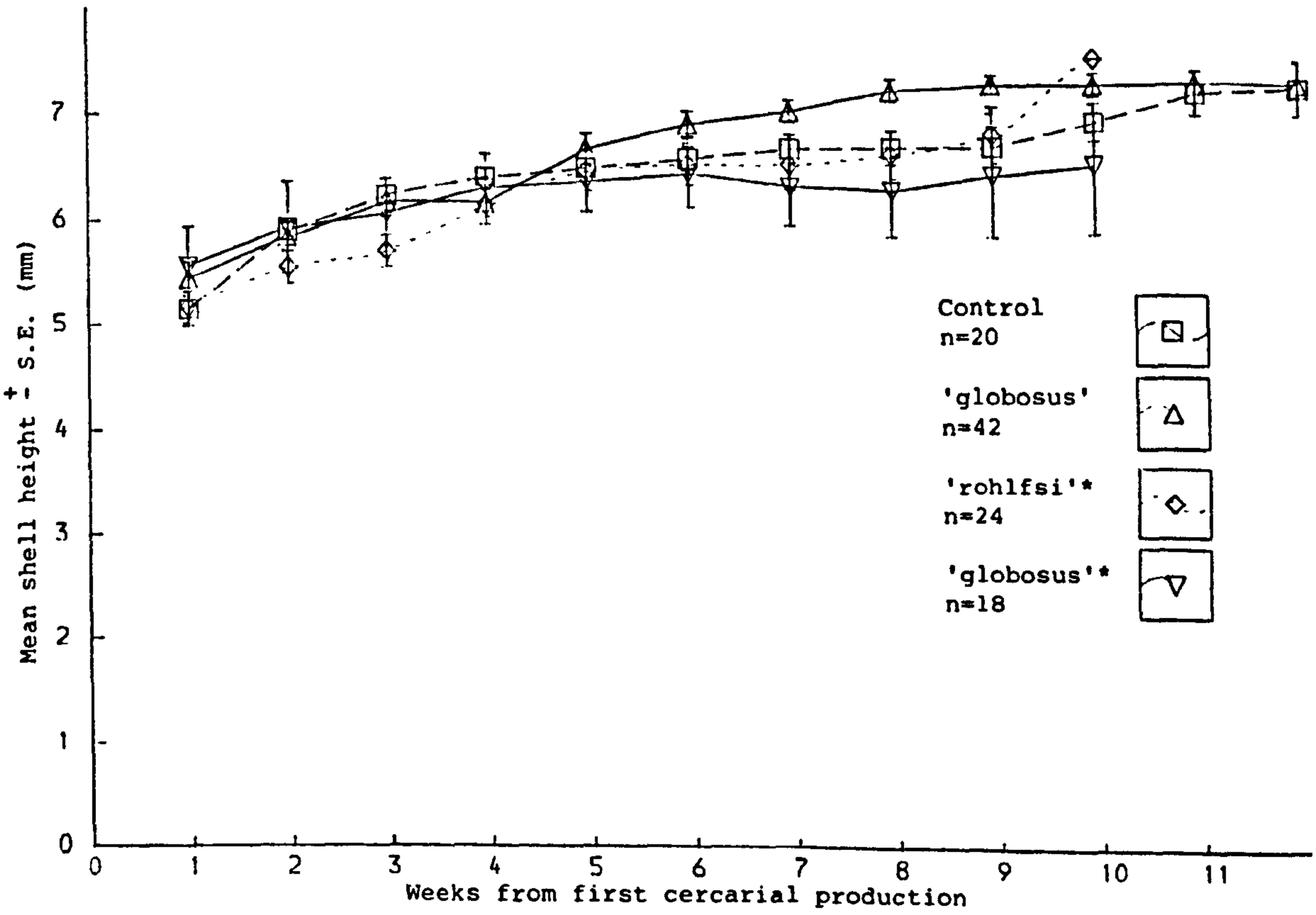


FIGURE 6.3

Growth of B. senegalensis infected with 'globosus' and 'rohlfsi' strains of S. haematobium and uninfected controls




(* data obtained before change in water supply)

## 2. Snail Egg Production.

### a) Total suppression of egg laying.

Table 6.1 shows the numbers of control and infected snails that, having reached maturity (calculated from the minimum size at egg laying in uninfected snails), laid egg masses at some stage during the period monitored. Evidence of total suppression of oviposition was only seen in B. globosus Ngala where only 6 out of 24 infected individuals (25%) laid eggs compared to 19 out of 20 uninfected controls (95%).

### b)Levels of egg production.

Analysis of the data showed that there was no effect of changing the water supply on the levels of egg production of each snail species. In order to obtain enough data for a full statistical analysis, results obtained from snails infected both before and after the change were therefore pooled for comparison with uninfected controls.

Table 6.2 summarises the egg-laying activity of all snails that produced egg masses during the monitored period. Infection with S. haematobium had a significant effect on reproductive activity of all three host species.

In the case of both B. globosus Ngala and B. rohlfsi, infection with their respective schistosome strains resulted in a significant reduction in the mean number of egg masses produced per day (see Table 6.2a and 6.2b), although these masses contained a similar number of embryos per mass as those from uninfected controls. There was thus a significant reduction in the actual reproductive output of infected snails,

**TABLE 6.1**

**The effect of infection with S. haematobium on oviposition in Bulinus species**

| | | n* | Oviposited n | Oviposited % | Suppression of oviposition $X^2$ | Suppression of oviposition p |
|---|---|---|---|---|---|---|
| B. globosus (Ngala) | Infected | 24 | 6 | 25.0 | 19.0273 | <0.001 |
| | Control | 20 | 19 | 95.0 | | |
| B. rohlfsi | Infected | 14 | 10 | 71.5 | 0.4102 | N.S. |
| | Control | 16 | 14 | 87.5 | | |
| B. senegalensis | Infected 'g' | 40 | 39 | 97.5 | 0.0013 | N.S. |
| | Control | 20 | 20 | 100.0 | | |
| | Infected 'r' | 29 | 28 | 96.5 | 0.0532 | N.S. |

* Snails that did not reach the normal minimum size for oviposition are not included.
'g'='globosus' strain 'r'='rohlfsi' strain.
N.S. = not siginificant at the 5% level of confidence

## TABLE 6.2

**Effects of infection on oviposition in snails that continued to lay eggs over the study period**

**A: B. globosus (Ngala)**

| | | n | Mean | S.D. | t | p |
|---|---|---|---|---|---|---|
| Embryos/day | Control | 19 | 1.2174 | 0.771 | -3.56 | 0.001 |
| | Infected | 32 | 0.5670 | 0.531 | | |
| Egg masses/day | Control | 19 | 0.1424 | 0.085 | -4.46* | <0.001 |
| | Infected | 33 | 0.0515 | 0.036 | | |
| Embryos/mass | Control | 19 | 8.8505 | 3.160 | 1.06* | N.S. |
| | Infected | 32 | 10.1128 | 5.373 | | |

**B: B. rohlfsi**

| | | n | Mean | S.D. | t | p |
|---|---|---|---|---|---|---|
| Embryos/day | Control | 14 | 0.9403 | 0.644 | -3.09* | 0.007 |
| | Infected | 26 | 0.3727 | 0.331 | | |
| Egg masses/day | Control | 14 | 0.4299 | 0.225 | -3.27* | 0.004 |
| | Infected | 26 | 0.2174 | 0.124 | | |
| Embryos/mass | Control | 14 | 2.0036 | 0.541 | -1.78* | N.S. |
| | Infected | 26 | 1.5885 | 0.934 | | |

TABLE 6.2 continued

**C: B. senagalensis infected with 'globosus' strain**

| | | n | Mean | S.D. | t | p |
|---|---|---|---|---|---|---|
| Embryos/day | Control | 20 | 2.5196 | 0.712 | -2.53 | 0.014 |
| | Infected | 56 | 2.0021 | 0.811 | | |
| Egg masses/day | Control | 20 | 0.6469 | 0.137 | -1.22 | N.S. |
| | Infected | 56 | 0.5978 | 0.161 | | |
| Embryos/mass | Control | 20 | 3.8740 | 0.571 | -3.02* | 0.004 |
| | Infected | 56 | 3.3220 | 0.977 | | |

**D: B. senegalensis infected with 'rohlfsi' strain**

| | | n | Mean | S.D. | t | p |
|---|---|---|---|---|---|---|
| Embryos/day | Control | 20 | 2.5196 | 0.712 | 1.29 | N.S. |
| | Infected | 28 | 2.8805 | 1.098 | | |
| Egg masses/day | Control | 20 | 0.6469 | 0.137 | 3.83* | <0.001 |
| | Infected | 28 | 0.8740 | 0.268 | | |
| Embryos/mass | Control | 20 | 3.8740 | 0.571 | -2.92* | 0.005 |
| | Infected | 28 | 3.2479 | 0.829 | | |

† = Student t-test
* : separate variance estimate used as variance ratio high
N.S. = not significant at the 5% level of confidence

with fewer embryos being produced.

Infection of B. senegalensis with the 'globosus' strain of S. haematobium also resulted in an overall reduction in egg-laying (Table 6.2c). In this case there was no significant reduction in the rate of egg mass deposition, but those masses laid contained significantly fewer embryos than masses produced by uninfected controls. B. senegalensis infected with the 'rohlfsi' strain of the parasite laid significantly more egg masses per day over the study period than uninfected controls, but again these masses contained smaller numbers of embryos (Table 6.2d). In this case the net effect was no significant reduction in the overall egg production of infected and uninfected snails.

Egg masses consisting of a gelatinous matrix with no embryos were produced by B. globosus Ngala and B. rohlfsi (Table 6.3). In both species, a significantly higher proportion of these blank egg masses were produced by infected snails compared with controls. The majority of snails that produced these 'blanks' also produced normal embryonated egg masses at some time in the study period. No other egg mass abnormalities such as polyembryony or cercarial inclusion were observed.

**3. Patterns of Egg Production.**

In both B. rohlfsi and B. senegalensis reproductive activity was observed before the start of monitoring in some individuals, therefore data are not available for a full analysis of the patterns of egg production with time. B.

## TABLE 6.3

**Effect of infection on the prevalence of 'blank' egg masses**

| | | Total masses n | Blanks n | Blanks % | $X^2$ | p |
|---|---|---|---|---|---|---|
| *B. globosus* (Ngala) | Infected | 270 | 40 | 14.81 | 21.6176 | <0.001 |
| | Control | 237 | 6 | 2.53 | | |
| *B. rohlfsi* | Infected | 471 | 32 | 6.79 | 16.9509 | <0.001 |
| | Control | 693 | 13 | 1.88 | | |

## TABLE 6.4

**Time elapsed from the start of experiments to first oviposition in *B. globosus* (Ngala)**

| | n | Mean (weeks) | S.D. | t | p |
|---|---|---|---|---|---|
| Infected | 33 | 16.0606 | 8.019 | 6.95 | <0.001 |
| Control | 19 | 5.3158 | 2.888 | | |

## TABLE 6.5

**Size of *B. globosus* at first oviposition**

| | n | Mean shell height (mm) | S.D. | t | p |
|---|---|---|---|---|---|
| Infected | 32 | 10.8594 | 1.166 | 7.15 | <0.001 |
| Control | 19 | 8.9737 | 0.716 | | |

(t= Student t-test with separate variance estimates)

globosus Ngala did not commence oviposition before the study period. This, combined with the greater longevity of this species, allowed a more detailed analysis of the patterns of egg production.

Table 6.4 shows the number of weeks that elapsed from the start of the study period to first ovipostion. Infection with S. haematobium significantly delayed oviposition (infected snails on average produced their first egg mass at 16 weeks while uninfected ones laid at 5 weeks). The time delay observed is not simply a reflection of a reduced growth rate of infected snails, since infected individuals were also significantly larger at first egg laying than controls (Table 6.5).

Fig. 6.4 shows the patterns of egg laying of infected and uninfected control B. globosus Ngala in terms of mean daily production of embryos per snail at weekly intervals from the start of the study period. This clearly shows the inhibition of egg laying in infected snails over the early weeks, with this reduced production being most marked from week 10 to 19.

**4. Egg Laying and Cercarial Production.**

Cercarial production data were available for a limited number of snails also monitored for egg laying. In the analysis of cercarial production from B. globosus Ngala infected with the 'globosus' strain of S. haematobium, two distinct patterns were recorded (Chapter 5). The first showed a single period of high production early in the infection, followed by a long period of low output until the snail's

FIGURE 6.4

Levels of egglaying at weekly intervals in B. globosus infected with the 'globosus' strain of S. haematobium and uninfected control snails

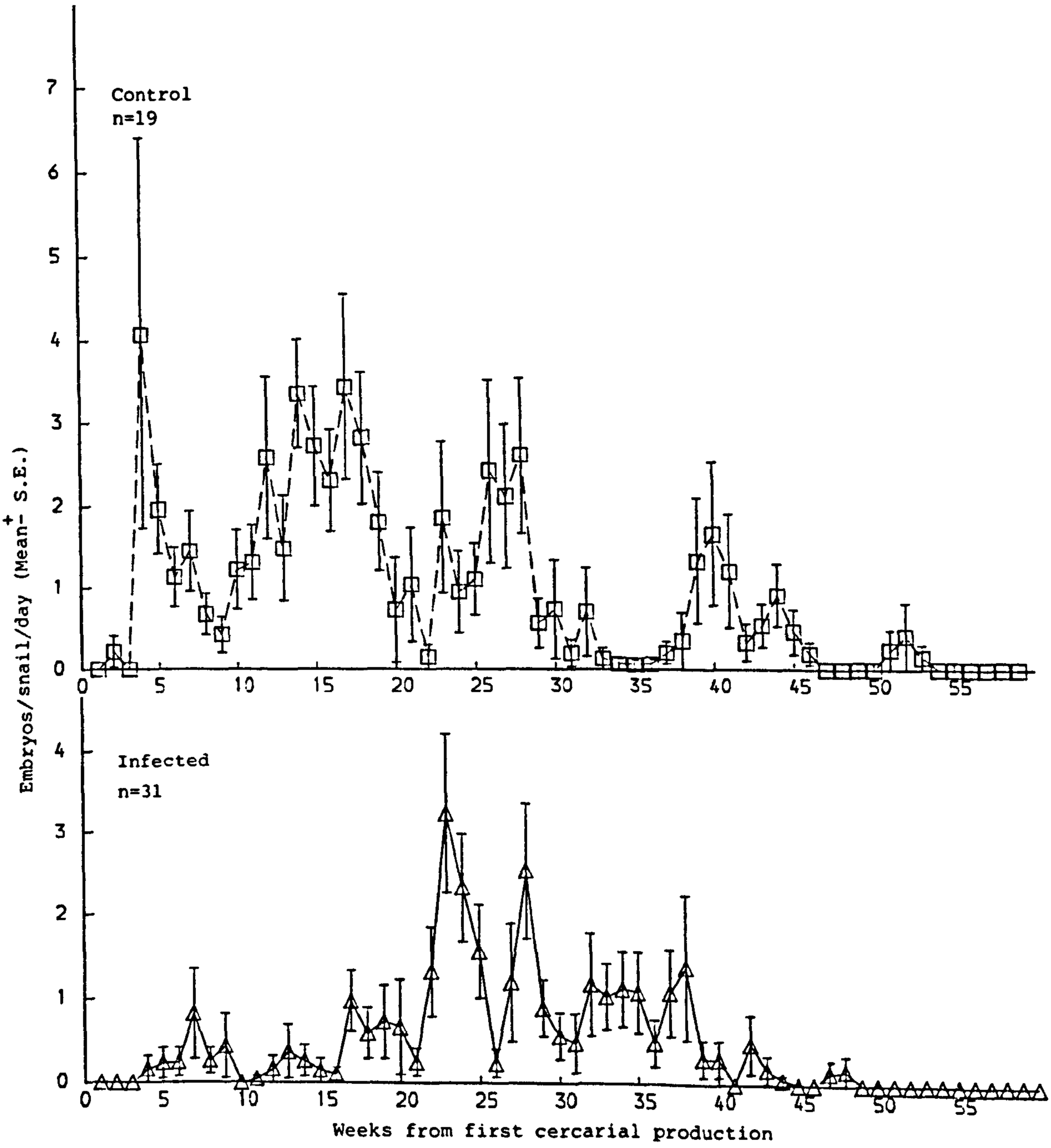
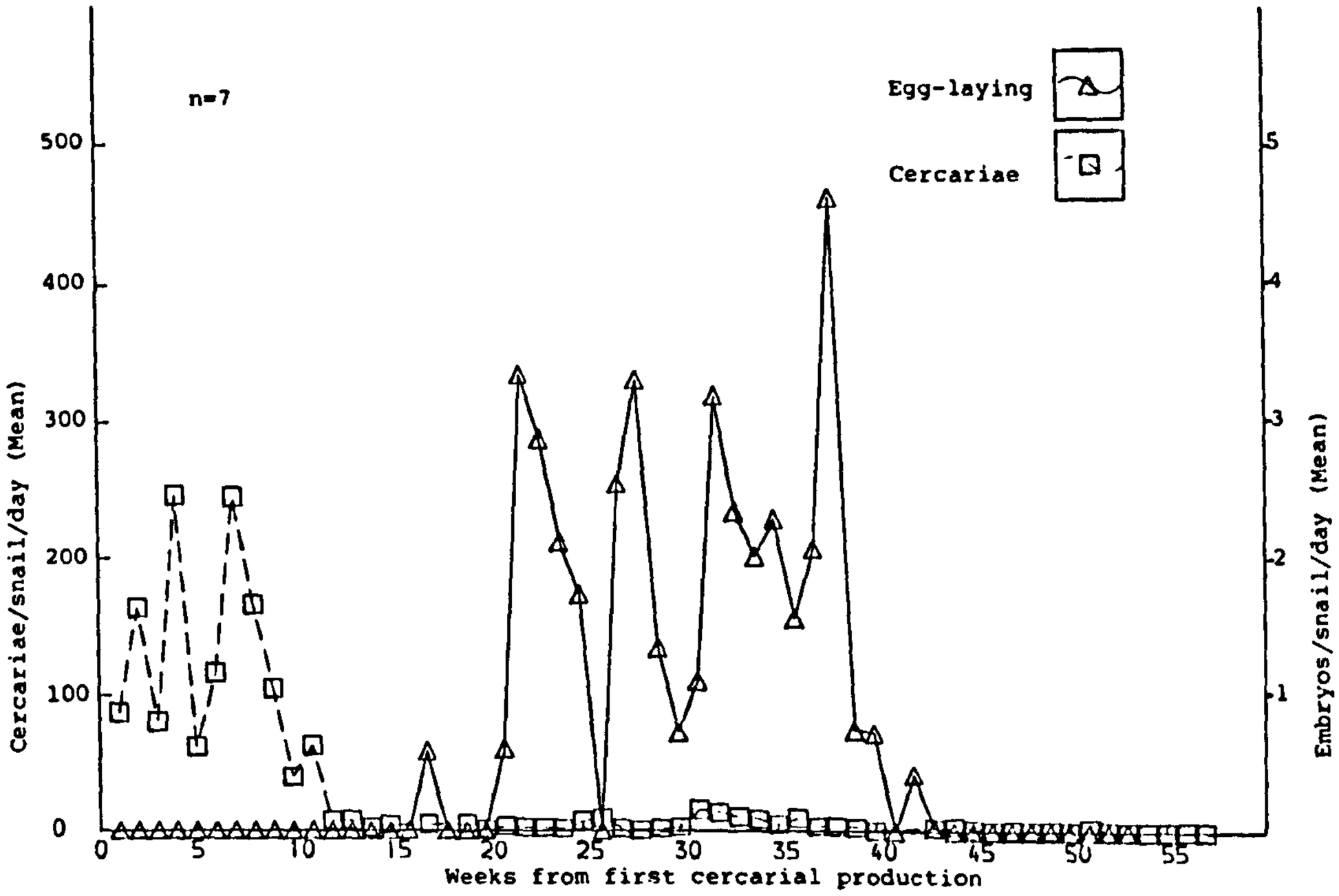


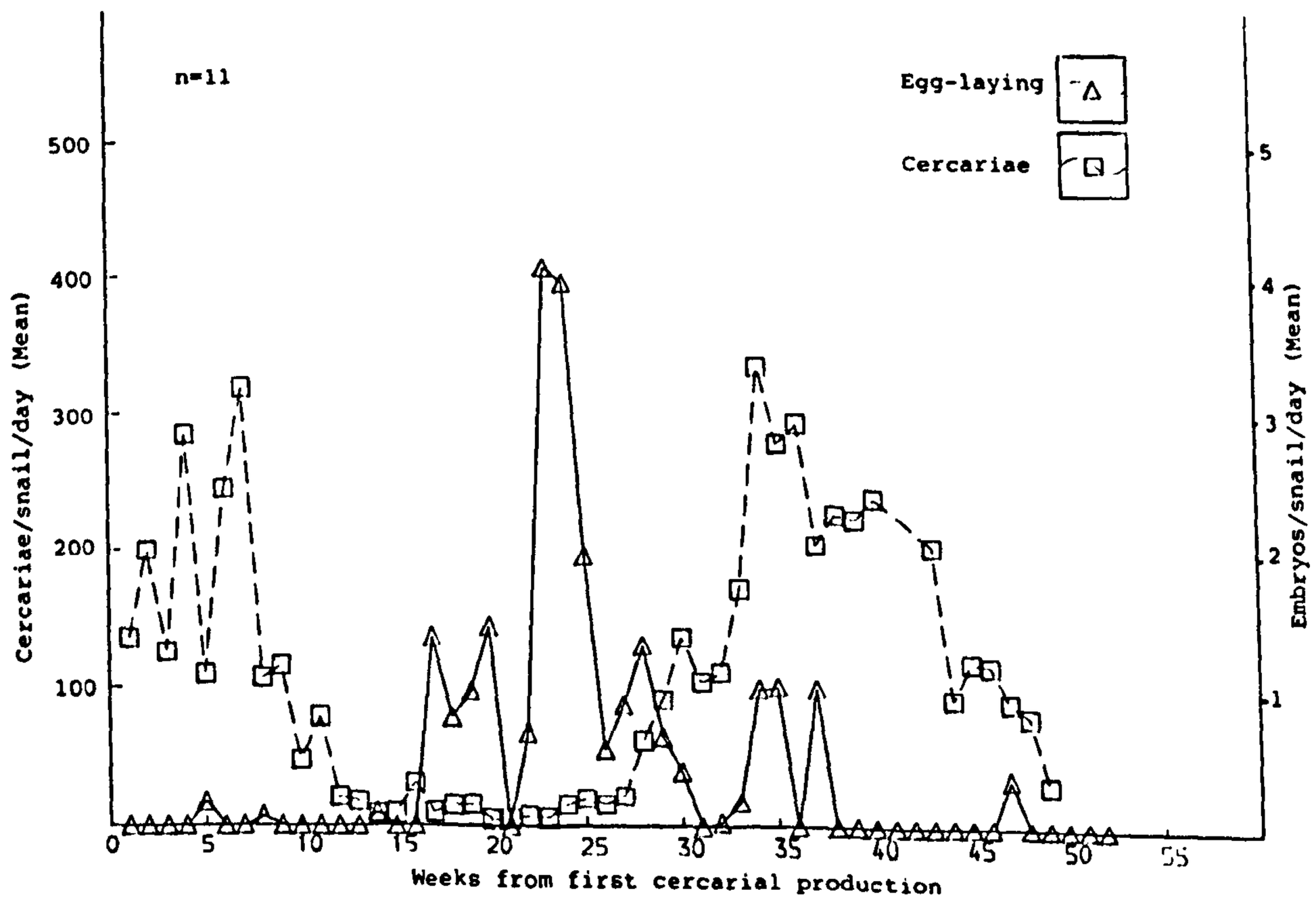
FIGURE 6.5

Egglaying and cercarial output from B. globosus infected with the 'globosus' strain of S. haematobium

A: snails showing a single peak in cercarial output




B: snails showing two peaks in cercarial output



death. A second group of snails showed two periods of high production of cercariae with a short period where only small numbers were shed. Figs. 6.5 shows the egg laying patterns of snails from these two groups superimposed on the cercarial shedding data. In both groups there is a clear antagonistic effect, with maximum egg laying by infected B. globosus Ngala during the period of low S. haematobium cercarial output.

## DISCUSSION

In all three Bulinus species investigated, infection with S. haematobium affected the growth and reproductive biology of the host, although the effects varied with host species.

In the majority of previous studies on the effects of schistosome infections on molluscan hosts, some acceleration in growth has been reported. This was particularly seen in S. mansoni infections of Biomphalaria species (Sturrock, 1966; Sturrock and Sturrock, 1970; Meuleman, 1982) although a reduction in the growth rate of Biomphalaria glabrata was reported when snails were exposed to infection with S. mansoni at a very early age (Sturrock and Sturrock, 1970). An increase in growth rate was observed in B. n. productus infected with S. haematobium (Sturrock, 1967) and Mohamed and Ishak (1982) reported giant growth of infected B. truncatus, yet other workers have found no significant effect of infection with this species on the growth of a variety of bulinid species (Chu et al.,1966; Lo, 1972).

The results presented in this study display the whole

range of effects of schistosome infection on host growth, from an acceleration in B. rohlfsi, to an inhibition in B. globosus Ngala. The high variability of growth rates of individual B. senegalensis, with no apparent effect of parasite infection may be a result of data being confused by the tendency of this species to undergo periods of voluntary aestivation, as discussed in Chapter 3. Precercarial periods in this species are, as a result, highly variable, and the use of the onset of cercarial production as a starting point for growth monitoring resulted in a wide spread of sizes of B. senegalensis, with a subsequent confusion in the interpretation of the growth curves. A more exhaustive study following snails individually from initial exposure to miracidia would be more appropriate for this species.

The inhibitory effect of schistosome infections on their molluscan hosts' reproductive activity has been widely reported. Although infection with S. haematobium affected reproduction in all three snail species investigated in the present study, there were interesting differences in these effects between species. B. globosus Ngala showed the greatest reduction in reproductive output following infection, with a significant proportion (75%) of infected snails failing to deposit eggs. This complete suppression was not observed in infected B. rohlfsi, however both this species, and those individual B. globosus Ngala where some egg-laying capacity remained, showed a marked reduction in oviposition. In neither species was there a significant reduction in the number of

embryos contained in egg masses laid.

Infection of B. senegalensis with S. haematobium did not result in a decrease in the number of egg masses deposited. In the case of snails infected with the 'rohlfsi' strain of parasite there was a significant increase in the rate of egg mass production, but infection with both parasite strains significantly reduced the mean number of embryos contained in each egg mass produced. Najarian (1961) found a similar situation in B. truncatus infected with S. haematobium, with the reduction in egg mass size being more marked than the reduction in the rate of egg mass deposition. Chu et al. (1966) and Lo (1970) also found that egg masses from infected snails contained fewer embryos.

As with other aspects of the host-parasite relationships studied, the different effects on the host's growth and reproduction observed between host species and parasite strain combinations do not seem to be the result of differences between the two parasite strains. The one species susceptible to both strains of schistosome, B. senegalensis, showed a similar response regardless of the strain of parasite involved. This was particularly true in the case of the effect of infection on reproduction, where the reduction in egg mass size rather than rate of oviposition occurred regardless of parasite strain, yet infection of the other host species showed the reverse, with egg mass deposition being inhibited and not the number of embryos per mass. Once again the different effects of S. haematobium observed seem to be a reflection of

differences in the biology of the bulinid host species.

Other studies have indicated that the timing of infection relative to the age or reproductive maturity of the snail is of importance (Sturrock, 1966; Sturrock and Sturrock, 1970; Sluiters et al., 1980; Meier and Meier-Brook, 1981). In the present study it was not possible to use snails of a known age at the time of infection although shell height was used as an indicator to ensure that only 'young' animals were used. This is only a rough indicator of age since not all snails grow at the same rate. Although the age differences between snail species were thus likely to be minimal, it was noted that the reproductive development of each was quite different. B. senegalensis commenced oviposition at a very early age, some when only 3mm in height, and in many cases were reproductively mature before exposure to miracidia. Although B. rohlfsi was never observed to lay eggs before the size used for exposure, experimental groups began egg laying within two to three weeks following infection, thus being reproductively mature before cercarial production started. In contrast B. globosus Ngala did not become reproductively active until a much later age, and after the start of the monitored period.

Each snail species was therefore being challenged to parasite infection at a different stage in its reproductive development: B. senegalensis at or around maturity; B. rohlfsi shortly before reproductive activity commenced, with the parasite usually maturing after first oviposition; and B. globosus Ngala at a very early stage in reproductive

development, with cercarial production occurring before the start of host egg production. The effects of infection on the reproduction of each species therefore fit in with the patterns shown by Meier and Meier-Brook (1981) with S. mansoni infected Biom. glabrata: if the period of parasite prepatency is completely or nearly over before the snails reach sexual maturity there is a total suppression of egg laying, whereas if snail sexual maturity is reached before the middle of prepatency then nearly all infected snails lay eggs, though in smaller numbers than uninfected individuals. This general pattern was also shown by Sturrock and Sturrock (1970), although Sturrock (1966) observed complete sterilization only if infection was acquired after the onset of egg laying. Results obtained in studies involving Bulinus species are not as clear. Lo (1979) found a general inhibition of egg laying in infected snails, with the levels of oviposition in groups exposed at different size classes showing similar patterns of variation as controls of the same size.

The immaturity of B. globosus Ngala may also be the reason for the observed inhibition of growth of infected snails. A similar retardation of growth was demonstrated when very young Biom. glabrata were infected with S. mansoni (Sturrock and Sturrock, 1970; Meier and Meier-Brook, 1981).

The production of abnormal egg masses has been observed by other workers (e.g. Etges and Gresso, 1965; Sturrock, 1966). Lo (1970) described several categories of egg abnormalities from Bulinus species, including masses consisting of a

gelatinous matrix with no embryos (as observed in this study). Lo (1970) also found that the prevalence of these 'blank' masses was higher in infected bulinid snails than uninfected ones. This agrees with the findings in B. globosus Ngala and B. rohlfsi in the present investigation.

The patterns of oviposition shown by B. globosus Ngala throughout the course of infection with S. haematobium show that the infection exerts its maximum effect on the host reproduction when cercarial production is also maximal. This is particularly evident during the first peak of cercarial output. Sturrock (1967) also found a link between S. haematobium output and egg production of infected B. n. productus, with a fall in oviposition as cercarial output increased.

This study confirms that S. haematobium has a marked effect on its molluscan host's growth and reproduction. Although the mechanisms involved cannot be identified from the data collected, the results fit the hypothesis that the parasite is affecting the host's endocrine system. In confirmation with other studies (Sturrock, 1966; Sturrock and Sturrock, 1970; Sluiters et al., 1980; Meier and Meier-Brook, 1981), differential effects of the parasite with host species show that the state of maturity of the reproductive system at the time of infection is of great importance in determining subsequent growth and reproduction. A more detailed investigation involving infection of snails at a known stage of reproductive development is required to show definitively

whether the observed differences are due solely to this factor.

The data presented in this chapter once more indicate that the species of Bulinus is of greater importance in the host-parasite relationship than the schistosome strain to which it is exposed, once an infection has become established.

## REFERENCES


Berrie, A.D., 1970. Snail problems in African Schistosomiasis. Advances in Parasitology 8 : 43-96.

Chu, K.Y., Sabbaghian, H. and Massoud, J., 1966. Host-parasite relationship of Bulinus truncatus and Schistosoma haematobium in Iran. 2: Effect of exposure dosage of miracidia on the biology of the snail host and the development of the parasites. Bulletin of the World Health Organisation 34 : 121-130.

Etges, F.J. and Gresso, W., 1965. Effects of Schistosoma mansoni infection upon fecundity of Australorbis glabratus. Journal of Parasitology 51 : 757-760.

Lo, C-T., 1972. Compatibility and host-parasite relationships between species of the genus Bulinus (Basommatophora: Planorbidae) and an Egyptian strain of Schistosoma haematobium (Trematoda: Digenea). Malacologia 11 : 225-280.

Meier, M. and Meier-Brook, C., 1981. Schistosoma mansoni: Effect on growth, fertility, and development of distal male organs in Biomphalaria glabrata exposed to miracidia at different ages. Zeitschrift fur Parasitenkunde 66 : 121-131.

Meuleman, E.A., 1972. Host-parasite interrelationships between the freshwater pulmonate Biomphalaria pfeifferi and the trematode Schistosoma mansoni. Netherlands Journal of Zoology 22 : 355-427.

Mohamed, A.M. and Ishak, M.M., 1982. Comparative studies on the effects of castration and schistosome infection on Bulinus truncatus. Journal of the Egyptian Society of Parasitology 12 : 499-512.

Najarian, H.H., 1961. Egg-laying capacity of the snail Bulinus truncatus in relation to infection with Schistosoma haematobium. Texas Reports on Biology and Medicine 19: 327-331.

Pan, C-T., 1963. Generalized and focal tissue responses in the snail Australorbis glabratus, infected with Schistosoma mansoni. Annals of the New York Academy of Science 113 : 475-485.

Pan, C-T., 1965. Studies on the host-parasite relationships between Schistosoma mansoni and Australorbis glabratus. American Journal of Tropical Medicine and Hygiene 14 : 931-976.

Sluiters, J.F., 1981. Development of Trichobilharzia ocellata in Lymnaea stagnalis and the effects of infection on the reproductive system of the host. Zeitschrift fur Parasitenkunde 64 : 303-319.

Sluiters, J.F. and Dogterom, G.E., 1984. The effect of infection of Lymnaea stagnalis with Trichobilharzia ocellata on the presence of, and reactivity to the ovulation hormone of the host. Zeitschrift fur Parasitenkunde 70 : 485-489.

Sluiters, J.F. and Gerearts, W.P.M., 1984a. Effects of elevated haemolymph levels of the female gonadotropic hormone on the cercarial production of Trichobilharzia ocellata in Lymnaea stagnalis. Zeitschrift fur Parasitenkunde 70 : 477-484.

Sluiters, J.F. and Gerearts, W.P.M., 1984b. Normal development of Trichobilharzia ocellata in Lymnaea stagnalis in the absence of the female gonadotropic hormone producing bodies of the snail host. Proceedings. Koniklijke Nederlandse akademie van Wetenschappen, Series C, Biol & Med. Sci. 87 : 231-234.

Sluiters, J.F., Brussaard-Wust, C.C.M. and Meuleman, E.A., 1980. The relationship between miracidial dose, production of cercariae, and reproductive activity of the host in the combination Trichobilharzia ocellata and Lymnaea stagnalis. Zeitschrift fur Parasitenkunde 63 : 13-26.

Sluiters, J.F., Roubos, E.W. and Joosse, J., 1984. Increased activity of the female gonadotropic hormone producing bodies in Lymnaea stagnalis infected with Trichobilharzia ocellata. Zeitschrift fur Parasitenkunde 70 : 67-72.

Sturrock, B.M., 1966. The influence of infection with Schistosoma mansoni on the growth rate and reproduction of Biomphalaria pfeifferi. Annals of Tropical Medicine and Parasitology 60 : 187-197.

Sturrock, B.M., 1967. The effect of infection with Schistosoma haematobium on the growth and reproduction rates of Bulinus (Physopsis) nasutus productus. Annals of Tropical Medicine and Parasitology 61 : 321-325.

Sturrock, B.M. and Sturrock, R.F., 1970. Laboratory studies on the host-parasite relationship of Schistosoma mansoni and Biomphalaria glabrata from St. Lucia, West Indies. Annals of Tropical Medicine and Parasitology 64 : 357-363.

Wright, C.A., 1966. The pathogenesis of helminths in the Mollusca. Helminthological Abstracts 35 : 207-224.

Wright, C.A., 1971. Flukes and Snails George Allen and Unwin Ltd., London. 168pp.

# CHAPTER 7

# STUDIES ON THE ABILITY OF BULINUS SENEGALENSIS CARRYING IMMATURE SCHISTOSOMA HAEMATOBIUM INFECTIONS TO SURVIVE PERIODS OF EXPERIMENTAL DESICCATION

## INTRODUCTION

The finding of Bulinus senegalensis in the area of the South Chad Irrigation Project (S.C.I.P.) Borno State, Nigeria, constituted the first confirmed record of this species outside the Senegambian region (Betterton, Fryer and Wright, 1983). Smithers (1956) demonstrated the involvement of this species in the transmission of Schistosoma haematobium in the Gambia, and suspected that the temporary rain filled pools which form this species' typical habitat may well have been overlooked by workers looking for transmission sites in other areas. Wright (1959) reviewed the available information on the distribution of this species, and similarly suspected that it occurred throughout the western Sahel region.

Early accounts of B. senegalensis emphasised the importance of laterite substrates in its distribution. More recently populations have been found in habitats on other substrates (Goll, 1981), and the typical habitats in the S.C.I.P. area are on clay or sandy clay (Betterton et al., 1983). In previous reports on the distribution of the species in Senegambia, snails were seen to be restricted to temporary pools. Furthermore B. senegalensis appears to have an

intrinsic requirement for aestivation. Even where populations were found in habitats where water levels were artificially maintained throughout the year, such as a pool formed by overspill fom a borehole and irrigation drains within S.C.I.P, B. senegalensis populations disappeared soon after the rains ended (Betterton et al., 1983).

The most probable stimulus for aestivation in natural habitats is an increase in ionic concentrations resulting from evaporation of the water body. Observations on natural populations of Bulinus species indicate that snails show a response to falling water levels long before the severe contraction of water bodies (e.g. Barlow, 1933; Webbe, 1962; Goll and Wilkins, 1984), leaving aestivating snails stranded around the periphery of pools. In the case of B. senegalensis in S.C.I.P. one possible stimulus for this aestivatory behaviour is the marked drop in water temperatures at the end of the rains (Betterton et al., 1983). When laboratory cultures of this species were first set up at maintenance temperatures of 26°C, considerable problems were experienced with a large proportion of snails persistently crawling out of the water (Chapter 2). Raising the temperature to 30°C reduced this problem considerably, except in the case of cultures that were allowed to become overcrowded.

The importance of the ability of molluscan intermediate hosts to survive periods of drought in the epidemiology of schistosomiasis and the development of control stategies has long been recognised. Blacklock and Thompson (1924) in a

series of simple desiccation experiments showed that B. physopsis c.f. globosa could survive up to a fortnight on slowly drying mud. Barlow (1933, 1935) in a series of extensive studies on the survival of schistosome intermediate hosts over periods of "winter closure" of irrigation channels in Egypt, found that a high proportion of snails could survive the period of desiccation. Both these and many later workers recognised that the practice of diverting or cutting off water from infested channels would not ensure the destruction of all host snails.

Various field observations have indicated that schistosome infections may be carried through periods of drought in aestivating snails (e.g. Barlow, 1935; Webbe and Msangi, 1958; Webbe, 1962). Gordon Davey and Peaston (1934) showed that Planorbis pfeifferi carrying a mature S. mansoni infection could survive 66 hours out of water in the laboratory, and still shed cercariae when returned to water. Other laboratory studies indicate that the stage of infection is of importance, with snails harbouring immature infections being more likely to survive periods of desiccation (e.g. Cridland, 1957; work reviewed by Barbosa and Olivier, 1958; Hira, 1968).

The importance of B. senegalensis in the transmission of S. haematobium in the Gambia has been well established (e.g. Smithers, 1956). Populations from S.C.I.P. have proven to be susceptible to infection by local strains of the parasite (Chapter 4), and its involvement in transmission is strongly suspected. In many villages the temporary rain-filled pools

that form the main habitats for this species are the major source of water contact. Since these pools are dry for several months of the year, the ability of B. senegalensis to survive the dry season, still carrying schistosome infections, is of considerable significance in the epidemiology of the disease.

Goll and Wilkins (1984) failed to find patent schistosome infections in B. senegalensis collected during the first two months after pools refilled, supporting Smithers' (1956) view that infections do not survive the period of aestivation. As part of a wider study on the epidemiology of S. haematobium in the S.C.I.P. area, a series of experiments was performed to test the ability of B. senegalensis with immature infections to survive periods of desiccation.

## MATERIALS AND METHODS

All individual B. senegalensis used in the present study were laboratory bred. Details of the routine culture conditions, experimental exposure of snails to S. haematobium miracidia and subsequent detection of patent infections are described in Chapter 2.

Aestivation slopes were produced by pouring a layer of saturated, sterilized alluvial mud (approx. 20mm deep) into "Lunch Pack" containers (175 X 115 X 41mm) (as described in Chapter 2). These were then allowed to stand for 24 hours, resting at a shallow angle so that the mud dried out slightly to form a stable slope.

Snails that were to be subjected to a period of

experimental desiccation were placed on aestivation slopes to which water had been added gradually to reach half way up the mud slope. These containers were then covered with coarse gauze to prevent the escape of snails, but to allow evaporation. After a period of 6 hours most of the water was removed with a pipette, and any snails that had crawled up the sides of the container were replaced on the wet mud. All free water was removed after 24 hours, and the slope allowed to dry completely.

Snails were revived from aestivation after the appropriate period by reflooding the slopes with water, and allowing them to stand undisturbed. Snails that emerged were transferred to a clean mud free container within 24 hours of reflooding. After this time the mud was carefully searched for further survivors, and any empty shells.

For each experiment, all snails were exposed to miracidia of S. haematobium from the same source at the same time and maintained identically until the start of the period of experimental desiccation. Unaestivated control snails were then kept in the same conditions while those being subjected to a period of drying were transferred to aestivation slopes. Control snails were screened for cercarial production from the 20th day after exposure at 2 to 4 day intervals as described in Chapter 2. Snails that had survived aestivation were similarly screened from 2 to 4 days after revival, allowing time for them to recover and feed before subjecting them to the stresses of handling and isolation.

**Experiment I**

Twenty-five B. senegalensis ranging in shell height from 2.0 to 5.0mm were each exposed to five miracidia from the 'rohlfsi' strain of S. haematobium (Chapter 5). These snails were maintained at 26±1°C to encourage individuals to crawl out of the water. On the 15th day following exposure, snails were measured, and all those that had left the water were transferred to an aestivation slope for a period of 28 days desiccation. The remaining snails were replaced in the culture container and kept as unaestivated controls.

**Experiment II**

One hundred B. senegalensis 2.0 to 6.0 mm in shell height were individually exposed to five miracidia from the 'globosus' strain of S. haematobium. These were maintained at 26±1°C, 25 snails to a container. On the 15th day following exposure, surviving snails were divided into two groups of similar size distribution. One group was transferred to an aestivation slope for 56 days desiccation, while the other was kept as unaestivated controls.

**Experiment III**

One hundred B. senegalensis 2.0 to 6.0mm in shell height were each exposed to five miracidia from the 'globosus' strain of S. haematobium. These snails were then kept 25 to a container at the higher temperature of 30±1°C. On the 15th day following exposure snails were divided as in Experiment II, with one group transferred to an aestivation slope for 56 days, and the other maintained at 30±1°C as controls.

**Experiment IV**

100 B. senegalenis 2.0 to 6.0mm in shell height were each exposed to five miracidia from the 'globosus' strain of S. haematobium. These snails were then placed 50 to a container to encourage individuals to crawl out of the water, being kept at 30±1°C. On the seventh day following exposure all snails were measured, and those that had left the water were transferred to aestivation slopes for 56 days. The remaining snails were kept as unaestivated controls.

**RESULTS**

When B. senegalensis were placed on aestivation slopes the majority crawled over the wet surface of the mud, eventually coming to rest with their shell apertures in close contact with the substrate. The simple act of crawling on the semi-liquid mud left snails partially buried, with many having only the top whorls of their shell visible above the surface. A few snails became buried in the mud, but these had apparently crawled to the edge of the container and pulled themselves down the side, using the plastic as a firm substrate. Other individuals crawled up the sides of the container, but when replaced on the mud, all crawled around and came to rest on, or just under, the surface.

When aestivation slopes were reflooded, snails that had only been attached loosely to the mud rose to the surface of the water, being buoyant due to air trapped in the shells. If alive, these individuals soon extended their head-foot and

became active. Snails fixed more firmly to the mud took longer to emerge, either floating away as the mud softened, or, having revived, freeing themselves from the substrate.

In the majority of cases, all survivors had emerged and were active within 3 to 6 hours of reflooding. Four individuals in Experiment II were not recovered until the mud was searched after 24 hours. All four died soon afterwards, and it is assumed that they had been trapped beneath the mud, unable to free themselves.

The results of all four experiments are summarised in Table 7.1, which gives the survival and infection rates of all aestivated and control snails.

**Experiment I**

Fig. 7.1 presents the size frequency distribution of the 25 B. senegalensis at the time of exposure to parasite miracidia, and of survivors on day 15. Three snails had died during this period. 15 snails were still in the water, feeding and active. These were kept as unaestivated controls. Seven individuals (all 3.5 to 4.0mm in height) had crawled out of the water, and were transferred to an aestivation slope for 28 days.

Five of the 15 (33.3%) control snails shed cercariae. All seven aestivated snails survived the period of desiccation, but one of these was damaged and survived only a few hours after emerging. Two of the six snails remaining (33.3%) developed patent infections.

Details of the precercarial periods and survival of

## TABLE 7.1

## Summary of results from aestivation experiments

### A: Aestivated snails

| Expt. | Number aestivated | Stage of infection (days) | Days dry | Survivors to: Aestivation; n (%) | Screening n (%) | Patent Infections n (%) |
|---|---|---|---|---|---|---|
| I | 7 | 15 | 28 | 7(100) | 6(85.7) | 2(33.3) |
| II | 48 | 15 | 56 | 20(41.7) | 16(80.0) | 2(12.5) |
| III | 45 | 15 | 56 | 11(24.4) | 11(100) | 0 |
| IV | 32 | 7 | 56 | 9(25.0) | 9(100) | 1(11.1) |

### B: Controls

| Expt. | n | Survived to first Screening n (%) | Patent Infections n (%) |
|---|---|---|---|
| I | 16 | 15 (93.7) | 5 (33.3) |
| II | 49 | 46 (93.9) | 12 (26.1) |
| III | 47 | 45 (95.7) | 6 (13.3) |
| IV | 63 | 50 (79.4) | 20 (40.0) |

FIGURE 7.1

Size frequency distributions of B. senegalensis in Experiment I at the start of the experiment and on day 15

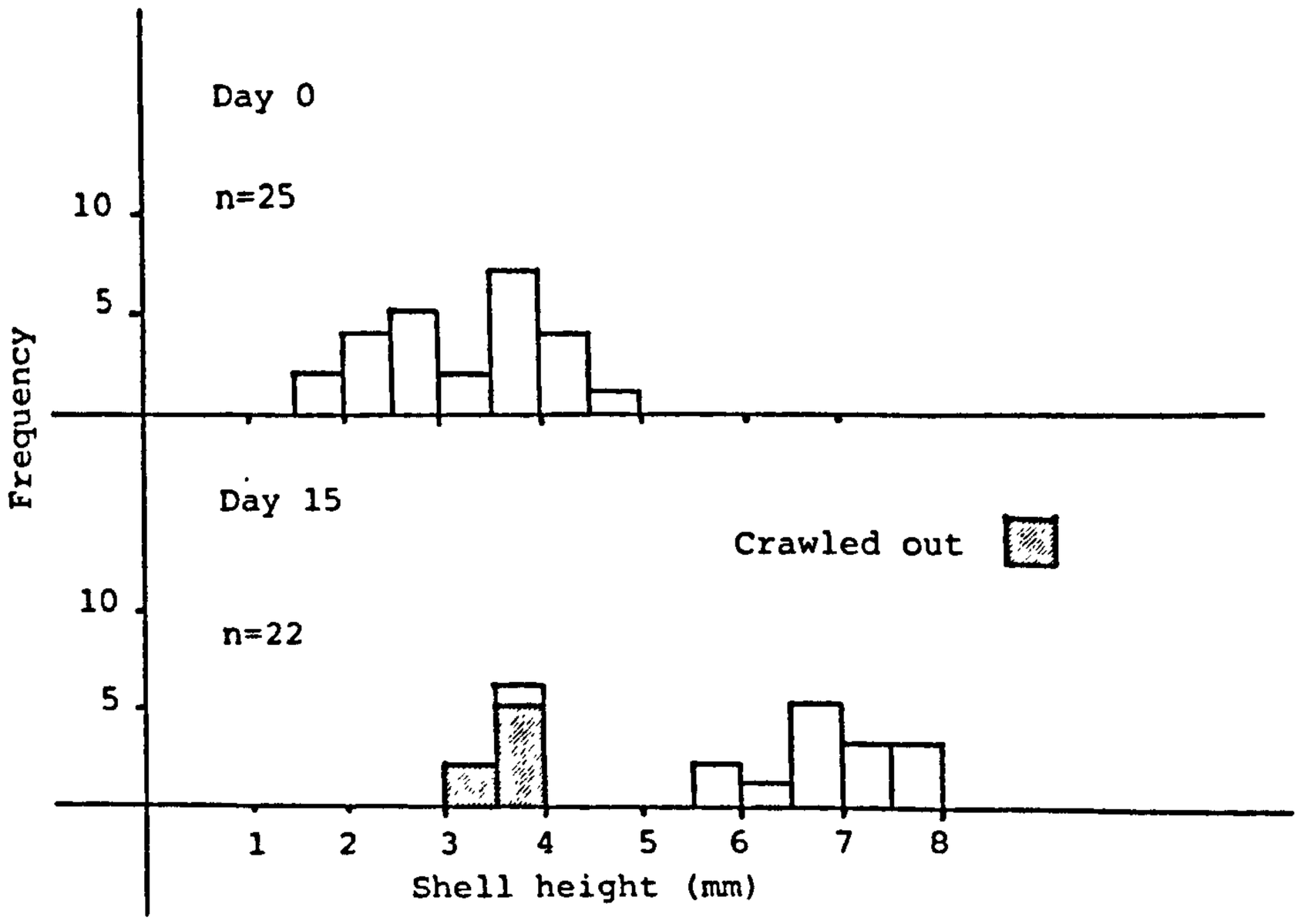



FIGURE 7.2

Size frequency distributions of B. senegalensis in Experiment II on day 15, showing those snails that survived 56 days of aestivation

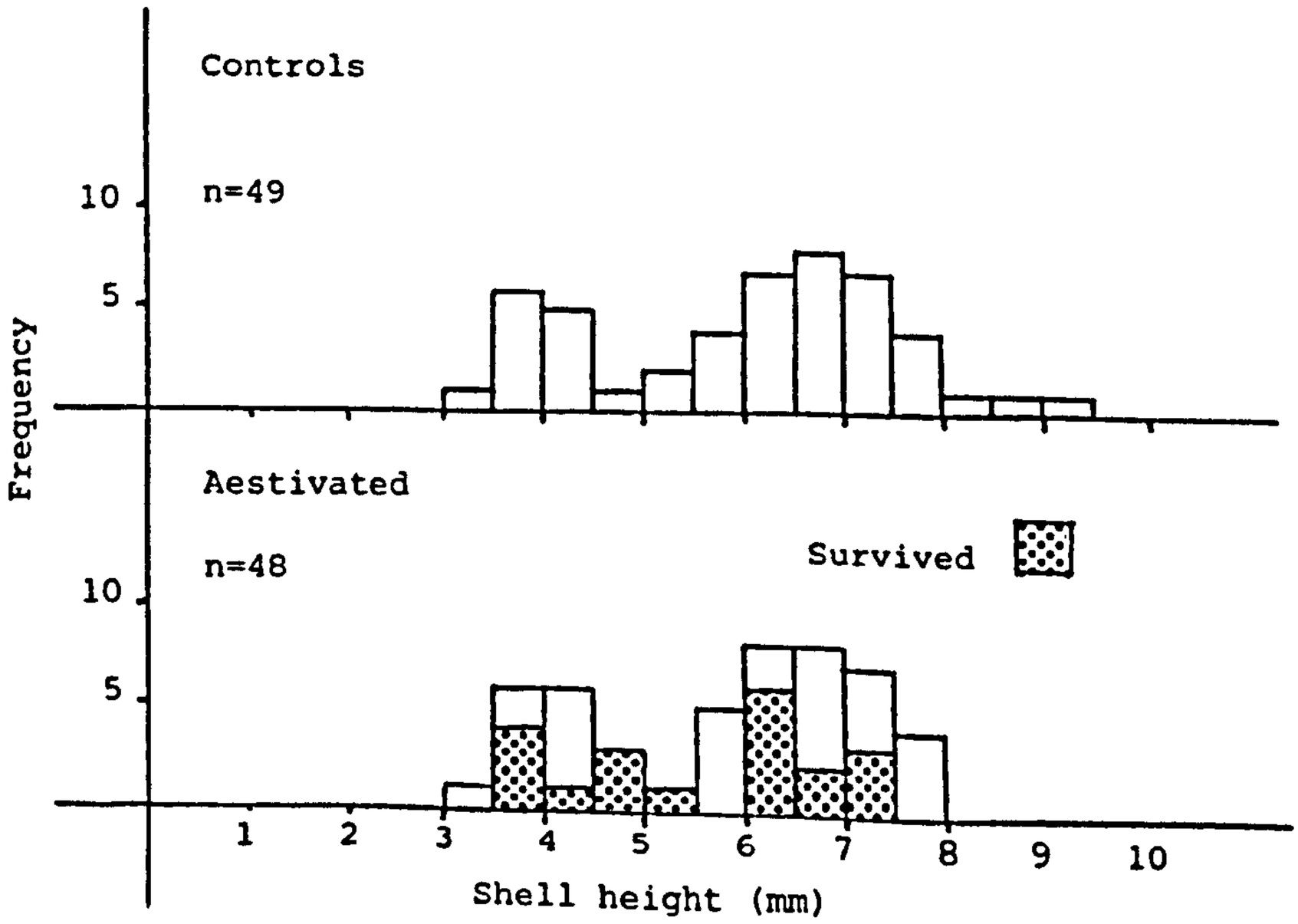


FIGURE 7.3

Size frequency distributions of B. senegalensis in Experiment III on day 15 showing those snails that survived 56 days of aestivation

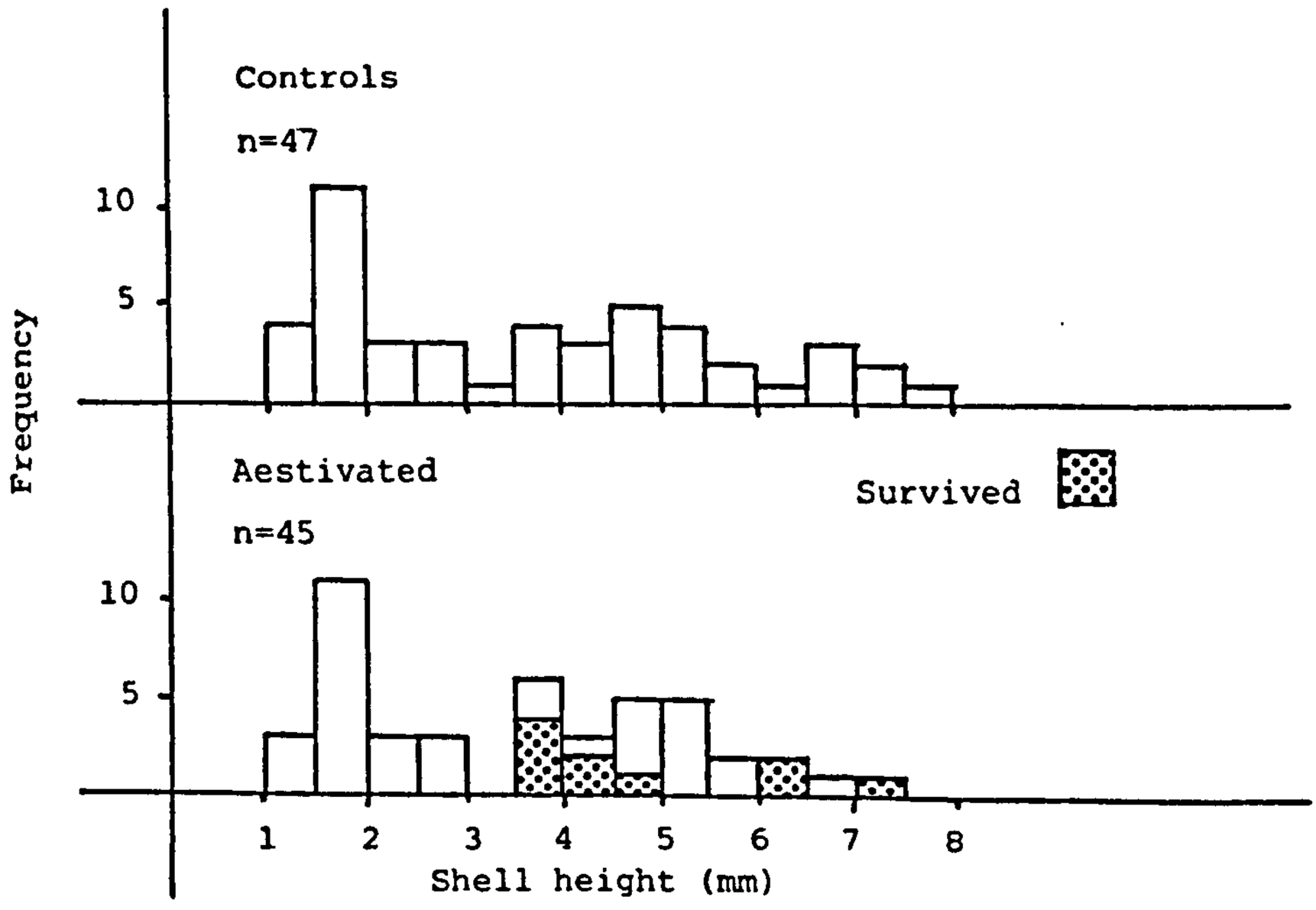
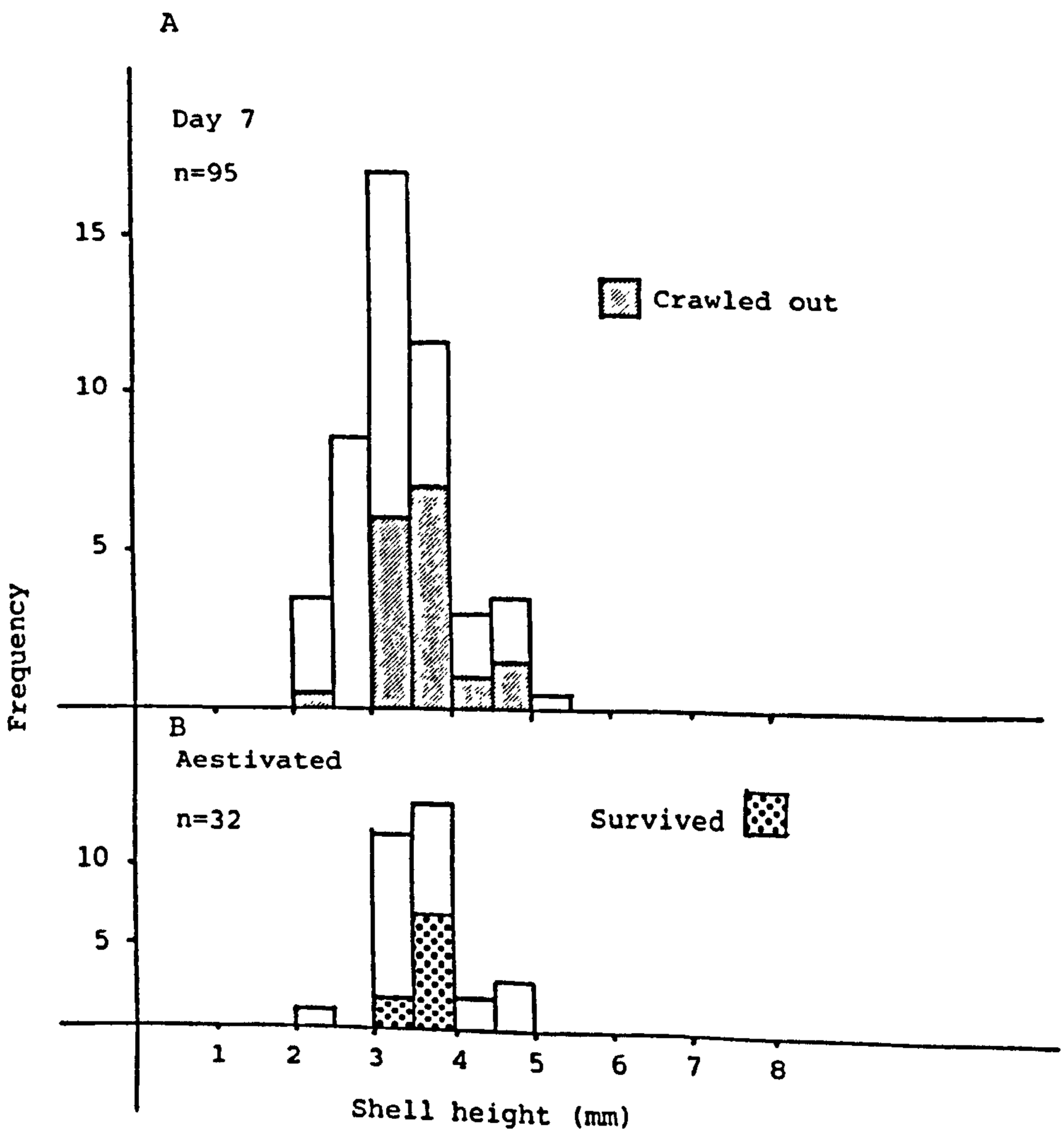



FIGURE 7.4

Size frequency distributions of B. senegalensis in Experiment IV. A: Day 7 B: Aestivated snails showing those that survived 56 days of aestivation



infected snails are given in Table 7.2.

**Experiment II**

Of the 100 B. senegalensis exposed to miracidia, 97 survived to day 15. These snails were divided into two groups with similar size distributions (Fig. 7.2), with 49 maintained as unaestivated controls, and 48 being transferred to an aestivation slope for 56 days.

Of the 46 control snails surviving to the first screening for the presence of cercariae, 12 developed patent infections (26.1%).

Twenty snails emerged alive from the aestivation slope (41.7%). Their size frequency distribution is also shown in Fig. 7.2. Four of these were only found when the mud was searched 24 hours after reflooding. They appeared to have been trapped beneath the mud, were very weak and lethargic when recovered and died within 24 hours.

Two of the surviving aestivated snails developed patent infections (12.5%). The precercarial periods and survival times of these and infected control snails are shown in Table 7.3.

**Experiment III**

Of the 100 snails exposed to miracidia, 92 survived to day 15. These snails were divided into two groups of similar size frequency (Fig. 7.3) with 47 kept as controls and 45 transferred for 56 days of desiccation.

Of the 45 control snails surviving for first screening, 6 (13.3%) developed patent infections.

## TABLE 7.2

**Details of parasite development and host survival of *B. senegalensis* infected in Experiment I**

| | Precercarial Period* (days) Min.(-A) | Max.(-A) | Days Patent | Survival from exposure (days) (-A) |
|---|---|---|---|---|
| **CONTROLS** | 25 | 25 | 71 | 96 |
| | 28 | 28 | 34 | 62 |
| | 39 | 42 | 56 | 98 |
| | 74 | 80 | 68 | 148 |
| | 74 | 80 | 32 | 112 |
| **AESTIVATED** | 74 (59) | 78 (63) | 85 | 163 (148) |
| | 85 (70) | 87 (72) | 78 | 165 (150) |

* Precercarial period: Min.= day of last negative screening + 1
Max.= day first cercariae detected
(-A)= - days aestivating

## TABLE 7.3

**Details of parasite development and host survival of *B. senegalensis* infected in Experiment II**

| | Precercarial Period* (days) Min.(-A) | Max.(-A) | Days Patent | Survival from exposure (days) (-A) |
|---|---|---|---|---|
| **CONTROLS** | 33 | 35 | 47 | 82 |
| | 41 | 45 | 36 | 81 |
| | 41 | 45 | 73 | 118 |
| | 41 | 45 | 45 | 90 |
| | 41 | 45 | 50 | 95 |
| | 41 | 45 | 35 | 80 |
| | 41 | 45 | 36 | 81 |
| | 46 | 47 | 34 | 81 |
| | 46 | 47 | 46 | 93 |
| | 46 | 47 | 37 | 84 |
| | 46 | 47 | 29 | 76 |
| | 49 | 55 | 34 | 89 |
| **AESTIVATED** | 90 (34) | 95 (39) | 29 | 124 (68) |
| | 106 (50) | 109 (53) | 10 | 119 (63) |

* Precercarial period: Min.= day of last negative screening + 1
Max.= day first cercariae detected
(-A)= - days aestivating

TABLE 7.4

Details of parasite development and host survival of *B. senegalensis* infected in Experiment IV

| | Precercarial Period* (days) Min.(-A) | Max.(-A) | Days Patent | Survival from exposure (days) (-A) |
|---|---|---|---|---|
| **CONTROLS** | 30 | 31 | 77 | 108 |
| | 30 | 31 | 42 | 73 |
| | 30 | 31 | 56 | 86 |
| | 32 | 33 | 54 | 87 |
| | 32 | 33 | 20 | 53 |
| | 34 | 35 | 52 | 87 |
| | 34 | 35 | 52 | 87 |
| | 36 | 37 | 99 | 136 |
| | 38 | 39 | 55 | 94 |
| | 38 | 39 | 69 | 108 |
| | 43 | 45 | 54 | 99 |
| | 46 | 49 | 80 | 129 |
| | 46 | 49 | 31 | 80 |
| | 50 | 51 | 78 | 129 |
| | 50 | 51 | 78 | 129 |
| | 50 | 51 | 43 | 94 |
| | 52 | 53 | 47 | 100 |
| | 61 | 67 | 108 | 175 |
| | 67 | 74 | 76 | 150 |
| | 77 | 85 | 44 | 129 |
| **AESTIVATED** | 89 (33) | 90 (34) | 68 | 158 (102) |

* Precercarial period: Min.= day of last negative screening + 1
Max.= day first cercariae detected

(-A)= - days aestivating

## TABLE 7.4

**Details of parasite development and host survival of *B. senegalensis* infected in Experiment IV**

| | Precercarial Period* (days) Min.(-A) | Max.(-A) | Days Patent | Survival from exposure (days) (-A) |
|---|---|---|---|---|
| **CONTROLS** | 30 | 31 | 77 | 108 |
| | 30 | 31 | 42 | 73 |
| | 30 | 31 | 56 | 86 |
| | 32 | 33 | 54 | 87 |
| | 32 | 33 | 20 | 53 |
| | 34 | 35 | 52 | 87 |
| | 34 | 35 | 52 | 87 |
| | 36 | 37 | 99 | 136 |
| | 38 | 39 | 55 | 94 |
| | 38 | 39 | 69 | 108 |
| | 43 | 45 | 54 | 99 |
| | 46 | 49 | 80 | 129 |
| | 46 | 49 | 31 | 80 |
| | 50 | 51 | 78 | 129 |
| | 50 | 51 | 78 | 129 |
| | 50 | 51 | 43 | 94 |
| | 52 | 53 | 47 | 100 |
| | 61 | 67 | 108 | 175 |
| | 67 | 74 | 76 | 150 |
| | 77 | 85 | 44 | 129 |
| **AESTIVATED** | 89 (33) | 90 (34) | 68 | 158 (102) |

* Precercarial period: Min.= day of last negative screening + 1
Max.= day first cercariae detected

(-A)= - days aestivating

Eleven of the aestivated snails emerged when the slope was reflooded, with a size frequency distribution as shown in Fig. 7.3. None of these snails developed patent infections.

**Experiment IV**

Of the 100 B. senegalensis exposed, 97 survived to day seven when kept at the high population density. Of these, 32 had left the water and were transferred for aestivation. The size frequency of all snails is shown in Fig 7.4.

Fifty of the control snails had survived to first screening for cercariae, 20 of these susequently developed patent infections (40%).

Nine of the aestivated snails survived with a size frequency distribution as shown in Fig. 7.4. Only one of these developed a patent infection (11.1%). The precercarial periods and survival times of this snail and the infected controls are shown in Table 7.4.

**DISCUSSION**

Mud slopes were used in the present study to provide snails with conditions for aestivation as close to nature as possible. The random crawling of the majority of B. senegalensis over the wet mud resulted in many of them becoming partially covered, with their shell apertures sealed by the drying mud. Similar behaviour has been observed in both the field and laboratory in a variety of planorbid snails by other workers (e.g. Barlow, 1933; Odei, 1966; Hira, 1968). Shiff (1964) observed that only those B. globosus that had their

shells well sealed with dry mud escaped predation by ants when they were stranded during the dry season.

Although active burrowing behaviour has been reported in B. truncatus (Chu, Bijan and Massoud, 1967) and B. tropicus (Stiglingh, 1971) the only snails that found their way beneath the mud surface in the present study had crawled down the edge of the container, using the firm plastic to pull themselves through the mud. Cridland (1967) suggested that snails in natural habitats may use plant roots in a similar manner. It is likely that the four snails that became trapped in the mud in Experiment II had pulled themselves below the surface in this way. Chu et al. (1967) observed that on reflooding snails that had burrowed, forming tunnels in the drying mud, simply floated to the surface. It is probable that escape from beneath the surface of mud depends to a great extent on the physical properties of the substrate.

Goll and Wilkins (1984) found that all survivors from naturally aestivating populations of B. senegalensis were immature and remarkably constant in size, with a mean shell height of just over 3.0mm. Observations on laboratory cultures of this species in the present studies indicate that at low temperatures (26°C) it is almost exclusively immature individuals that leave the water spontaneously, as occurred in Experiment I. All seven snails in this case survived the short period of 28 days desiccation. In Experiment II, where snails also kept at 26°C were subjected to a longer period of desiccation, whether or not they had previously left the water,

individuals ranging from 4.0 to 7.5mm aestivated sucessfully. In Experiment III, conducted in the same manner but at the higher temperature (30°C), again snails covering a wide size range survived. These results thus indicate that it is not solely immature snails that are capable of surviving periods of drought.

Although the proportion of B. senegalensis leaving the water in cultures was greatly reduced by raising the temperature to 30°C, under conditions of high population density a significant number of snails crawled out. In Experiment IV individuals showing this behaviour covered the whole size range of the population. When subjected to desiccation, however, mortality was high and the only surviving snails were 3.5 to 4.0mm in height.

The above observations are based on very limited data, and further experiments with more replicates are required before any firm conclusions can be drawn. The results obtained do, however, support the hypothesis that at low temperatures (as occurs at the end of the rainy season in natural habitats within S.C.I.P. (Betterton et al., 1983)) immature B. senegalensis actively leave the water body and become stranded on the drying mud in a favourable position for aestivation. Goll and Wilkins (1984), in addition to finding only immature snails surviving aestivation, also observed that no snails were found at the start of the rains when only the centre portion of pools was flooded. They suggested that the site of aestivation was around the periphery, as had been suggested by Webbe (1962)

for populations of B. nasutus in small pools in Tanzania. There are considerable adaptive advantages associated with aestivating high up the shoreline of temporary habitats. Snails situated away from the centre of pools would remain dormant during early, unreliable rains, only emerging when pools had become well established. In addition, snails that crawl out of the water while levels are still high are more likely to encounter rooted vegetation than those left lower down the shoreline as the habitat contracts. Annecke and Peacock (1951) recovered aestivating Bulinus species from dry tufts of grass and between plant roots as long as 18 months after pools had dried. Barbosa and Olivier (1958), reviewing a series of studies on Biomphalaria species in Brazil, reported that survival through periods of drought was improved when snails were protected by vegetation. This has also been shown by Cridland (1967) and Hira (1968).

Although Smithers (1956) and Goll and Wilkins (1984) concluded that S. haematobium infections did not survive in naturally aestivating populations of B. senegalensis, other authors have shown that snails with immature schistosome infections can survive periods of drought. In field studies Barlow (1935) recovered molluscan hosts carrying S. mansoni and S. haematobium infections from the dry beds of canals that had been closed for a month. Webbe (1962) found that natural populations of B. nasutus productus had carried immature S. haematobium infections over 98 days of desiccation, and considered that this was probably a common occurrence. Webbe

and Msangi (1958) collected B. nasutus shedding "mammalian type cercariae" 21 days after a pool, that had been dry for 5 months, refilled. Since this was shorter than the normal development time for laboratory infections, it was thought possible that these were immature infections carried over the period of drought.

Laboratory investigations have also shown that both molluscan host and schistosome can survive short periods of desiccation. Gordon et al. (1934) showed that Planorbis pfeifferi infected with S. mansoni survived for 66 hours out of water, shedding cercariae again when reimmersed. Hira (1968) found that B. globosus with mature S. haematobium infections only survived 11 to 12 days desiccation, a much shorter period than uninfected individuals. In the same study, snails with immature infections (2 to 9 days) could survive 30 days, carrying the infection through this period of aestivation. Other workers have found that snails harbouring mature infections do not survive periods of desiccation (e.g. Cridland, 1957) although individuals with immature parasites are capable of doing so (Barbosa and Olivier, 1958).

Results from the present investigation have shown that B. senegalensis carrying S. haematobium infections 7 to 15 days old can survive periods of enforced aestivation in the laboratory. This was the case with individuals infected with both 'rohlfsi' and 'globosus' strains of parasite. The maximum period of desiccation involved was only 56 days, considerably shorter than would be experienced by populations in their

natural habitats, which may be dry for 6 to 7 months. This period of 56 days is, however, longer than has been reported in previous studies involving infected Bulinus species, and a significant proportion of snails aestivated successfully (32%).

It seems probable that mortality during aestivation would be higher in a section of a population carrying a developing schistosome infection than in unparasitized snails. In Experiment I, where all snails survived the relatively short period of aestivation, infection rates were identical in aestivated and control snails. In all three subsequent experiments the proportion of surviving aestivated snails that developed patent infections was lower than in controls. Although the results from each individual experiment, with the very small number of snails involved, show no significant difference, when the data from all three is combined the infection rate in the control group is significantly higher than in the surviving aestivated snails ($X^2=4.8576$ $p<0.05$), indicating differential mortality.

Hira (1968) found that immature S. haematobium infections were dormant over at least part of the period of experimental aestivation of B. nasutus. In some cases the sum of the predesiccation period and the time between returning to water and production of cercariae was less than the normal developmental time for the parasite. This indicated that there was at least some parasite development during aestivation.

Precercarial periods of S. haematobium in B. senegalensis are extremely variable compared to those seen in other Bulinus

species carrying the same parasite strain (Chapter 4). This is thought to be due to inhibition of parasite development in individuals that leave the water for various periods during this phase. This characteristic of the host-parasite relationship makes any comparison of development rates in aestivated and control animals extremely difficult, especially given the small number of B. senegalensis that both survived the period of desiccation, and developed a patent infection. All that can be concluded from the present study is that the total time from exposure to miracidia to first cercarial production, minus the period of desiccation, falls within the same range as the observed precercarial periods of controls (Tables 7.2, 7.3 and 7.4).

The high mortality rate of snails during aestivation, combined with the low infection rates of S. haematobium in exposed B. senegalensis necessitates the use of extremely large numbers of snails in order to obtain sufficient infected aestivated snails for a detailed study. This investigation was part of a much wider study, and the supply of both molluscs and parasite was limited. Further large scale experiments are required to follow up the findings of the experiments presented here.

The possibility of S. haematobium infections surviving in aestivating B. senegalensis would greatly extend the period when cercariae are present in transmission sites where this is the only intermediate host. Field studies have shown that this bulinid is only active in village pools within the S.C.I.P.

area from mid-July to late-September (Betterton et al., 1983). With a probable minimum precercarial period of 3 weeks (Chapter 4), if no parasites had survived the dry season cercariae would only be present for about 7 weeks of each year. The potential extension of this period by up to 3 weeks (if fully mature infections had survived) would be of considerable significance in the epidemiology of the disease.

Theoretically, if there is no S. haematobium survival in aestivating B. senegalensis, in areas where this is the only bulinid present drug treatment of all infected members of the local community during the dry season, and subsequent screening and treatment of all persons entering the area, could halt transmission. However, the existence of infections in snails emerging from aestivation would provide a source of reinfection. Any control programmes in these areas must therefore take the possibility of infections being carried over periods of drought in intermediate hosts into consideration. Further investigations are therefore required to determine the survival of both host and parasite, with infections at various stages of development, over long periods of aestivation. Data must also be obtained on the effects of the host's aestivation on the development of the parasite.

**REFERENCES**


Annecke, S. and Peacock, P.N.B., 1951. Bilharziasis in the Transvaal. South African Medical Journal 25 : 689-692.

Barbosa, F.S. and Olivier, L., 1958. Studies on the snail vectors of Bilharziasis mansoni in North-eastern Brazil. Bulletin of the World Health Organisation 18 : 895-908.

Barlow, C.H., 1933. The effect of the "winter rotation" of water upon snails involved in the spread of schistosomiasis in Egypt, 1930-1931 and 1931-1932. American Journal of Hygiene 17 : 724-742.

Barlow, C.H., 1935. Further studies on revival, after drying, of the snail hosts of the human schistosomes of Egypt. American Journal of Hygiene 22 : 376-391.

Betterton, C., Fryer, S.E. and Wright, C.A., 1983. Bulinus senegalensis (Mollusca: Planorbidae) in northern Nigeria. Annals of Tropical Medicine and Parasitology 77 : 143-149.

Blacklock, D.B. and Thompson, M.G., 1924. Human schistosomiasis due to S. haematobium in Sierra Leone. Annals of Tropical Medicine and Parasitology 18 : 211-237.

Chu, K.Y., Bijan, H. and Massoud, J., 1967. The ability of Bulinus truncatus, Biomphalaria alexandrina and Lymnaea gedrosiana to survive out of water in the laboratory. Annals of Tropical Medicine and Parasitology 61 : 1-5.

Cridland, C.C., 1957. Ecological factors affecting the numbers of snails in temporary bodies of water. Journal of Tropical Medicine and Hygiene 60 : 287-293.

Cridland, C.C., 1967. Resistance of Bulinus (Physopsis) globosus, Bulinus (Ph) africanus, Biomphalaria pfeifferi and Lymnaea natalensis to experimental desiccation. Bulletin of the World Health Organisation 36 : 507-513.

Goll, P.H., 1981. Mixed populations of Bulinus senegalensis and B. forskali (Mollusca: Planorbidae) in the Gambia. Transactions of the Royal Society of Tropical Medicine and Hygiene 35 : 576-578.

Goll, P.H. and Wilkins, H.A., 1984. Field studies on Bulinus senegalensis Muller and the transmission of Schistosoma haematobium infection in a Gambian community. Tropinmedizin und Parasitologie 35 : 29-36.

Gordon, R.M., Davey, T.H. and Peaston, H., 1934. The transmission of human bilharziasis in Sierra Leone, with an account of the life-cycle of the schistosomes concerned, S. mansoni and S. haematobium. Annals of Tropical Medicine and Parasitology 28 : 323-418.

Hira, P.R., 1968. Studies on the capability of the snail transmitting urinary schistosomiasis in Western Nigeria to survive dry conditions. West African Medical Journal 17 : 153-160.

Odei, M.A., 1966. The behaviour and aestivating ability of Bulinus (Physopsis) globosus Morelet (Mollusca, Bulininae) under drought conditions. Ghana Journal of Science 7 :50-54.

Shiff, C.J., 1964. Studies on Bulinus (Physopsis) globosus in Rhodesia. III. Bionomics of a natural population existing in a temporary habitat. Annals of Tropical Medicine and Parasitology 58 : 240-255.

Smithers, S.R., 1956. On the ecology of schistosome vectors in the Gambia, with evidence of their role in transmission. Transactions of the Royal Society of Tropical Medicine and Hygiene 50 : 354-365.

Stiglingh, I., 1971. Bulinus tropicus (Krauss) and digging behaviour. Limnological Society of South Africa Newsletter 16 : 42-46.

Webbe, G., 1962. The transmission of Schistosoma haematobium in an area of Lake Province, Tanganyika. Bulletin of the World Health Organisation 27 : 59-85.

Webbe, G. and Msangi, A.S., 1958. Observations on three species of Bulinus on the East coast of Africa. Annals of Tropical Medicine and Parasitology 52 : 302-314.

Wright, C.A., 1959. A note on the distribution of Bulinus senegalensis. West African Medical Journal 8 : 142-148.

# CHAPTER 8

# STUDIES ON THE MORPHOLOGY AND CROSSBREEDING ABILITY OF TWO POPULATIONS OF *BULINUS GLOBOSUS* FROM NORTHERN NIGERIA

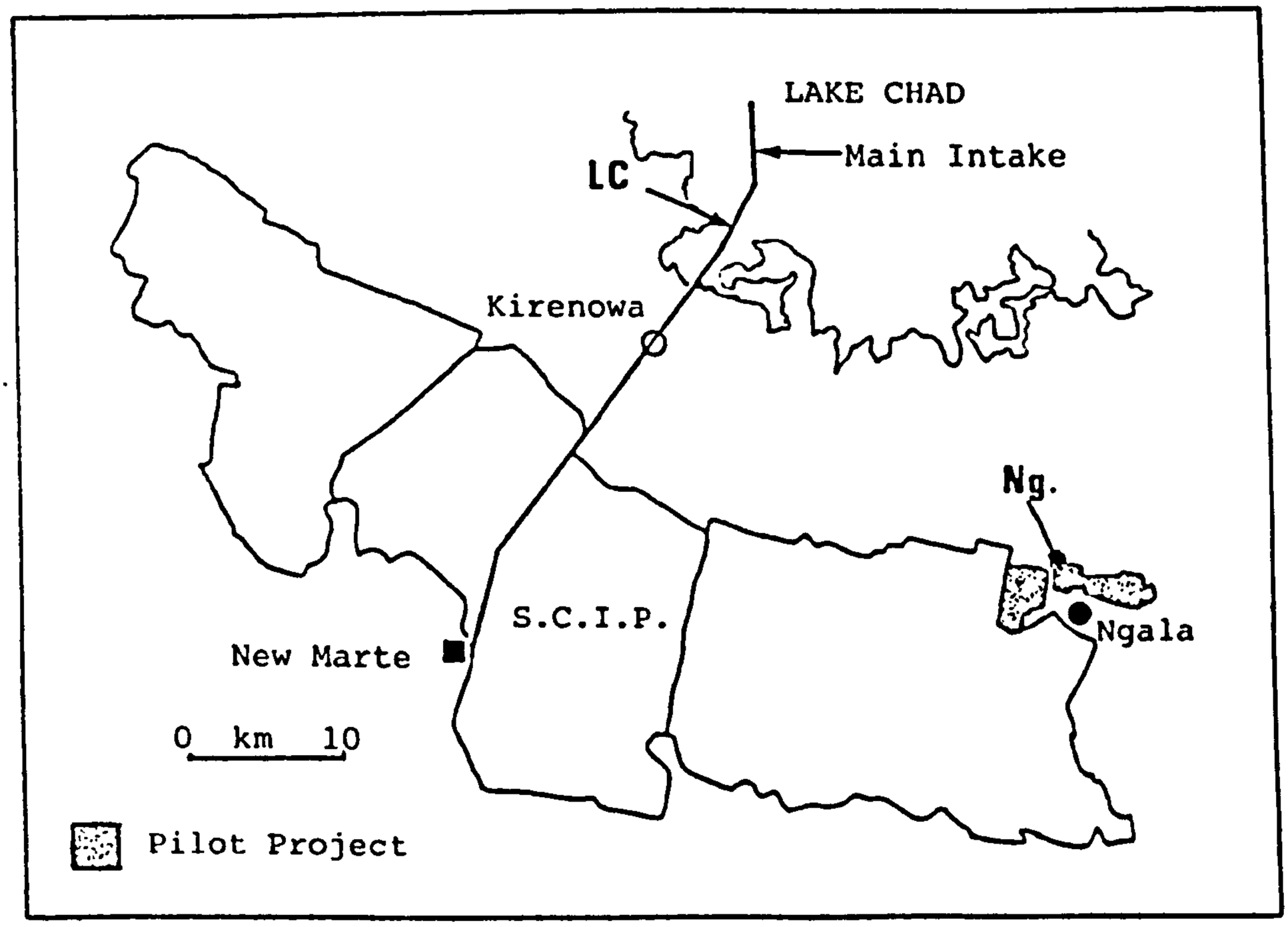
## INTRODUCTION

In initial surveys of potential intermediate hosts of schistosomes in the area of the South Chad Irrigation Project (S.C.I.P.), northern Nigeria, a member of the *Bulinus africanus* species group found in the main intake channel was reported as *B. umbilicatus* (Betterton, Fryer and Wright, 1983). This identification was based on the examination of a few shells by the British Museum (Natural History). Further collection of material revealed a second population of *africanus* group snails within the small Pilot Project at Ngala, some 40km from the S.C.I.P. intake channel (see Fig 8.1). Subsequent examination of live specimens by the British Museum led to the conclusion that both populations were *B. globosus* (Betterton, 1984), a species that had been reported from within S.C.I.P. in earlier surveys (Menu, Dove, Noamesi and Dazo, 1973; Noamesi and Morcos, 1974; Odei, 1977).

Betterton (1984) dealt with these two populations separately, referring to the population from the main intake channel of S.C.I.P. (a continuation of Lake Chad) as *B. globosus* var. Lake Chad, and that from the Pilot Project as *B. globosus* var. Ngala. Field observations indicated marked differences between the two, the most notable of which was the

FIGURE 8.1

Location of collection sites of B. globosus populations 'Lake Chad' & 'Ngala'



complete lack of trematode infections in the Lake Chad population, despite their presence in other bulinids at the same location, whereas B. globosus Ngala was found to be infected with a variety of trematodes including Schistosoma haematobium. In subsequent laboratory infection experiments (Chapter 4) B. globosus Lake Chad proved refractory to infection with a local strain of S. haematobium that developed in the Ngala snails.

The basis of the classification of the genus Bulinus is a revision by Mandahl-Barth (1957, 1958) based on characters of both the shell and anatomy. Mandahl-Barth (1965), after examination of more material from all over Africa, considered that several of the specific and sub-specific characters he had used in his classification were less constant and therefore less reliable than had been originally assumed. Brown (1980) considered that few species are defined entirely satisfactorily, with reproductive barriers only rarely having been tested by experiment. B. globosus is the most widely distributed member of the africanus species group and it is well recognised that several local forms exist (e.g. Mandahl-Barth, 1965; Hira, 1968; Berrie, 1973; Coker and Kuma, 1974).

Analyses of enzyme groups by isoelectric focussing has shown variations between populations of B. globosus from various locations throughout their range (Wright and Rollinson, 1979). A similar analysis of enzymes in a very small sample of snails from Lake Chad and Ngala showed differences in the occurrence of enzyme types of three main enzyme groups: glucose

phosphate isomerase (GPI), acid phosphatase (AcP) and hydroxybutyrate dehydrogenase (HBDH), (Rollinson, pers. comm.). On setting up cultures of Bulinus species from S.C.I.P. at U.C.N.W., Bangor, differences were observed between the two B. globosus populations particularly in the relative mortality of newly hatched snails kept under identical culture conditions (see Chapter 2). Examination of embryonic shells by scanning electron microscopy revealed differences in the micro-ornamentation of individuals from the two populations (Lund, 1983). However, the number of shells examined was small and examination of further material would be required to confirm this observation.

The above differences between B. globosus populations from Lake Chad and Ngala, which had been observed both in the field and the laboratory, prompted a further comparative study looking at anatomical characteristics and utilizing the observed differences in enzyme patterns as genetic markers in crossbreeding experiments.

## MATERIALS AND METHODS

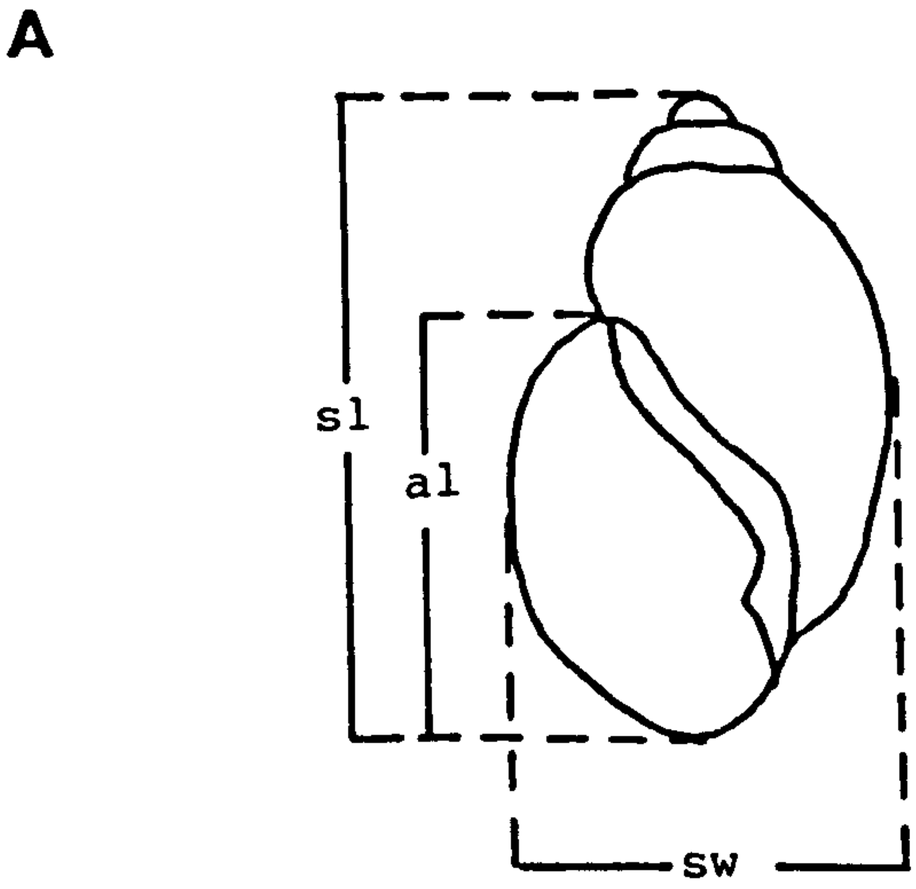
All the snails used in this study were bred and reared in the laboratory as described in Chapter 2.

### 1. Shell Measurements

Measurements were taken of each specimen's shell length (sl), shell width(sw) and aperture length (al) (Fig. 8.2a), to the nearest 0.1mm by means of vernier callipers.

FIGURE 8.2

Measurements taken of A: Shell and B: Copulatory organs in B. globosus
sl=shell length; sw=shell width; al=aperture length; psl=penis sheath length; psw=penis sheath width; ppl=preputium length; ppw =preputium width



**2. Examination of Radulae.**

Snails were relaxed by placing them in water with a few crystals of menthol floating on the surface, and leaving them in closed containers until there was no response to pinching the extended foot with forceps (Brown, 1980).

The whole buccal mass was dissected from relaxed snails and fixed in 2% glutaraldehyde. Specimens were then prepared for scanning electron microscopy by dehydration followed by critical point drying after which the buccal masses were stuck to aluminium stubs with the radulae uppermost. Finally the specimens were coated with gold using a sputter coater.

**3. Examination of Copulatory Organs.**

A high proportion of snails relaxed using menthol everted their male copulatory organs. Since the measurements required could not be made on such specimens, sagatal was utilized as the narcotic for all snails in this part of the study. Specimens were placed in a 0.08% solution of sagatal for 4-6 hours, until no response was made to the pinching of the extended foot with forceps.

The male copulatory organ was dissected from each relaxed snail without fixation, and gently straightened in a drop of glycerine on a glass microscope slide. After covering with a cover slip, drawings were made using a camera lucida attachment on a compound microscope, with as little delay as possible. Measurements were then taken from the drawing, taking into account any bends in the organ (Brown, 1980). The length of both preputium and penis sheath was recorded (ppl, psl) as were

their widths at the widest point (ppw, psw) (see Fig. 8.2b).

**4. Crossbreeding Experiments.**

In an initial trial experiment, pairs consisting of a mature Lake Chad and Ngala snail were placed in containers and allowed to remain together for several weeks. Occasional observations were made in an attempt to record any copulatory behaviour.

In the second experiment, five B. globosus from each population were isolated before reaching sexual maturity (<4.5mm sl), thus reducing the chance of any previous sperm transfer from copulation with other snails in the culture trays. The high mortality of individuals below this size made isolation of younger snails impracticable.

At the first sign of oviposition, or when snails reached 9.0mm sl (The majority of snails became reproductively active in culture trays at 8.0-9.0mm), pairs consisting of one Lake Chad and one Ngala snail were set up and left in the same container for one week. After this period, any egg masses were destroyed, and snails reisolated in clean containers. All subsequent offspring were reared in batches from each isolated snail. Approximately 12 weeks after the initial pairing, all snails (adults and young) were sent to Dr Rollinson at the British Museum (Natural History) for an analysis of enzymes of the glucose phosphate isomerase (GPI) and hydroxybutyrate dehydrogenase (HBDH) groups by isoelectric focussing.

## RESULTS

### 1. General Appearance of Snails.

In laboratory bred snails, reared under identical conditions there were clear differences in the external appearance of B. globosus from the Lake Chad and Ngala populations.

Although there were no gross differences in the appearance of the shells, the body tissues of snails from the two populations differed in their coloration (Plate 8.1). B. globosus Lake Chad were uniformly dark in both the head-foot and mantle tissue, where the latter was visible through the shell. B. globosus Ngala, in contrast, had pink tissues with little pigmentation except for a dark area on the posterior edge of the foot, and dense dark blotches of pigment on the mantle. Snails from both populations had small white flecks uniformly distributed over the surface of the head-foot and tentacles.

### 2. Radulae.

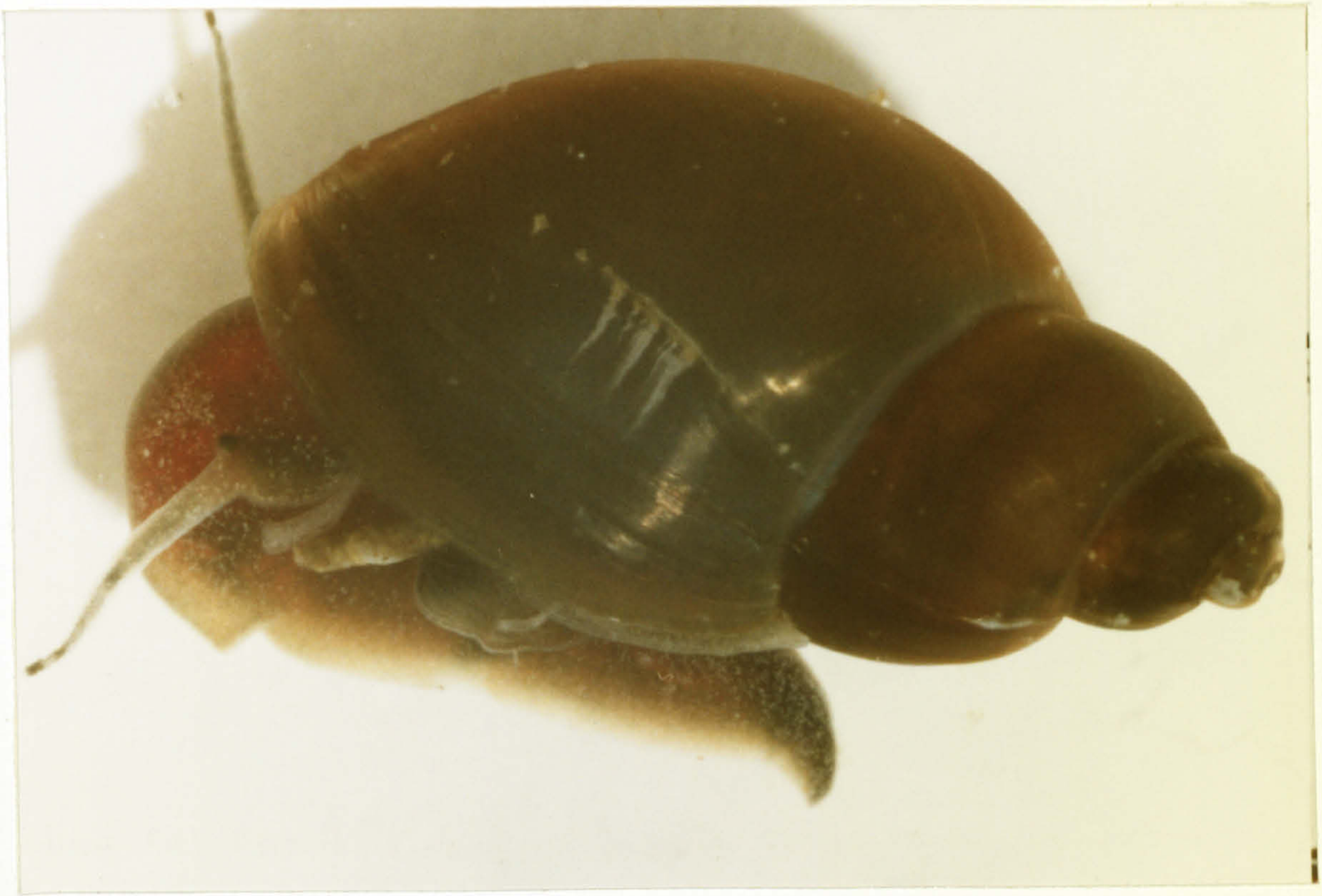
Radulae were examined from 8 B. globosus Lake Chad and 14 Ngala snails. There was considerable variation in the shape of radular teeth between specimens, with no clear differences between the two populations.

Plate 8.2 shows details of median and first lateral teeth from three snails of each population. Although the shape of the cusps is similar in all specimens, there was great variation in the occurrence of denticles between cusps of both median and lateral teeth. While some specimens lacked

## PLATE 8.1

General appearance of B. globosus Lake Chad (A) and Ngala (B). Note the difference in mantle pigmentation and everted copulatory organ in the Ngala specimen (arrow). (Both snails approximately 10mm shell height).

A



B

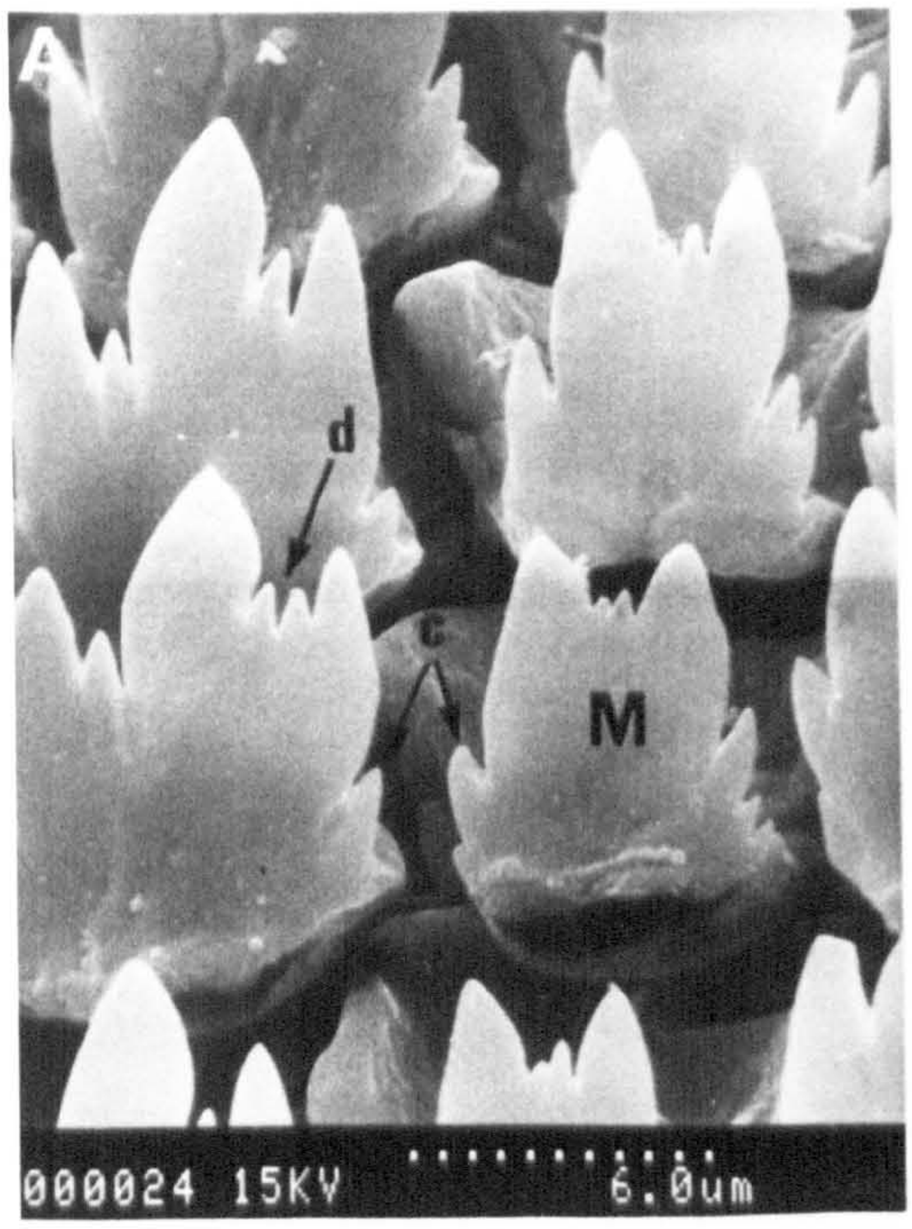
## PLATE 8.2

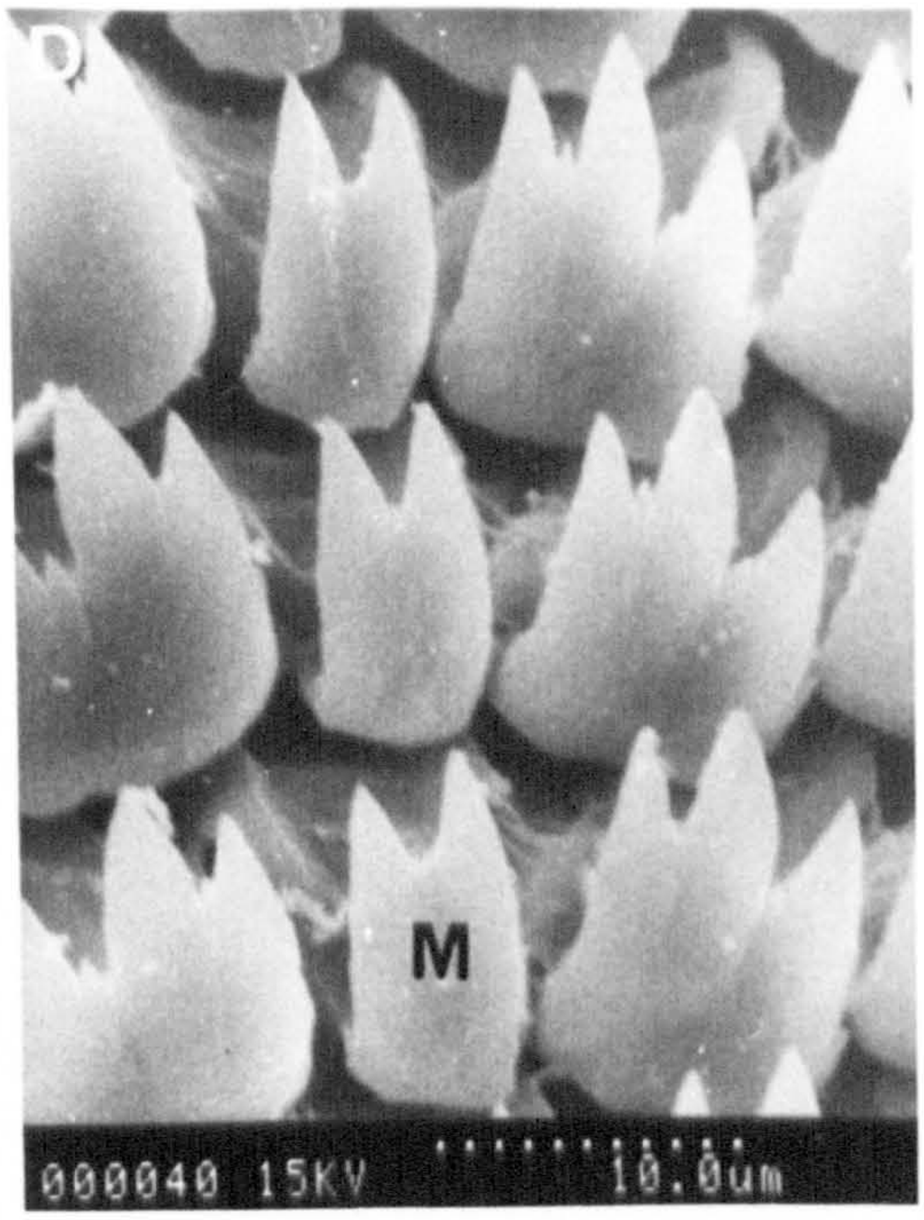
Scanning electron micrographs of radulae from B. globosus Lake Chad (A,B,C) and Ngala (D,E,F).

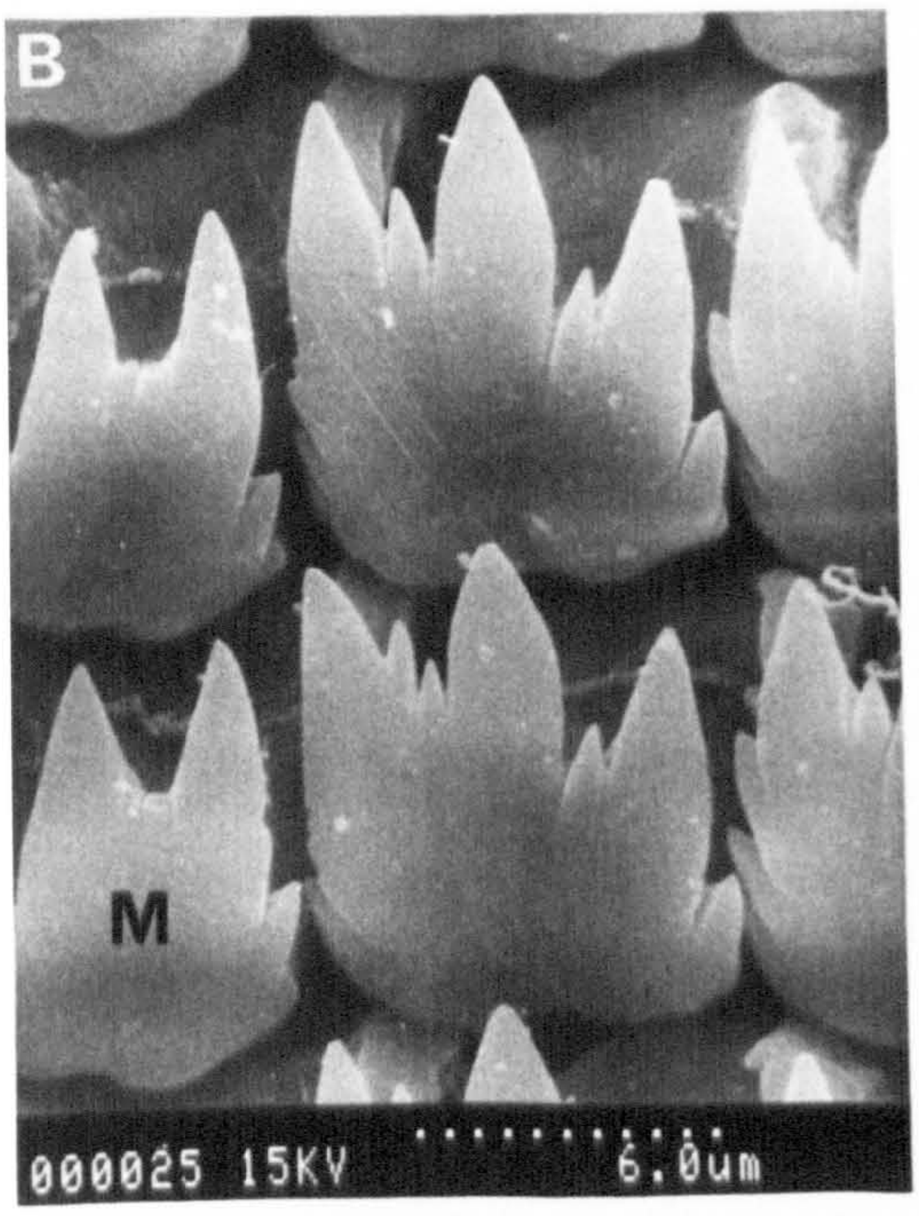
M: Median tooth d: denticles c: cusps on hook body

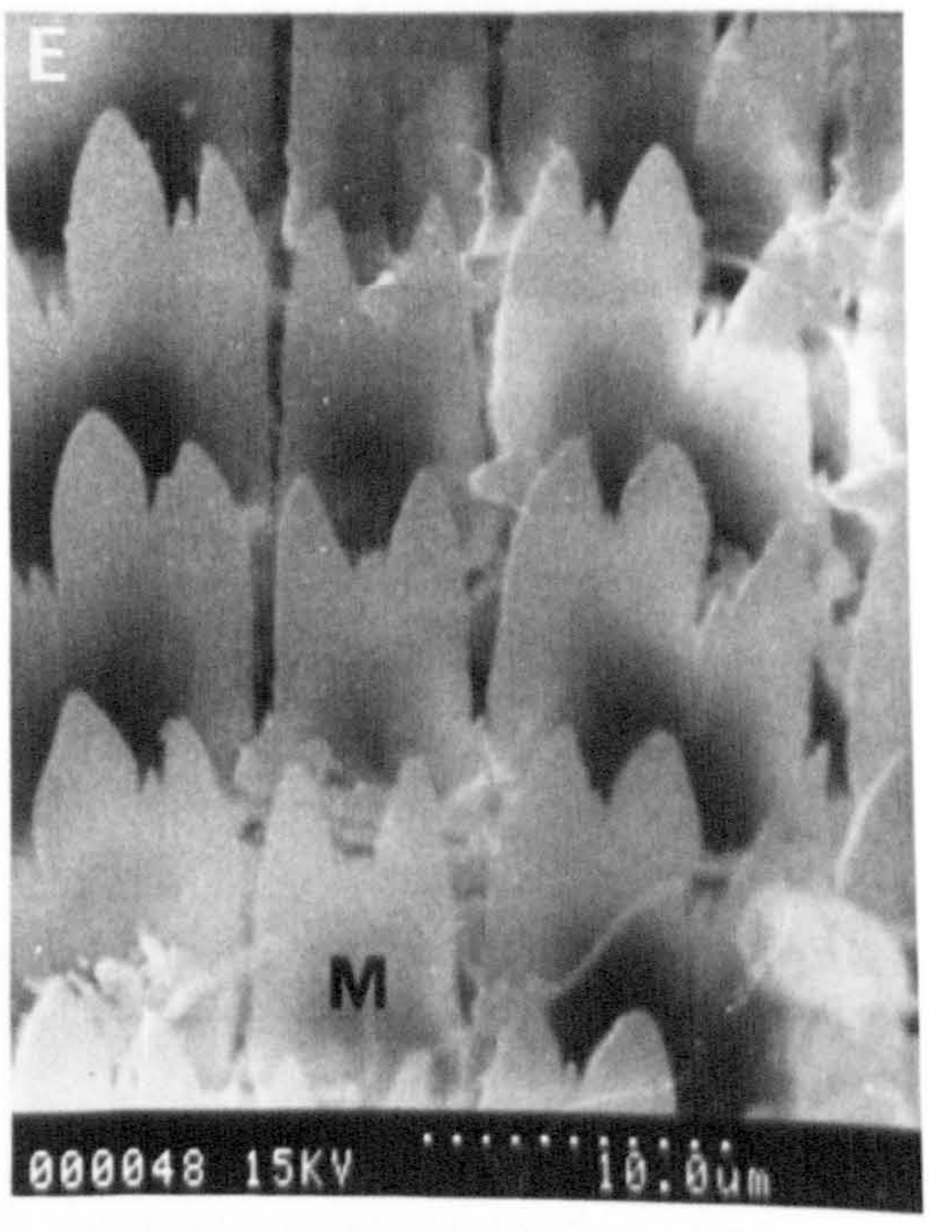
Lake Chad

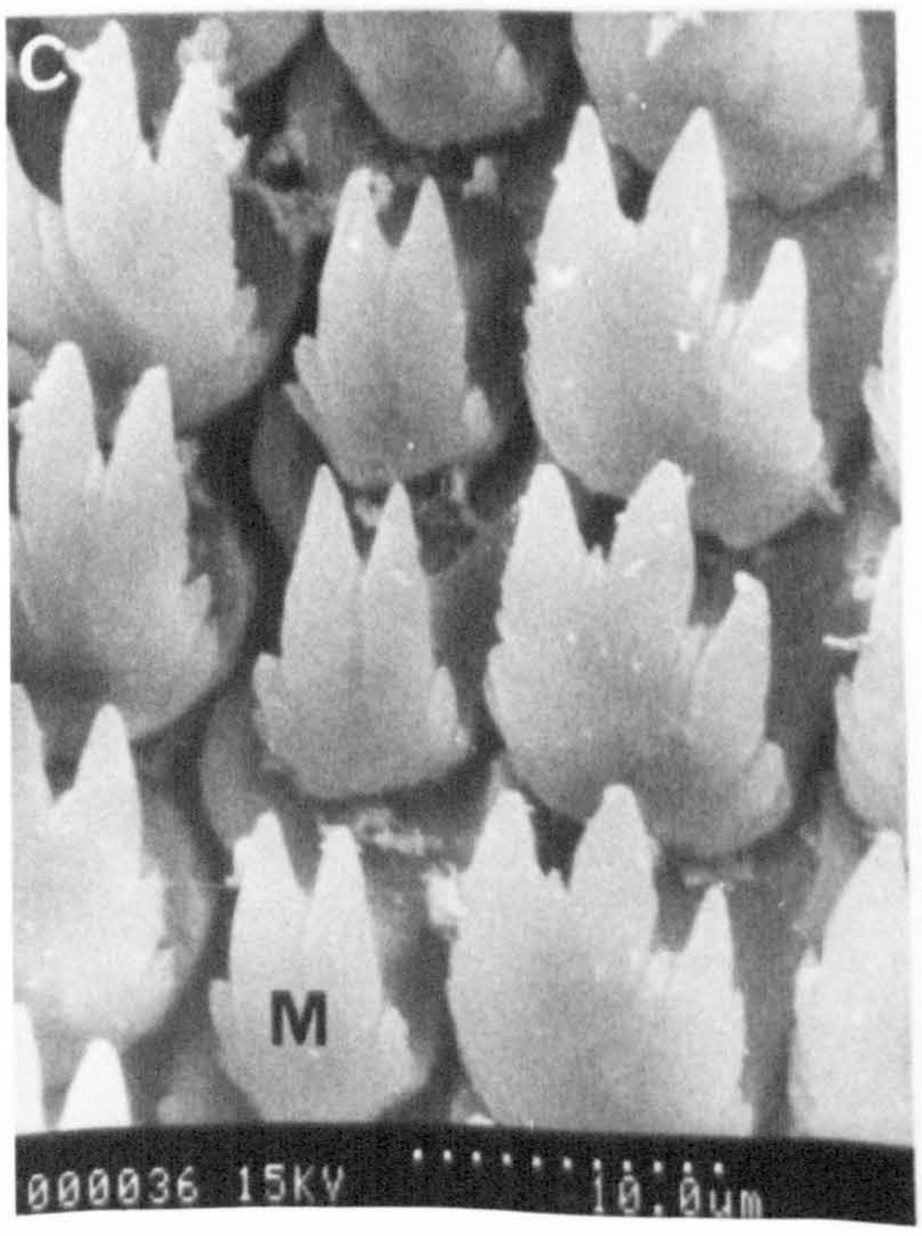
Ngala

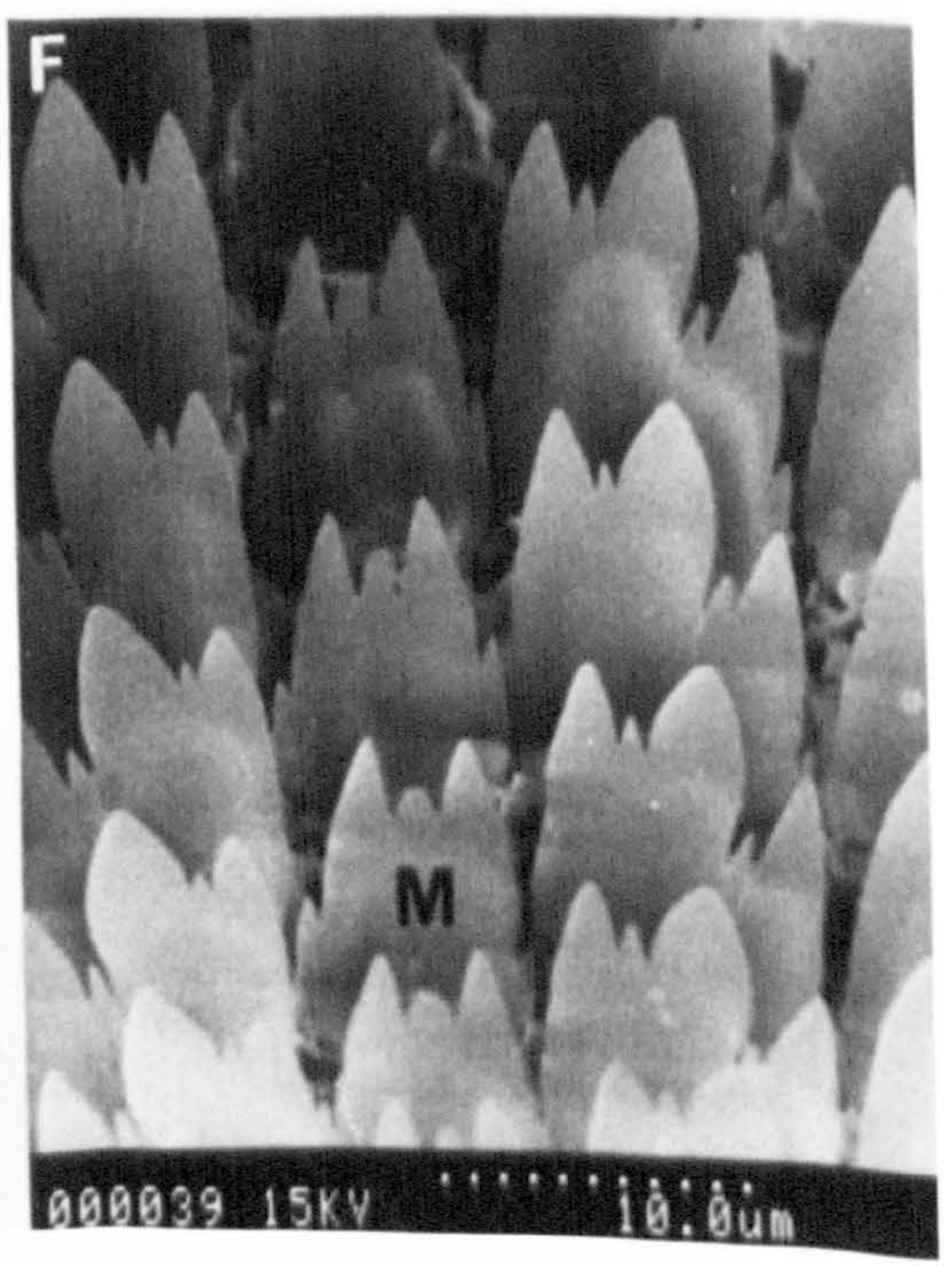


denticles, others had one or two clearly visible. There was also considerable variation in the occurrence and shape of extra cusps on the edges of the main hook body of the teeth (visible on A,B,C and F).

**3. Shell Measurements.**

Table 8.1 shows the mean dimensions of the shells of snails used in the examination of copulatory organs.

A similar size-range of specimens was selected from the two populations with a minimum shell length of 8.6mm and maximum of 13.0mm. There was therefore no significant difference between the mean shell length of the samples of B. globosus from Lake Chad and Ngala.

Although no significant difference was observed in either shell width or aperture length, B. globosus Lake Chad showed a significantly higher mean ratio of shell length to aperture length than the Ngala sample. Apertures of Lake Chad snails were therefore on average relatively smaller for a given shell length.

**4. Relaxation of Snails.**

Relaxation of snails using menthol as a narcotic resulted in a high proportion of the Ngala specimens everting their copulatory organ (see snail in Plate 8.1). The frequency of such eversions in both menthol and sagatal is shown in Table 8.2.

Even with the small number of snails involved in relaxations using menthol, the results showed that the frequency of eversions was significantly higher in the sample

TABLE 8.1

Shell dimensions of B. globosus samples from the Lake Chad and Ngala populations

| | | n | Mean | S.D. | Student's t-test t | d.f. | p |
|---|---|---|---|---|---|---|---|
| Shell length (mm) | L.C. | 20 | 11.320 | 1.290 | 1.02 | 38 | N.S. |
| | Ng. | 20 | 10.945 | 1.023 | | | |
| Shell width (mm) | L.C. | 20 | 7.165 | 0.817 | 0.77 | 38 | N.S. |
| | Ng. | 20 | 6.970 | 0.779 | | | |
| Aperture length (mm) | L.C. | 20 | 7.895 | 0.859 | -0.67 | 38 | N.S. |
| | Ng. | 20 | 8.080 | 0.882 | | | |
| Shell length / Shell width | L.C. | 20 | 1.584 | 0.117 | 0.22 | 38 | N.S. |
| | Ng. | 20 | 1.576 | 0.103 | | | |
| Shell length / Aperture length | L.C. | 20 | 1.439 | 0.132 | 2.17 | 38 | 0.036 |
| | Ng. | 20 | 1.360 | 0.095 | | | |

L.C.= Lake Chad population
Ng. = Ngala population
N.S.= not significant at 5% level of confidence

TABLE 8.2

Eversion of copulatory organs by B. globosus (Lake Chad and Ngala) during relaxation in menthol and sagatal

| | MENTHOL | | | SAGATAL | | |
|---|---|---|---|---|---|---|
| | n | Everted n | % | n | Everted n | % |
| Lake Chad | 12 | 3 | 25.0 | 24 | 3 | 12.5 |
| Ngala | 8 | 7 | 87.5 | 27 | 7 | 26.0 |

from the B. globosus Ngala population than observed in the Lake Chad sample ($X^2$=5.2082, p<0.025). Using sagatal as a narcotic resulted in a significant decrease in the proportion of Ngala snails showing eversion ($X^2$=7.4803, p<0.01), although no significant change was seen in Lake Chad specimens ($X^2$= 0.225, p>0.5). The difference in the frequency of the eversion of copulatory organs between the two populations was no longer significant when sagatal was used as the narcotic ($X^2$=0.7260, p>0.25).

**5. Copulatory Organs.**

On dissection of snails there was a noticeable difference in both the size and general appearance of the male copulatory organs of specimens of B. globosus from Lake Chad and Ngala populations.

All Lake Chad specimens examined showed dark pigmentation of the preputium, with a contrast between this and the very pale, almost white penis sheath and vas deferens. In Ngala snails the preputium, penis sheath and vas deferens were all a similar yellow/white colour.

The difference in the size of copulatory organs of snails from each population can be seen in Table 8.3. Both preputium and penis sheath were significantly longer in the sample of snails from Ngala, as was the width of the preputium at its widest point. Only the maximum width of the penis sheath showed no significant difference between the sample from the Lake Chad and Ngala populations.

The sample of B. globosus Ngala also showed a

## TABLE 8.3

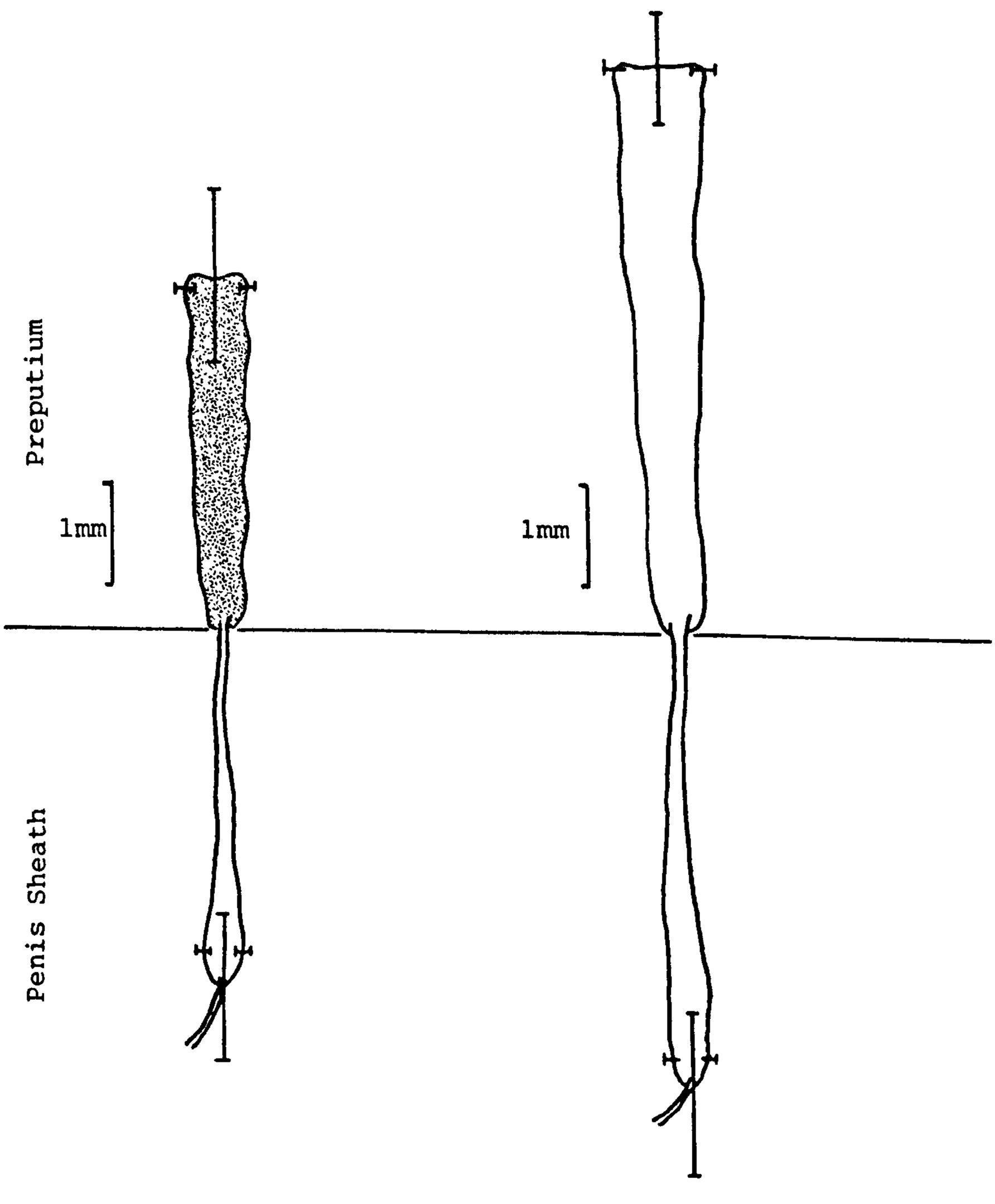
**Copulatory organ dimensions of B. globosus samples from the Lake Chad and Ngala populations**

| | | n | Mean (mm) | S.D. | Student's t-test t | d.f. | p |
|---|---|---|---|---|---|---|---|
| Preputium length (ppl) | L.C. | 20 | 3.635 | 0.900 | -9.14 | 31.73* | <0.0001 |
| | Ng. | 20 | 5.800 | 0.558 | | | |
| Preputium width (ppw) | L.C. | 20 | 0.572 | 0.136 | -6.27 | 38 | <0.0001 |
| | Ng. | 20 | 0.870 | 0.163 | | | |
| Penis sheath length (psl) | L.C. | 20 | 3.802 | 0.774 | -3.94 | 38 | <0.0001 |
| | Ng. | 20 | 4.827 | 0.869 | | | |
| Penis sheath width (psw) | L.C. | 20 | 0.425 | 0.070 | -0.44 | 38 | N.S. |
| | Ng. | 20 | 0.435 | 0.073 | | | |
| psl / ppl | L.C. | 20 | 1.084 | 0.257 | 3.69 | 31.15* | 0.001 |
| | Ng. | 20 | 0.836 | 0.154 | | | |
| psw / ppw | L.C. | 20 | 0.773 | 0.196 | 5.29 | 28.61* | <0.0001 |
| | Ng. | 20 | 0.511 | 0.102 | | | |
| ppl+psl | L.C | 20 | 7.437 | 1.411 | -7.86 | 38 | <0.0001 |
| | Ng. | 20 | 10.627 | 1.141 | | | |

L.C.= Lake Chad population
Ng. = Ngala population
N.S.= not significant at the 5% level of confidence
* = separate variance estimate used

FIGURE 8.3

Diagramatic representation of copulatory organs from B. globosus samples, drawn to mean measurements with standard deviations indicated



significantly higher ratio of both length and width of penis sheath to preputium, than was seen in the sample of Lake Chad snails. In all specimens from both populations the preputium was equal in width to, or wider than, the penis sheath at its widest point. Table 8.4 shows the distribution of specimens by relative length of preputium and penis sheath. Again there is a highly significant difference between the samples from the two populations, with the majority of Ngala specimens having a preputium greater in length than the penis sheath, with the opposite being the case in the Lake Chad sample.

Fig. 8.3 shows the relative dimensions of the copulatory organs of B. globosus from the Lake Chad and Ngala samples diagrammatically. In each case the preputium and penis sheath are drawn to scale using the mean measurements for each sample, with the standard deviations indicated.

**6. Changes in the Dimensions of Shell and Copulatory Organs with Growth.**

Table 8.5 shows that shell length is significantly positively correlated with both shell width and aperture length, and that this is true for both B. globosus populations.

There is also a significant positive correlation between shell length and the total length of preputium and penis sheath in both samples, indicating a growth of the copulatory organ over the size range studied. The observed correlations for the length of preputium and penis sheath separately with shell length indicate that in both populations it is the penis sheath that is increasing in length. There is no evidence of any

## TABLE 8.4

**Relative size of preputium and penis sheath in B. globosus samples from the Lake Chad and Ngala populations**

| | n | ppl>psl | ppl<psl |
|---|---|---|---|
| Lake Chad* | 19 | 8 | 11 |
| Ngala | 20 | 16 | 4 |

$X^2$=4.4189 d.f.=1 0.025<p<0.05

* one snail had a preputium the same length as its penis sheath

## TABLE 8.5

**Correlation between shell length and other shell and copulatory organ dimensions in B. globosus Lake Chad and Ngala**

| | LAKE CHAD | | NGALA | |
|---|---|---|---|---|
| | R | p | R | p |
| Aperture length | 0.6796 | 0.0005 | 0.7453 | <0.0001 |
| Shell width | 0.7827 | <0.0001 | 0.7466 | <0.0001 |
| Preputium length | 0.3381 | N.S. | 0.3765 | N.S. |
| Preputium width | 0.4033 | 0.0389 | 0.3204 | N.S. |
| Penis sheath length | 0.5079 | 0.0111 | 0.7394 | 0.0001 |
| Penis sheath width | 0.1930 | N.S. | 0.2820 | N.S. |
| psl/ppl | 0.1069 | N.S. | 0.5349 | 0.0075 |
| ppl + psl | 0.4945 | 0.0133 | 0.7473 | <0.0001 |

R = Correlation coefficient
N.S.= not significant at the 5% level of confidence

increase in the width of either part of the copulatory organ with increasing shell length.

The observed significant correlation between shell length and the ratio of penis sheath to preputium length in the sample of B. globosus Ngala confirms the finding that the penis sheath is increasing in length at a greater rate than the preputium.

**7. Crossbreeding.**

Observations of B. globosus Lake Chad and Ngala snails paired when mature, and left together for a long period, revealed two cases of copulatory activity. In the first the Ngala snail had everted its male copulatory organ into the female opening of the Lake Chad snail, and in the second observed mating the Lake Chad partner was taking the male role.

The results of the controlled crossbreeding experiments are summarised in Table 8.6. Enzyme analyses carried out by Rollinson produced clear evidence of cross-fertilization occurring in two of the five pairs.

In crosses B and D the Lake Chad snail died shortly after the pair had been separated and before any eggs had been deposited. In the absence of information on the enzyme profiles of these individuals, it could only safely be inferred that (since the $F_1$ generation from eggs laid by the Ngala parent are identical to that snail) there is no evidence of cross-fertilization having taken place.

In cross A, although the Ngala parent died before the end of the experiment, there were enough young produced by it to obtain data on its enzyme profile. Since the $F_1$ progeny from

TABLE 8.6

**Summary of results of experimental crossbreeding of B. globosus from Lake Chad and Ngala**

| Cross | Parent Origin | No. of $F_1$ Examined | Origin of $F_1$ from analysis of enzymes |
|---|---|---|---|
| A | Lake Chad | 14 | Cross-fertilized |
| | Ngala (D) | 15 | Self-fertilized |
| B | Lake Chad (D) | 0 | ---- |
| | Ngala | 1 | Identical to parent* |
| C | Lake Chad | 1 | Self-fertilized |
| | Ngala | 6 | Self-fertilized |
| D | Lake Chad (D) | 0 | ---- |
| | Ngala | 5 | Identical to parent* |
| E | Lake Chad | 5 | Cross-fertilized |
| | Ngala | 5 | Self-fertilized |

D Snail died before examination

* since no information is available on the Lake Chad member of these crosses, no conclusions can be drawn

the Lake Chad snail contained enzyme types from both the Lake Chad parent, and those shown by the surviving Ngala progeny, it may be concluded that they were produced by cross-fertilization. The Ngala $F_1$ however contained none of the Lake Chad enzymes and therefore must have been produced by self fertilization. A similar conclusion could be reached in the case of cross E, where both parents were available for enzyme analysis.

In cross C, both members of the pair survived and produced offspring. In the case of both $F_1$ from the Lake Chad and the Ngala parent, enzyme profiles were identical to the snail that had produced them, showing that all were a result of self-fertilization.

**DISCUSSION**

Observations of populations of B. globosus from Lake Chad and Ngala in the field (Betterton, 1984) and in the laboratory (Chapter 4) had shown that there were significant differences in the susceptibility of these snails to infection with trematodes, including S. haematobium. The results of the present study support the hypothesis that these two populations differ both morphologically and physiologically, as had been indicated in very limited studies of embryonic shell microsculpture (Lund, 1983) and enzyme profiles (Rollinson, pers. comm.). However, despite these differences snails from the two populations were capable of successful crossbreeding in the laboratory.

The differences in external appearance of individual B. globosus from Lake Chad and Ngala were not as clear in field-caught specimens as in laboratory-bred animals. Snails bred and reared in culture were relatively free from the encrusting fauna and flora encountered in the field. In addition, the low concentration of salts in the water used in culture (Chapter 2), led to the formation of relatively thin and translucent shells, with body tissues clearly visible beneath. Any possible differences in water quality or diet of snails in their natural habitats were eliminated in the laboratory-cultured populations. The contrast between the uniformly dark tissues of the head-foot and mantle of Lake Chad snails and the pink tissues with dark markings on the mantle of Ngala individuals was very clear. Close examination of some Lake Chad snails did reveal relatively darker areas on the mantle, but these did not contrast with their background.

The shells of both B. globosus populations under study showed some variation in their general shape. Although there was a significant difference between the mean ratios of shell length to aperture length between the Lake Chad and Ngala samples, there is no clear separation of the two groups when these parameters are plotted (Fig. 8.4). All specimens showed truncation of the columella and the spirally arranged nodules on the upper shell whorls typical of the africanus species group. Lund's study (1983) of the micro-ornamentation of embryonic shells from the two populations showed fine ridges joining the pits on Ngala specimens, whereas these ridges were

FIGURE 8.4

Scattergram of aperture lengths by shell length of B. globosus samples from Lake Chad and Ngala populations

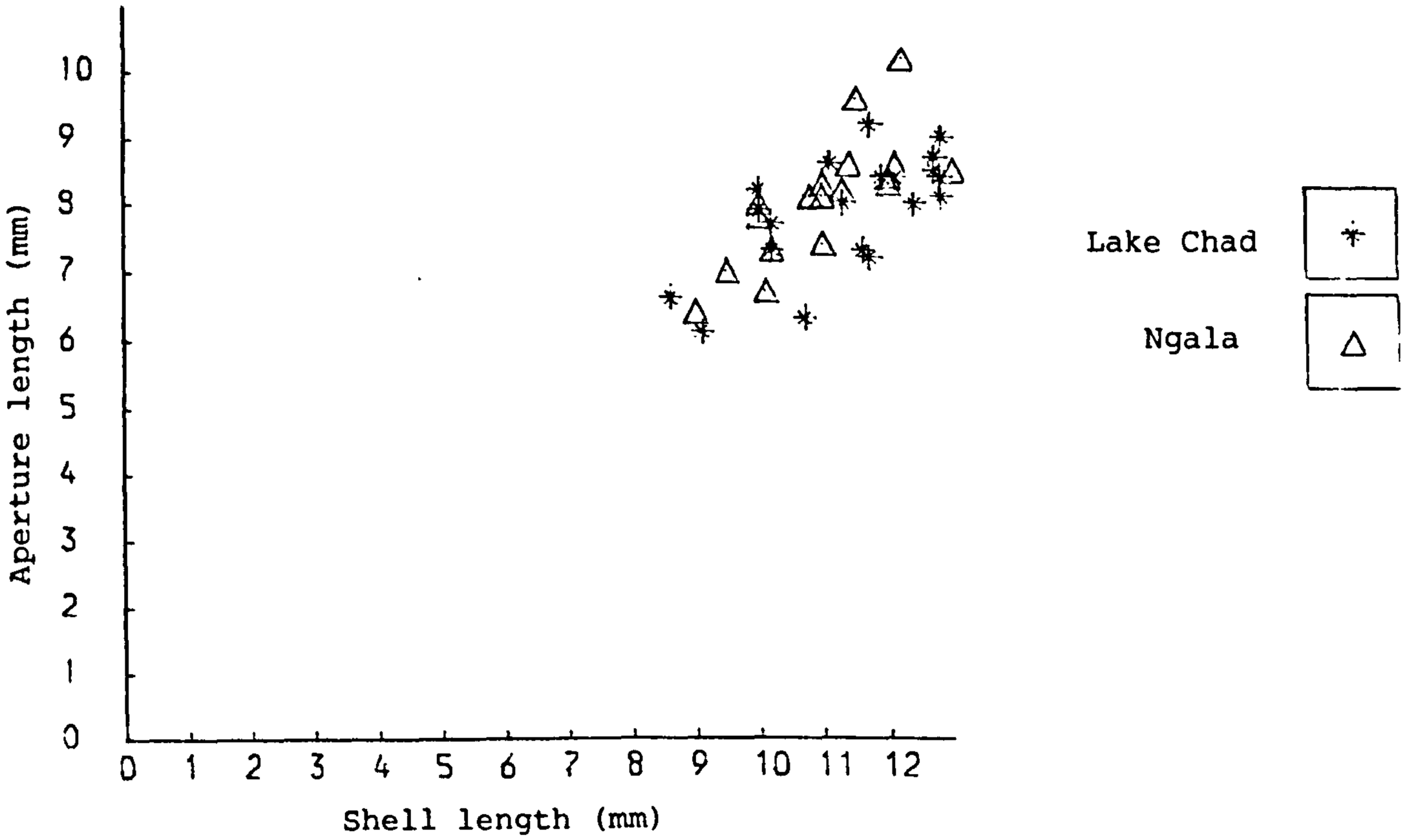
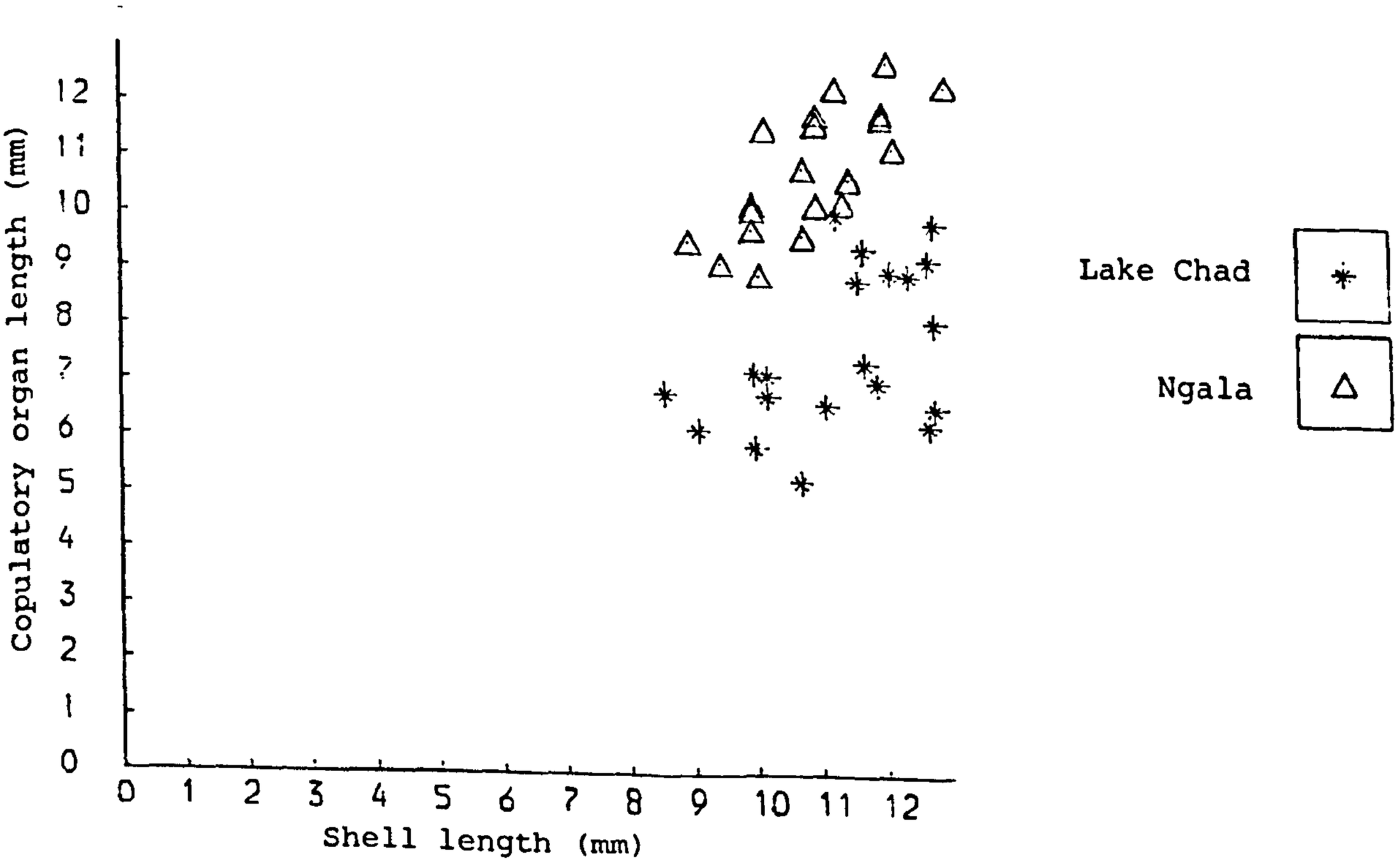



FIGURE 8.5

Scattergrams of total copulatory organ length by shell lengths of B. globosus samples from Lake Chad and Ngala populations



absent on shells from Lake Chad snails. Since only a few shells were examined it is not clear whether these patterns are typical of the two populations.

The shape of the radular teeth is important in the taxonomy of members of the B. truncatus/tropicus complex (Schutte, 1965; Brown, 1980,1982) and is rarely used in the identification of africanus group snails. Considerable variation is known to exist even between mesocones of the same radula, and some changes in shape are associated with an individual's growth (Brown, 1982). In the present study there was considerable variation between radulae within the two B. globosus populations. Although examination of a larger sample may show minor differences between the radular teeth of Lake Chad and Ngala snails, there is no evidence of consistent differences in the material examined so far.

The morphology of the copulatory organ is generally considered to be the most important taxonomic characteristic of members of the B. africanus group (Mandahl-Barth, 1957,1958; Brown, 1966,1980). Although the measurement of the various parts of bulinid copulatory organs is often considered unreliable, due to the variations in the degree of tissue contraction with different techniques (e.g. Wu, 1972) and possible effects of parasitism (Brown, 1966,1980), both these problems were reduced to a minimum in the present comparative study. All material was treated identically, and the laboratory bred specimens were free from parasite infections.

The observed differences in the dimensions of the

preputium and penis sheath between the samples of B. globosus Lake Chad and Ngala were highly significant, and there is a good separation of the two populations when the combined length of these two portions of the copulatory organ are plotted against the shell length of individuals (Fig. 8.5).

Particular importance is attached to the relative proportion of preputium and penis sheath in members of the africanus group. B. globusus is described as having a small penis sheath, narrower and shorter than the preputium (Mandahl-Barth, 1957, 1958; Brown, 1980) although there is some variation between populations (Brown, 1980). Although all specimens in the present study had a preputium equal in width to, or wider than, the penis sheath at its broadest point, a large number of specimens had a penis sheath longer than the preputium (Table 8.4), thus not strictly fitting the description of B. globosus.

The observed ratio of penis sheath to preputium length of B. globosus Lake Chad is higher than that reported in studies of populations from other regions, with the preputium longer than penis sheath in the majority of specimens examined. The mean value of psl/ppl in the sample of Ngala snails (0.836) agrees with other studies of B. globsosus, including those of Angolan snails by Wright (1963) (0.83) and Brown (1966) (ranging from 0.81 to 0.96 in various size classes), although it is higher than that of the Tanzanian population studied by Pringle, Otieno and Chimtawi (1971), (0.72).

Although neither Wright (1963) nor Brown (1966) found any

significant change in the relative length of penis sheath and preputium with snail size, data from B. globosus Ngala sampled in the present study show a significant positive correlation resulting from the growth of the penis sheath, while there is no significant change in the length of the preputium over the size range involved. If this proved to be the case on examination of a larger sample covering a wider size range, it would reduce the usefulness of this ratio in the taxonomy of the group.

In addition to these observed anatomical differences between specimens of B. globosus from Lake Chad and Ngala populations, the reported differences in the frequency of the eversion of the copulatory organ with the two narcotics, menthol and sagatal, indicate physiological differences.

Freshwater habitats are both insular and unstable in nature, particularly in arid zones. Many workers on the taxonomy of Bulinus have recognised that the isolation of snail habitats leads to the development of genetically distinct populations, whereas the temporary nature of many water bodies probably works against the formation of new species (e.g. Hubendick, 1954, 1962; Wright, 1961; Mandahl-Barth, 1965; Berrie, 1970). B. globosus, the most widely distributed member of the africanus species group, is known to exist in several distinct local forms (Mandahl-Barth, 1965; Berrie, 1973) with some forms where the copulatory organ is intermediate in form with that typical of B. africanus (e.g. Wright, 1963). Wright and Rollinson (1979) showed differences in the occurrence of

enzyme types in B. globosus from different populations over its range. Hira (1968) reported microgeographical races of this species, showing differences in both morphology and their host-parasite relationship with S. haematobium, in an area around Ibadan, Nigeria. Coker and Kuma (1974) described differences between populations of B. globosus from the three main vegetation zones of southern Ghana.

Although reported from pools in the S.C.I.P. area by previous workers (Menu et al., 1973; Noamesi and Morcos, 1974; Odei, 1977) Betterton (1984 a) failed to find B. globosus in these habitats, despite their presence in similar water bodies some 20km to the south. It seems probable that this species is at the northern limit of its range, only able to exist in these unstable habitats in years when rainfall is high (Betterton, 1984 a). Lake Chad forms an atypical freshwater habitat in the region, which, despite large annual fluctuations in water level, provides a relatively stable habitat. Historically populations of B. globosus in the lake are likely to have survived periods of unfavourable climatic conditions when those in temporary water bodies died out. Despite its proximity to Lake Chad, the relatively new irrigation channels in the Ngala Pilot Project would appear to have been colonised by B. globosus from a different origin, possibly the seasonal river Ebeji which has occasionally been used as a source of water for the scheme (Betterton, 1984 a). Ngala and Lake Chad snails have therefore been isolated from each other for a considerable period, with subsequent evolutionary divergence

producing the marked differences in various characteristics as observed in the present investigation. However, the proven capability for individuals from the two locations to cross-fertilize successfully indicates that they are not reproductively isolated.

Reproductive barriers between defined species of Bulinus have only rarely been tested by laboratory cross-breeding experiments (Brown, 1980). Pringle et al. (1971) in their study of B. globosus and the closely related B. nasutus from Tanzania observed snails of the two species attempting to copulate, but found no evidence of hybridisation. Wu (1972) carried out crossbreeding experiments between an albino population of B. tropicus and other members of the polyploid series of tropicus and truncatus group snails. His results indicated cases of both mutual cross-fertilization and one-way cross-fertilization. There appeared to be an increasing difficulty in cross-fertilization as the geographical separation of populations increased.

In the present study, although pairs of snails were observed copulating with both B. globosus Lake Chad and Ngala acting as the male partner, evidence from the controlled experiments proved cross-fertilization in one direction only, with the Ngala parent fertilizing the ova of the Lake Chad snail. As with other bulinids, copulation in B. globosus is known to be unilateral (Rudolph, 1979) and it is quite possible that in the relatively short time snails were paired, only copulations with the Ngala snail transferring sperm occurred.

Further pairings are required to discover whether sperm transfer from Lake Chad to Ngala snails is possible and whether fertilization may be successful.

Further experiments involving crossbreeding of the two populations of B. globosus from Lake Chad and Ngala would be of great interest. In Wu's studies (1972) crosses between diploid and polyploid snails, when successful, produced only sterile progeny. The fertility of the $F_1$ generation in the present study has yet to be investigated.

Detailed studies of the susceptibility to S. haematobium infection of the $F_1$ generation from the known susceptible B. globosus Ngala and the refractory Lake Chad snails may yield information on mechanisms involved in the intermediate host-parasite relationship.

## REFERENCES


Berrie, A.D., 1970. Snail problems in African schistosomiasis. Advances in Parasitology 8 : 43-96.

Berrie, A.D., 1973. Snails, schistosomes and systematics: some problems concerning the genus Bulinus. In The Systematics Association, special volume No. 5. Taxonomy and Ecology. Ed. V.H.Heywood : pp173-188.

Betterton, C., 1984. Ecological studies on the snail hosts of schistosomiasis in the South Chad Irrigation Project Area, Borno State, northern Nigeria. Journal of Arid Environments 7 : 43-57.

Betterton, C., Fryer, S.E. and Wright, C.A., 1983. Bulinus senegalensis (Mollusca: Planorbidae) in northern Nigeria. Annals of Tropical Medicine and Parasitology 77 : 143-149.

Brown, D.S., 1966. On certain morphological features of Bulinus africanus and B. globosus (Mollusca: Pulmonata) and the distribution of these species in south eastern Africa. Annals of the Natal Museum 18 : 401-415.

Brown, D.S., 1980. Freshwater Snails of Africa and their Medical Importance. Taylor and Francis Ltd. London. 487pp.

Brown, D.S., 1982. The radular mesocone as a source of taxonomic characters in Bulinus (Basommatophora: Planorbidae). Malacologia 22 : 505-508.

Coker, W.Z. and Kuma, E., 1974. A study of the genetic heterogeneity in Bulinus globosus populations in southern Ghana: electrophoretic patterns of esterase enzymes in snail extracts. Ghana Journal of Science 14 : 19-22.

Hira, P.R., 1968. Microgeographical races of Bulinus (Physopsis) globosus, the intermediate host of Schistosoma haematobium in Ibadan, Nigeria. West African Medical Journal 17 : 86-88.

Hubendick, B., 1954. Viewpoints on species discrimination with special attention to medically important snails. Proceedings of the Malacological Society of London 31 : 6-11.

Hubendick, B., 1962. Aspects of the diversity of the freshwater fauna. Oikos 13 : 249-261.

Lund, S.J.S., 1983. A comparison of shell growth of gastropod embryos. Unpublished Honours Project, School of Animal Biology, U.C.N.W., Bangor

Mandahl-Barth, G., 1957. Intermediate hosts of Schistosoma. African Bulinus and Biomphalaria. 2. Bulinus. Bulletin of the World Health Organisation 17 : 1-65.

Mandahl-Barth, G., 1958. Intermediate hosts of Schistosoma. African Biomphalaria and Bulinus. World Health Organisation Monograph No. 37: Geneva.

Mandahl-Barth, G., 1965. The species of the genus Bulinus, intermediate hosts of Schistosoma. Bulletin of the World Health Organisation 33 : 33-44.

Menu, J.P., Dove, J.E., Noamesi, G.K. and Dazo, B.C., 1973. Health Component in South Chad Irrigation Project - Feasibility Study. Report on missions Sept. 1972 - March 1973. World Health Organisation unpublished document **AFR/PHA/117** 92pp.

Noamesi, G.K. and Morcos,G., 1974. Health Component in South Chad Irrigation Project - Feasibility Study. Follow-up studies on schistosomiasis and malaria. World Health Organisation unpublished document **AFR/SCHIST/30** 20pp.

Odei, M.A., 1977. Health Component in South Chad Irrigation Project - Feasibility Study. Follow-up studies on the snail hosts of schistosomiasis. World Health Organisation unpublished document **AFR/SCHIST/37** 24pp.

Pringle, G., Otieno, L.H. and Chimtawi, M.B., 1971. Notes on the morphology, susceptibility to Schistosoma haematobium and genetic relationships of Bulinus (Physopsis) globosus and B. (P.) nasutus from north eastern Tanzania. Annals of Tropical Medicine and Parasitology **65** : 211-219.

Rudolph, P.H., 1979. An analysis of copulation in Bulinus globosus (Gastropoda, Planorbidae). Malacologia **19** : 147-156.

Schutte, C.H.J., 1965. Notes on the radular mesocone as a criterion for distinguishing between truncatus and tropicus groups of the genus Bulinus (Mollusca: Basommatophora). Annals and Magazine of Natural History 8 : 409-419.

Wright, C.A., 1961. Taxonomic problems in the molluscan genus Bulinus. Transactions of the Royal Society of Tropical Medicine and Hygiene 55 : 225-231.

Wright, C.A., 1963. The freshwater gastropod mollusca of Angola. Bulletin of the British Museum (Natural History) Zoology 10: 449-528.

Wright, C.A. and Rollinson, D., 1979. Analysis of enzymes of the Bulinus africanus group (Mollusca: Planorbidae) by isoelectric focussing. Journal of Natural History 13 : 263-273.

Wu, S.-K., 1972. Comparative studies on a polyploid series of the African snail genus Bulinus (Basommatophora: Planorbidae). Malacological Review 5 : 95-164.

# APPENDIX A

## Questionnaire used in the survey at New Marte Secondary School

SCHOOL NO.:

NAME:

AGE:

RELIGION:

ETHNIC GROUP:

SCHOOL HOUSE:

FATHER'S OCCUPATION:

LIVESTOCK KEPT:

MOSQUITO NET?

SHOES?

RECENT MEDICAL COMPLAINTS:

NOTES:

HOME VILLAGE:

LOCAL GOVERNMENT:

PREVIOUS PLACES OF RESIDENCE:

VACATION RESIDENCE:

HOUSING TYPE:

SANITATION:

TAP:

VILLAGE WATER SUPPLY:

WASHING PLACES:
Dry season:
Wet season:

SWIMMING:
Dry season:
Wet season:
School:
With other children?

# APPENDIX B

## Breakdown of cercarial production from infected bulinids

### TABLE B.1

The number of S. haematobium cercariae produced from B. rohlfsi on sample days each week from first cercarial emergence.

| Week | Number of Snails | NUMBER OF CERCARIAE PRODUCED Mean/ Day | S.D. | S.E. | Range Min. | Range Max. | Median |
|---|---|---|---|---|---|---|---|
| 1 | 18 | 13.89 | 22.05 | 5.20 | 1 | 83 | 4.00 |
| 2 | 29 | 8.86 | 10.59 | 1.97 | 0 | 46 | 6.00 |
| 3 | 28 | 12.43 | 21.16 | 4.00 | 0 | 70 | 2.50 |
| 4 | 26 | 16.15 | 23.72 | 4.65 | 0 | 95 | 6.83 |
| 5 | 23 | 23.65 | 33.82 | 7.05 | 0 | 146 | 10.00 |
| 6 | 17 | 24.82 | 40.06 | 9.72 | 0 | 151 | 4.25 |
| 7 | 9 | 28.67 | 27.65 | 9.22 | 0 | 74 | 16.00 |
| 8 | 8 | 13.25 | 16.67 | 5.89 | 0 | 45 | 6.50 |
| 9 | 5 | 10.20 | 10.21 | 4.56 | 2 | 27 | 8.00 |
| 10 | 5 | 4.80 | 5.02 | 2.24 | 0 | 12 | 2.50 |
| 11 | 2 | 0.50 | * | * | * | * | * |
| 12 | 1 | 2.00 | * | * | * | * | * |
| OVERALL | 29 | 15.60 | 24.75 | 1.89 | 0 | 151 | 6.00 |

(* Insufficient data)

## TABLE B.2

**The number of *S. haematobium* cercariae produced from *B. senegalensis*, infected with 'rohlfsi' strain, on sample days each week from first cercarial emergence.**

| Week | Number of Snails | NUMBER OF CERCARIAE PRODUCED Mean/ day | S.D. | S.E. | Range Min. | Max. | Median |
|---|---|---|---|---|---|---|---|
| 1 | 20 | 7.25 | 19.41 | 4.34 | 0 | 88 | 1.39 |
| 2 | 20 | 15.10 | 37.19 | 8.32 | 0 | 170 | 3.50 |
| 3 | 20 | 24.30 | 30.44 | 6.31 | 1 | 99 | 8.50 |
| 4 | 18 | 37.67 | 41.91 | 9.88 | 0 | 120 | 22.50 |
| 5 | 10 | 74.20 | 75.13 | 23.76 | 3 | 197 | 43.00 |
| 6 | 14 | 54.93 | 92.51 | 24.72 | 2 | 359 | 23.00 |
| 7 | 9 | 58.56 | 76.61 | 25.54 | 3 | 204 | 11.00 |
| 8 | 10 | 41.60 | 39.47 | 12.48 | 2 | 109 | 23.50 |
| 9 | 7 | 144.14 | 221.43 | 83.69 | 1 | 623 | 74.00 |
| 10 | 4 | 35.25 | 52.73 | 26.36 | 2 | 113 | 3.50 |
| 11 | 4 | 20.25 | 27.03 | 13.52 | 0 | 60 | 10.50 |
| 12 | 2 | 6.50 | * | * | * | * | * |
| OVERALL | 20 | 38.48 | 73.73 | 6.28 | 0 | 623 | 10.00 |

( * Insufficient data)

TABLE B.3

The number of S. haematobium cercariae produced from B. senegalensis, infected with 'globosus' strain, on sample days each week from first cercarial emergence.

| Weeks | Number of Snails | NUMBER OF CERCARIAE PRODUCED Mean/ day | S.D | S.E | Range Min. | Max. | Median |
|---|---|---|---|---|---|---|---|
| 1 | 20 | 5.45 | 10.42 | 2.33 | 1 | 48 | 1.41 |
| 2 | 20 | 21.25 | 32.51 | 7.27 | 0 | 115 | 5.00 |
| 3 | 20 | 75.45 | 179.96 | 40.24 | 2 | 783 | 17.50 |
| 4 | 17 | 66.53 | 87.04 | 21.11 | 2 | 228 | 10.25 |
| 5 | 14 | 28.43 | 51.27 | 13.70 | 0 | 183 | 6.17 |
| 6 | 8 | 91.62 | 137.53 | 48.62 | 0 | 395 | 4.50 |
| 7 | 6 | 36.33 | 43.66 | 17.82 | 0 | 120 | 20.50 |
| 8 | 4 | 44.75 | 50.52 | 25.26 | 1 | 103 | 5.50 |
| 9 | 4 | 68.75 | 98.34 | 49.17 | 0 | 211 | 9.00 |
| 10 | 3 | 130.00 | 221.70 | 128.00 | 2 | 386 | 98.00 |
| 11 | 3 | 6.00 | 5.57 | 3.22 | 0 | 11 | 7.00 |
| 12 | 1 | 3.00 | * | * | * | * | * |
| OVERALL | 20 | 44.90 | 100.03 | 9.13 | 0 | 783 | 7.00 |

(* Insufficient data)

## TABLE B.4

**The number of *S. haematobium* cercariae produced from *B. globosus* (Ngala) on sample days each week from first cercarial emergence.**

| Week | Number of Snails | NUMBER OF CERCARIAE PRODUCED Mean/ day | S.D. | S.E. | Range Min. | Max. | Median |
|---|---|---|---|---|---|---|---|
| 1 | 25 | 105.44 | 80.39 | 16.08 | 15 | 265 | 82.00 |
| 2 | 24 | 160.96 | 133.24 | 27.20 | 3 | 476 | 116.50 |
| 3 | 25 | 140.16 | 176.59 | 35.32 | 9 | 903 | 97.00 |
| 4 | 25 | 254.84 | 208.24 | 41.65 | 18 | 770 | 180.00 |
| 5 | 25 | 108.36 | 105.90 | 21.18 | 4 | 361 | 62.00 |
| 6 | 25 | 185.04 | 144.88 | 28.97 | 8 | 516 | 143.00 |
| 7 | 25 | 249.36 | 218.16 | 43.63 | 4 | 718 | 156.00 |
| 8 | 24 | 126.29 | 101.31 | 20.68 | 2 | 350 | 97.50 |
| 9 | 23 | 124.04 | 119.25 | 24.87 | 3 | 449 | 85.00 |
| 10 | 24 | 79.79 | 127.55 | 26.04 | 3 | 616 | 32.50 |
| 11 | 24 | 101.75 | 109.29 | 22.31 | 0 | 508 | 73.50 |
| 12 | 24 | 73.54 | 146.98 | 30.00 | 0 | 501 | 12.50 |
| 13 | 22 | 98.14 | 197.17 | 42.04 | 0 | 702 | 14.83 |
| 14 | 24 | 36.04 | 81.45 | 16.63 | 0 | 261 | 2.50 |
| 15 | 20 | 71.00 | 147.74 | 33.04 | 0 | 562 | 8.00 |
| 16 | 8 | 128.50 | 124.70 | 44.09 | 1 | 393 | 82.00 |
| 17 | 22 | 81.77 | 187.72 | 40.02 | 0 | 662 | 9.00 |
| 18 | 8 | 155.62 | 192.02 | 67.89 | 0 | 490 | 42.50 |
| 19 | 23 | 66.35 | 160.02 | 33.37 | 0 | 595 | 4.00 |
| 20 | 23 | 48.70 | 134.72 | 28.09 | 0 | 581 | 1.25 |
| 21 | 23 | 98.83 | 262.24 | 54.68 | 0 | 890 | 2.00 |
| 22 | 23 | 85.52 | 267.91 | 55.86 | 0 | 1098 | 3.00 |
| 23 | 23 | 77.30 | 238.18 | 49.66 | 0 | 1071 | 2.75 |
| 24 | 23 | 88.30 | 239.14 | 49.86 | 0 | 977 | 1.75 |
| 25 | 23 | 30.00 | 46.86 | 9.77 | 0 | 146 | 8.00 |
| 26 | 23 | 48.03 | 127.93 | 26.68 | 0 | 593 | 10.00 |
| 27 | 21 | 64.14 | 181.52 | 39.61 | 0 | 795 | 3.00 |
| 28 | 22 | 80.95 | 178.78 | 38.12 | 0 | 777 | 2.17 |
| 29 | 22 | 68.23 | 117.16 | 24.98 | 0 | 392 | 16.50 |
| 30 | 20 | 83.25 | 141.06 | 31.54 | 0 | 592 | 16.50 |
| 31 | 22 | 80.09 | 114.18 | 24.34 | 2 | 410 | 35.00 |
| 32 | 20 | 74.90 | 93.13 | 20.82 | 0 | 367 | 41.00 |
| 33 | 19 | 107.79 | 143.57 | 32.94 | 3 | 506 | 77.00 |
| 34 | 19 | 200.37 | 302.73 | 69.45 | 3 | 1046 | 16.00 |
| 35 | 17 | 172.47 | 240.75 | 58.39 | 2 | 901 | 111.75 |
| 36 | 16 | 186.75 | 236.91 | 59.23 | 3 | 881 | 107.50 |
| 37 | 17 | 122.82 | 184.64 | 44.78 | 0 | 659 | 77.00 |
| 38 | 14 | 131.43 | 174.02 | 46.51 | 0 | 585 | 10.50 |
| 39 | 14 | 130.71 | 187.27 | 50.05 | 0 | 574 | 15.50 |
| 40 | 14 | 137.64 | 178.80 | 47.79 | 0 | 507 | 7.50 |
| 41 | 3 | 44.33 | 62.08 | 35.84 | 7 | 116 | 10.00 |
| 42 | 3 | 23.33 | 19.22 | 11.10 | 6 | 44 | 20.00 |
| 43 | 14 | 128.43 | 145.34 | 38.84 | 0 | 356 | 55.50 |
| 44 | 13 | 59.00 | 79.04 | 21.92 | 0 | 237 | 14.00 |

TABLE B.4 continued

| Week | Number of Snails | NUMBER OF CERCARIAE PRODUCED Mean/ day | S.D. | S.E. | Range Min. | Max. | Median |
|---|---|---|---|---|---|---|---|
| 45 | 11 | 134.82 | 221.11 | 66.67 | 0 | 761 | 87.00 |
| 46 | 12 | 84.50 | 109.14 | 31.51 | 0 | 310 | 20.50 |
| 47 | 10 | 53.10 | 73.53 | 23.51 | 0 | 187 | 4.50 |
| 48 | 10 | 40.90 | 62.91 | 19.90 | 0 | 152 | 2.50 |
| 49 | 10 | 28.50 | 49.03 | 15.50 | 0 | 154 | 3.50 |
| 50 | 9 | 15.22 | 25.06 | 8.35 | 0 | 76 | 1.25 |
| 51 | 9 | 8.78 | 11.08 | 3.69 | 0 | 30 | 3.00 |
| 52 | 9 | 2.67 | 6.91 | 2.30 | 0 | 21 | 0.25 |
| 53 | 6 | 7.83 | 18.22 | 7.44 | 0 | 45 | 0.50 |
| 54 | 5 | 7.40 | 16.55 | 7.40 | 0 | 37 | 4.62 |
| 55 | 6 | 5.17 | 12.67 | 5.17 | 0 | 31 | 3.10 |
| 56 | 6 | 3.50 | 7.61 | 3.11 | 0 | 19 | 0.50 |
| 57 | 5 | 0.80 | 1.79 | 0.80 | 0 | 4 | 0.50 |
| 58 | 3 | 5.67 | 9.81 | 5.67 | 0 | 17 | 4.25 |
| 59 | 3 | 1.67 | 2.08 | 1.20 | 0 | 4 | 1.00 |
| 60 | 3 | 4.33 | 4.93 | 2.85 | 1 | 10 | 2.00 |
| OVERALL | 25 | 99.79 | 167.35 | 5.30 | 0 | 1098 | 19.00 |

## TABLE B.5

**The number of S.haematobium cercariae produced from B. globosus (Ngala), showing a single peak in output, on sample days each week from first cercarial emergence.**

| Week | Number of Snails | NUMBER OF CERCARIAE PRODUCED Mean/ day | S.D. | S.E. | Range Min. | Max. | Median |
|---|---|---|---|---|---|---|---|
| 1 | 7 | 88.00 | 34.42 | 13.01 | 38 | 139 | 93.00 |
| 2 | 6 | 163.50 | 87.98 | 35.92 | 35 | 268 | 166.00 |
| 3 | 7 | 81.57 | 57.83 | 21.86 | 26 | 155 | 43.00 |
| 4 | 7 | 246.43 | 202.00 | 76.35 | 18 | 555 | 180.00 |
| 5 | 7 | 62.86 | 54.73 | 20.68 | 14 | 166 | 39.00 |
| 6 | 7 | 116.14 | 78.46 | 29.66 | 45 | 228 | 80.00 |
| 7 | 7 | 245.29 | 203.59 | 76.95 | 12 | 583 | 187.00 |
| 8 | 7 | 165.57 | 107.40 | 40.59 | 56 | 335 | 132.00 |
| 9 | 7 | 105.00 | 63.45 | 23.98 | 3 | 210 | 98.00 |
| 10 | 7 | 39.86 | 40.71 | 15.39 | 7 | 128 | 29.00 |
| 11 | 7 | 63.27 | 50.48 | 19.08 | 1 | 129 | 63.00 |
| 12 | 7 | 7.43 | 9.80 | 3.70 | 0 | 28 | 4.00 |
| 13 | 7 | 8.00 | 4.86 | 1.84 | 4 | 15 | 5.33 |
| 14 | 7 | 2.29 | 2.81 | 1.06 | 0 | 8 | 1.25 |
| 15 | 6 | 4.33 | 5.32 | 2.17 | 0 | 12 | 1.50 |
| 16 | 1 | * | * | * | * | * | * |
| 17 | 6 | 5.00 | 6.07 | 2.48 | 0 | 14 | 1.50 |
| 18 | 1 | * | * | * | * | * | * |
| 19 | 7 | 5.00 | 7.72 | 2.92 | 0 | 19 | 1.00 |
| 20 | 7 | 1.14 | 1.68 | 0.63 | 0 | 4 | 0.37 |
| 21 | 7 | 3.43 | 4.39 | 1.66 | 0 | 12 | 1.25 |
| 22 | 7 | 2.57 | 1.81 | 0.68 | 0 | 6 | 2.33 |
| 23 | 7 | 2.14 | 2.85 | 1.08 | 0 | 8 | 1.75 |
| 24 | 7 | 1.86 | 2.61 | 0.99 | 0 | 6 | 0.37 |
| 25 | 7 | 7.71 | 5.02 | 1.90 | 2 | 17 | 7.00 |
| 26 | 7 | 9.86 | 11.45 | 4.33 | 0 | 34 | 8.00 |
| 27 | 7 | 1.71 | 1.25 | 0.47 | 0 | 3 | 2.00 |
| 28 | 7 | 0.71 | 0.95 | 0.36 | 0 | 2 | 0.37 |
| 29 | 7 | 2.14 | 1.95 | 0.74 | 0 | 5 | 2.00 |
| 30 | 7 | 3.14 | 3.48 | 1.32 | 0 | 8 | 2.00 |
| 31 | 7 | 16.57 | 15.41 | 5.83 | 2 | 41 | 9.00 |
| 32 | 6 | 14.17 | 16.21 | 6.62 | 0 | 41 | 4.50 |
| 33 | 6 | 11.17 | 10.32 | 4.21 | 3 | 30 | 4.50 |
| 34 | 6 | 9.17 | 4.53 | 1.85 | 3 | 16 | 9.00 |
| 35 | 5 | 5.20 | 4.55 | 2.03 | 2 | 13 | 4.00 |
| 36 | 5 | 10.00 | 8.12 | 3.63 | 4 | 24 | 6.50 |
| 37 | 5 | 3.60 | 1.82 | 0.81 | 1 | 6 | 3.75 |
| 38 | 5 | 3.80 | 3.19 | 1.43 | 0 | 8 | 3.00 |
| 39 | 4 | 2.75 | 3.59 | 1.80 | 0 | 8 | 1.50 |
| 40 | 4 | 0.75 | 1.50 | 0.75 | 0 | 3 | 0.50 |
| 41 | 1 | * | * | * | * | * | * |
| 42 | 1 | * | * | * | * | * | * |
| 43 | 5 | 3.00 | 5.61 | 2.51 | 0 | 13 | 0.75 |
| 44 | 5 | 3.60 | 3.91 | 1.75 | 0 | 9 | 3.00 |

TABLE B.5 continued

| Week | Number of Snails | Mean/ day | NUMBER OF CERCARIAE PRODUCED S.D. | S.E. | Range Min. | Max. | Median |
|---|---|---|---|---|---|---|---|
| 45 | 3 | 2.00 | 1.73 | 1.00 | 1 | 4 | 1.75 |
| 46 | 4 | 0.50 | 0.58 | 0.23 | 0 | 1 | 0.50 |
| 47 | 4 | 1.25 | 1.50 | 0.75 | 0 | 3 | 0.50 |
| 48 | 4 | 0.75 | 0.96 | 0.48 | 0 | 2 | 0.50 |
| 49 | 4 | 1.00 | 1.41 | 0.71 | 0 | 3 | 0.50 |
| 50 | 4 | 0.25 | 0.50 | 0.25 | 0 | 1 | 0.17 |
| 51 | 4 | 2.75 | 2.50 | 1.25 | 0 | 6 | 2.50 |
| 52 | 4 | 0.75 | 0.96 | 0.48 | 0 | 2 | 0.50 |
| 53 | 4 | 0.00 | 0.00 | 0.00 | 0 | 0 | 0.00 |
| 54 | 3 | 0.00 | 0.00 | 0.00 | 0 | 0 | 0.00 |
| 55 | 4 | 0.00 | 0.00 | 0.00 | 0 | 0 | 0.00 |
| 56 | 4 | 0.50 | 0.58 | 0.29 | 0 | 1 | 0.50 |
| 57 | 3 | 0.00 | 0.00 | 0.00 | 0 | 0 | 0.00 |

(*Insufficient data)

## TABLE B.6

The number of *S. haematobium* cercariae produced from *B. globosus* (Ngala) , showing two peaks in output, on sample days each week from first cercarial emergence.

| Week | Number of Snails | NUMBER OF CERCARIAE PRODUCED Mean/ day | S.D. | S.E. | Range Min. | Max. | Median |
|---|---|---|---|---|---|---|---|
| 1 | 12 | 135.92 | 89.51 | 25.84 | 40 | 265 | 89.50 |
| 2 | 12 | 199.67 | 139.33 | 40.22 | 51 | 467 | 123.50 |
| 3 | 12 | 125.42 | 87.49 | 25.26 | 9 | 293 | 99.50 |
| 4 | 12 | 285.17 | 185.51 | 53.55 | 38 | 605 | 264.00 |
| 5 | 12 | 109.75 | 110.61 | 31.93 | 14 | 341 | 54.50 |
| 6 | 12 | 245.58 | 137.31 | 39.64 | 53 | 492 | 246.00 |
| 7 | 12 | 318.50 | 227.15 | 65.57 | 57 | 718 | 195.50 |
| 8 | 12 | 107.00 | 82.50 | 23.81 | 15 | 296 | 86.50 |
| 9 | 11 | 115.45 | 120.41 | 36.31 | 3 | 409 | 79.00 |
| 10 | 12 | 47.42 | 50.42 | 14.55 | 6 | 149 | 22.50 |
| 11 | 12 | 78.17 | 62.32 | 17.99 | 0 | 169 | 58.00 |
| 12 | 12 | 19.83 | 13.58 | 3.92 | 1 | 45 | 17.50 |
| 13 | 10 | 16.60 | 12.51 | 3.96 | 1 | 41 | 15.50 |
| 14 | 12 | 7.50 | 10.72 | 3.10 | 0 | 36 | 2.50 |
| 15 | 9 | 8.56 | 7.42 | 2.47 | 0 | 26 | 7.75 |
| £16 | 3 | 30.33 | 26.63 | 15.38 | 1 | 53 | 37.00 |
| 17 | 11 | 9.10 | 6.12 | 1.85 | 0 | 19 | 8.75 |
| £18 | 3 | 14.00 | 24.25 | 14.00 | 0 | 42 | 10.50 |
| 19 | 12 | 14.58 | 26.70 | 7.71 | 0 | 96 | 4.50 |
| 20 | 12 | 4.08 | 5.66 | 1.64 | 0 | 17 | 0.50 |
| 21 | 12 | 4.33 | 7.62 | 2.20 | 0 | 22 | 0.71 |
| 22 | 12 | 6.67 | 9.38 | 2.71 | 0 | 34 | 3.17 |
| 23 | 12 | 5.17 | 4.63 | 1.34 | 0 | 16 | 3.50 |
| 24 | 12 | 14.83 | 44.88 | 12.95 | 0 | 157 | 1.50 |
| 25 | 12 | 19.25 | 30.36 | 8.76 | 0 | 100 | 6.50 |
| 26 | 12 | 15.08 | 14.62 | 4.22 | 0 | 37 | 7.50 |
| 27 | 11 | 21.45 | 41.32 | 12.46 | 0 | 142 | 7.00 |
| 28 | 12 | 62.08 | 95.77 | 27.65 | 0 | 276 | 4.50 |
| 29 | 12 | 92.00 | 123.37 | 35.61 | 0 | 392 | 30.50 |
| 30 | 12 | 136.92 | 162.81 | 47.00 | 15 | 592 | 63.50 |
| 31 | 12 | 105.08 | 128.48 | 37.09 | 10 | 410 | 54.00 |
| 32 | 12 | 111.58 | 104.27 | 30.10 | 9 | 367 | 84.00 |
| 33 | 11 | 172.27 | 160.64 | 48.43 | 3 | 506 | 132.00 |
| 34 | 11 | 335.09 | 342.46 | 103.26 | 3 | 1046 | 231.00 |
| 35 | 10 | 279.10 | 266.94 | 84.41 | 3 | 901 | 239.50 |
| 36 | 10 | 293.50 | 244.43 | 77.30 | 11 | 881 | 240.00 |
| 37 | 10 | 204.80 | 206.04 | 65.16 | 0 | 659 | 105.00 |
| 38 | 8 | 227.50 | 177.82 | 62.87 | 10 | 585 | 190.00 |
| 39 | 8 | 223.75 | 204.91 | 72.44 | 4 | 574 | 123.00 |
| 40 | 8 | 239.75 | 177.67 | 62.82 | 3 | 507 | 180.00 |
| 41 | 1 | * | * | * | * | * | * |
| 42 | 1 | * | * | * | * | * | * |
| 43 | 7 | 203.43 | 121.75 | 46.02 | 55 | 352 | 203.00 |

TABLE B.6 continued

| Week | Number of Snails | NUMBER OF CERCARIAE PRODUCED Mean/ day | S.D. | S.E. | Range Min. | Max. | Median |
|---|---|---|---|---|---|---|---|
| 44 | 6 | 93.00 | 81.52 | 33.28 | 14 | 237 | 81.50 |
| 45 | 6 | 119.33 | 69.67 | 28.44 | 36 | 209 | 88.50 |
| 46 | 6 | 116.67 | 88.26 | 36.03 | 20 | 233 | 56.50 |
| 47 | 4 | 90.75 | 78.74 | 39.37 | 13 | 187 | 56.50 |
| 48 | 4 | 80.50 | 81.67 | 40.84 | 2 | 152 | 19.00 |
| 49 | 4 | 31.50 | 26.20 | 13.10 | 5 | 55 | 14.00 |

£ Limited data set.
* Insufficient data

# APPENDIX C

## Individual graphs of daily cercarial output at weekly intervals for each infected bulinid monitored (see Chapter 5)

**KEY:**

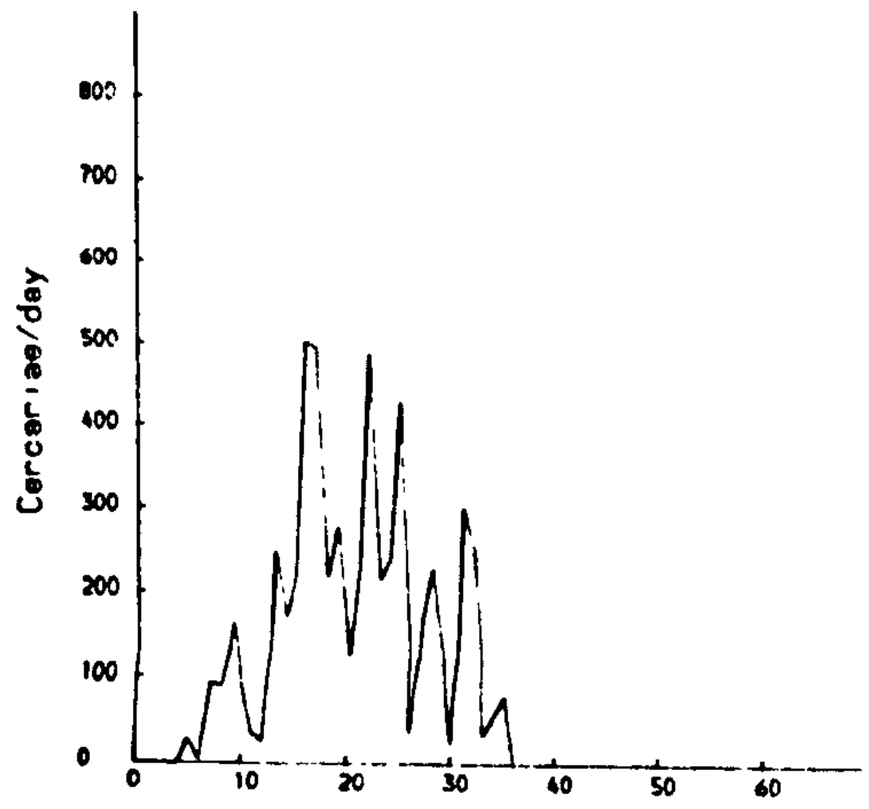
SBG = B. globosus Ngala infected with the 'globosus' strain of S. haematobium.

SBR= B. rohlfsi infected with the 'rohlfsi' strain of S.haematobium.

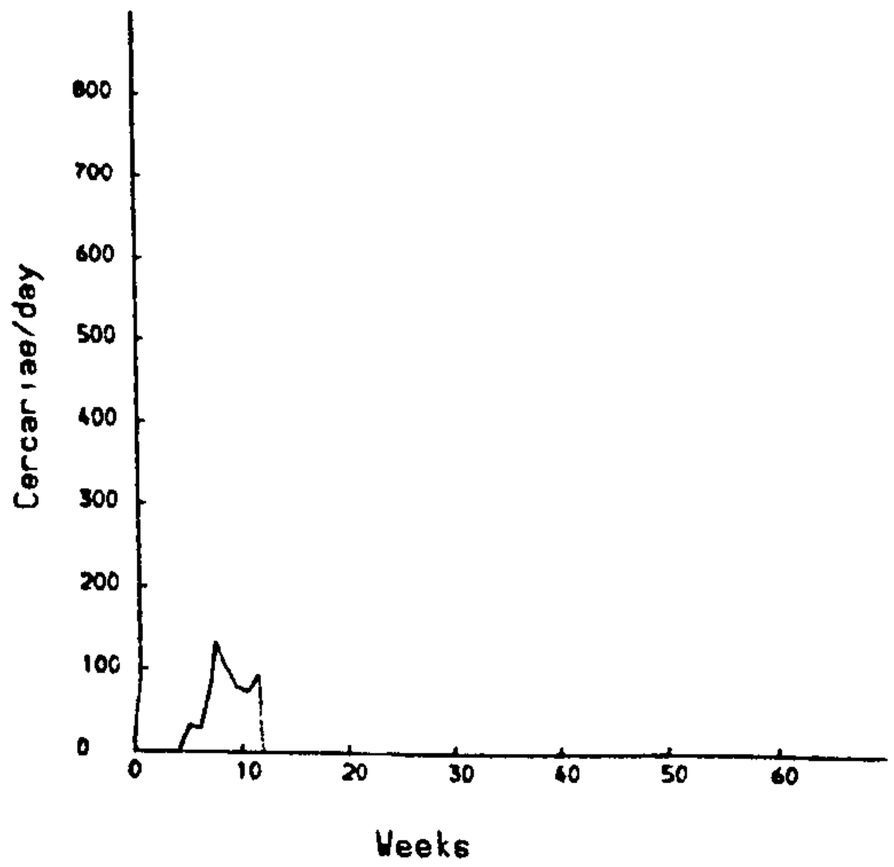
SBS (G) = B. senegalensis infected with the 'globosus' strain of S. haematobium.

SBS (R) = B. senegalensis infected with the 'rohlfsi' strain of S. haematobium.

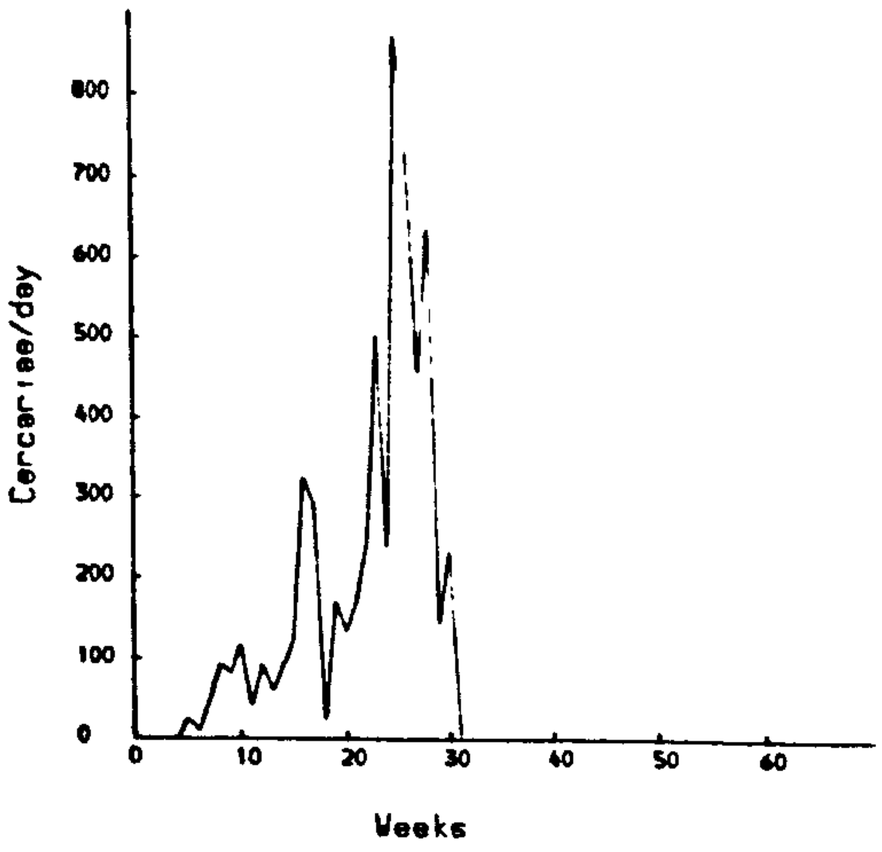
SBG 4



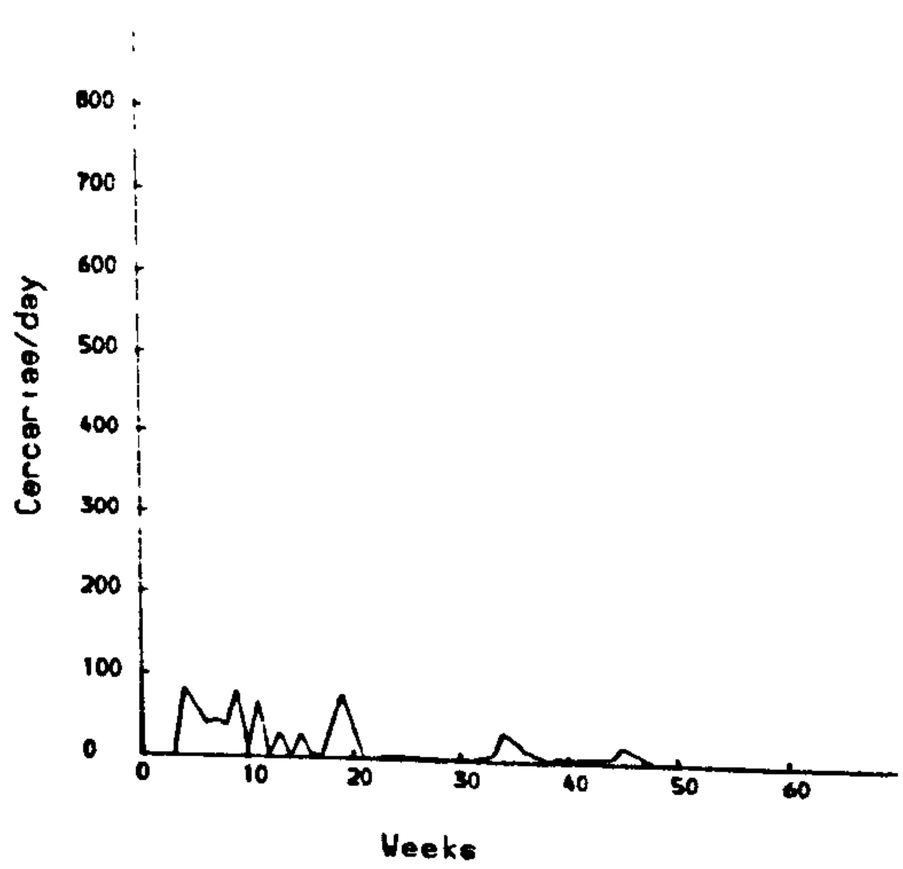

SBG 5



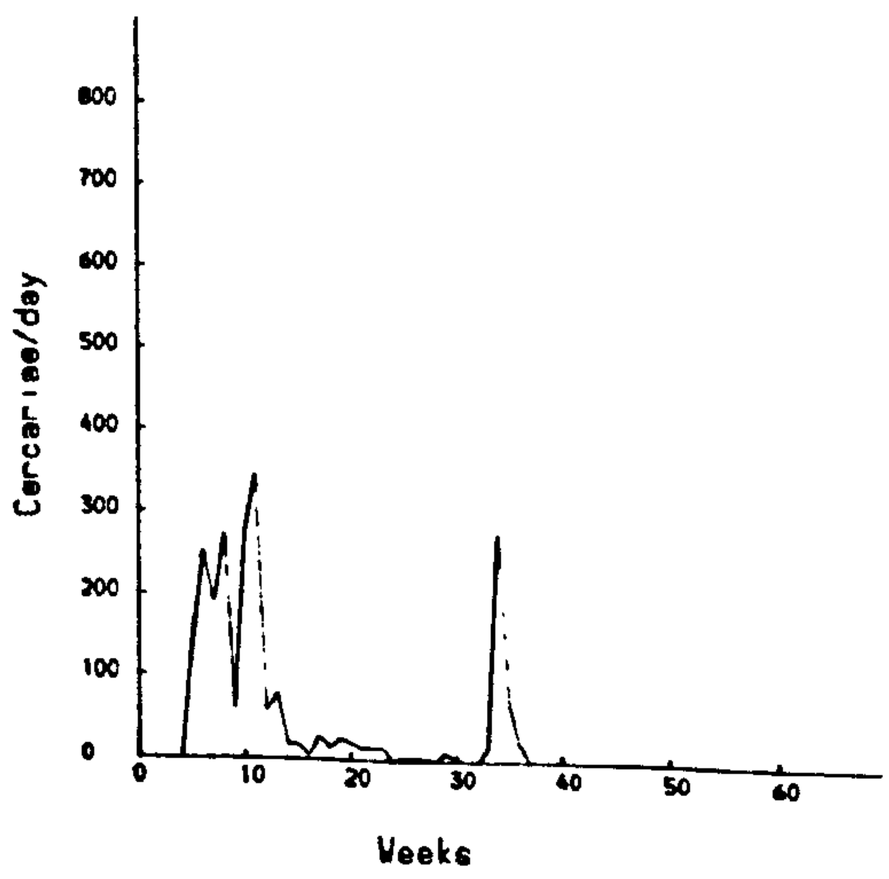

SBG 6



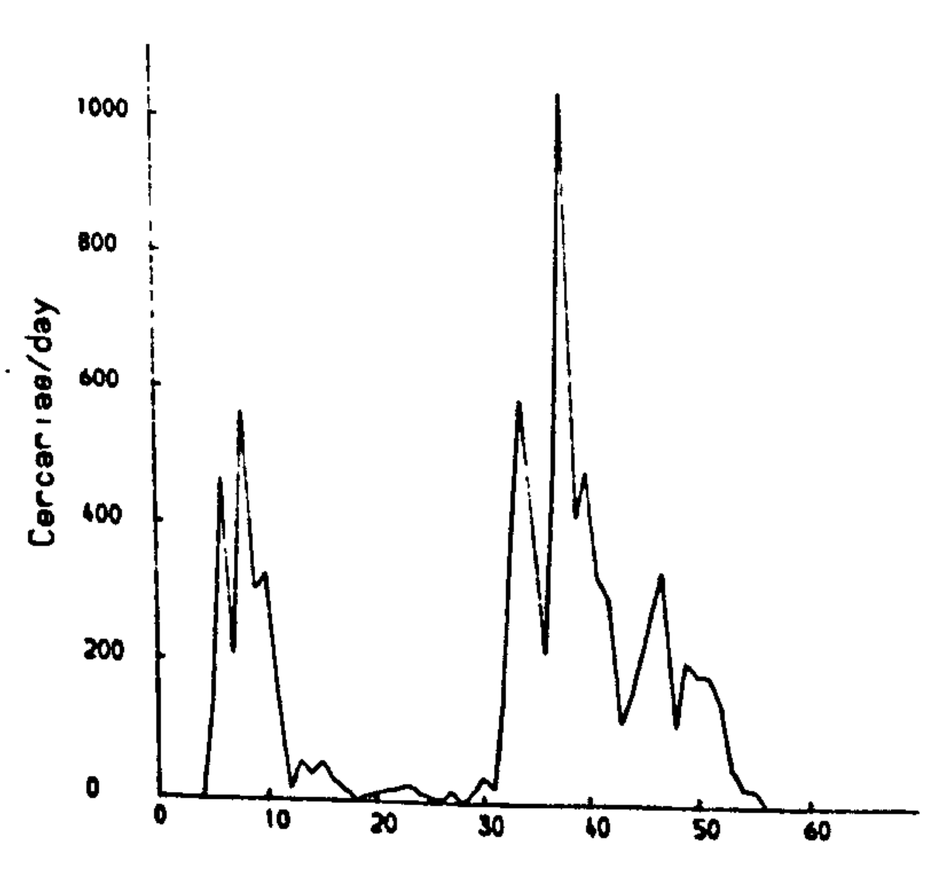

SBG 8

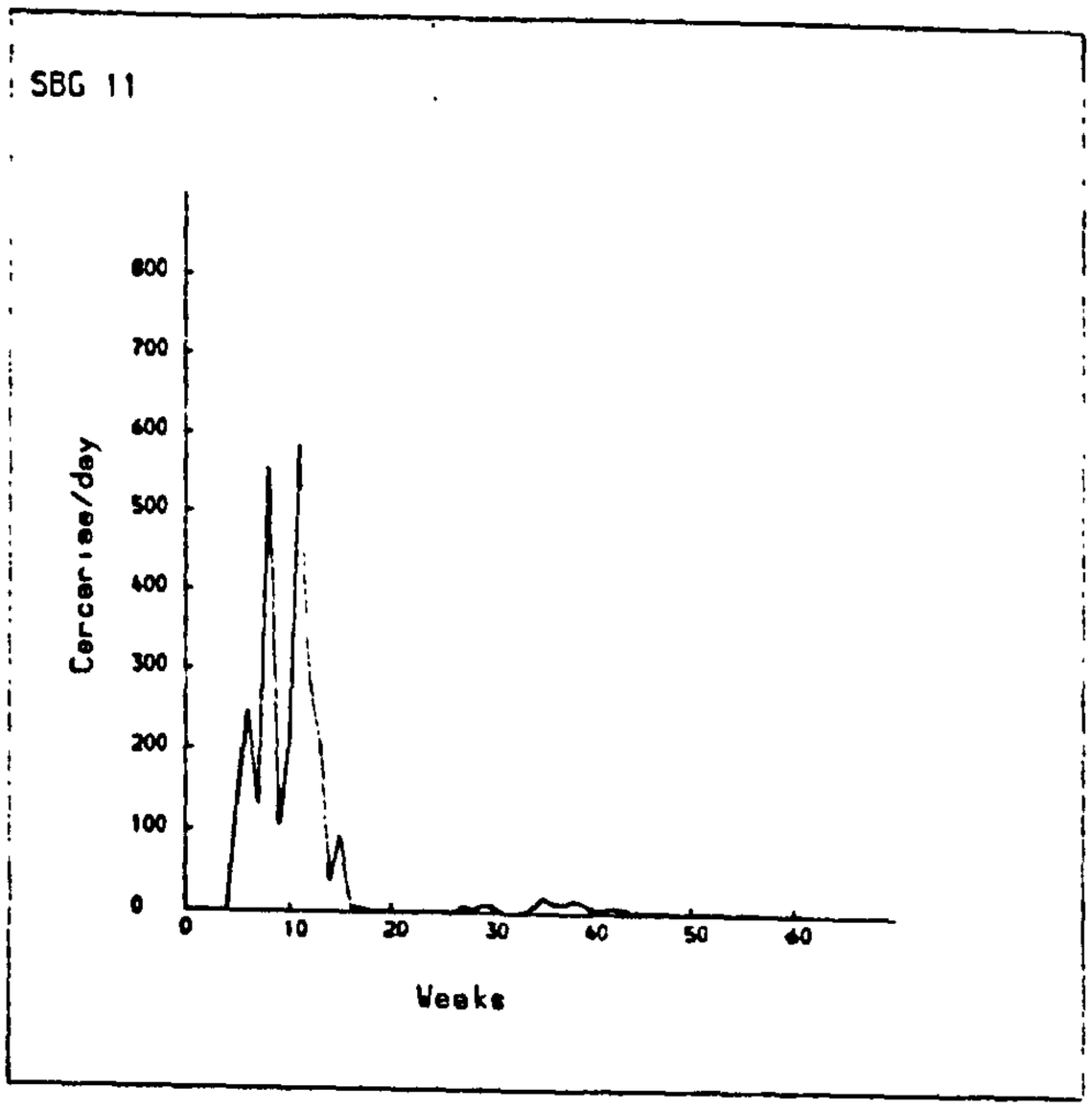


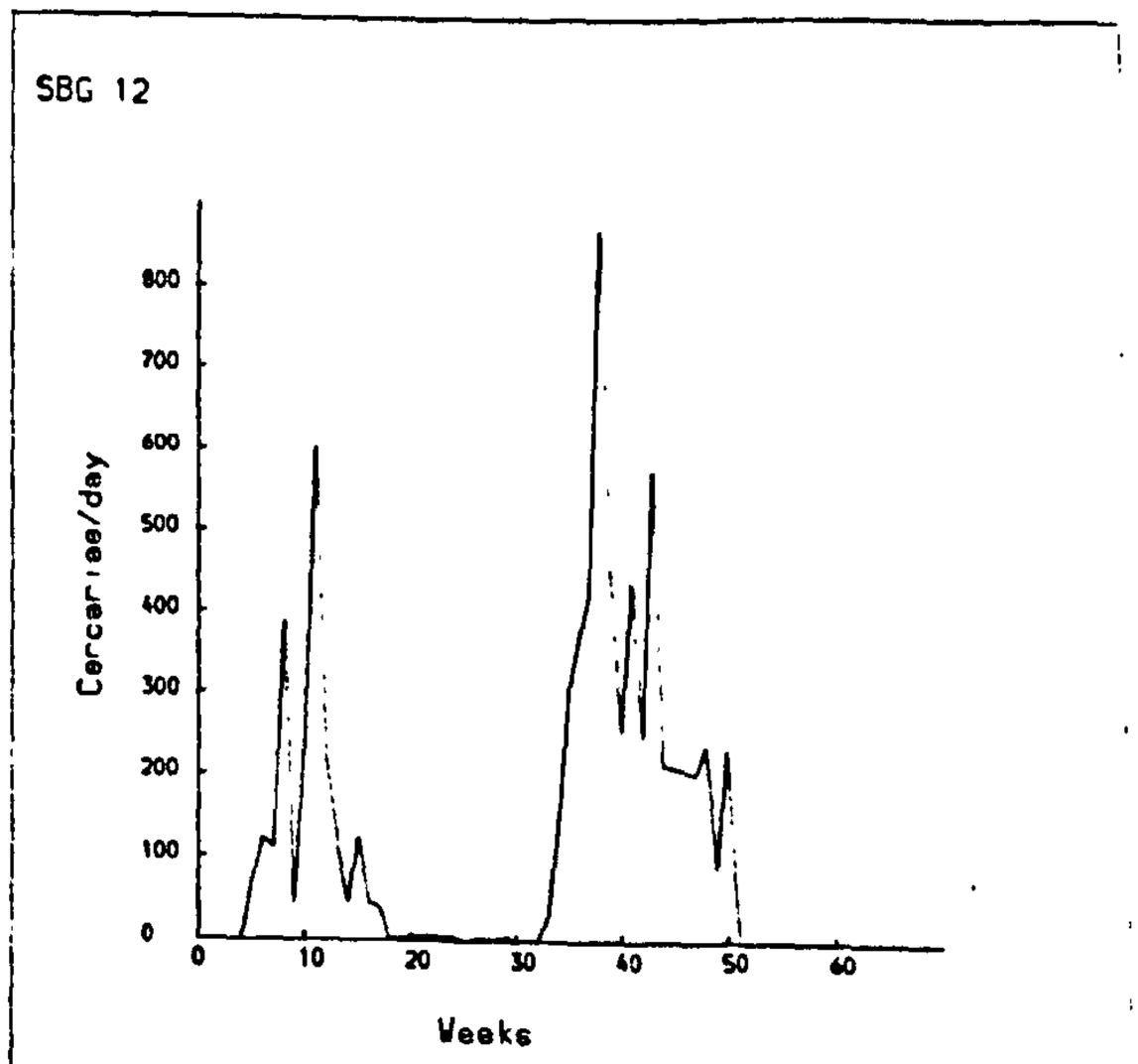

SBG 9

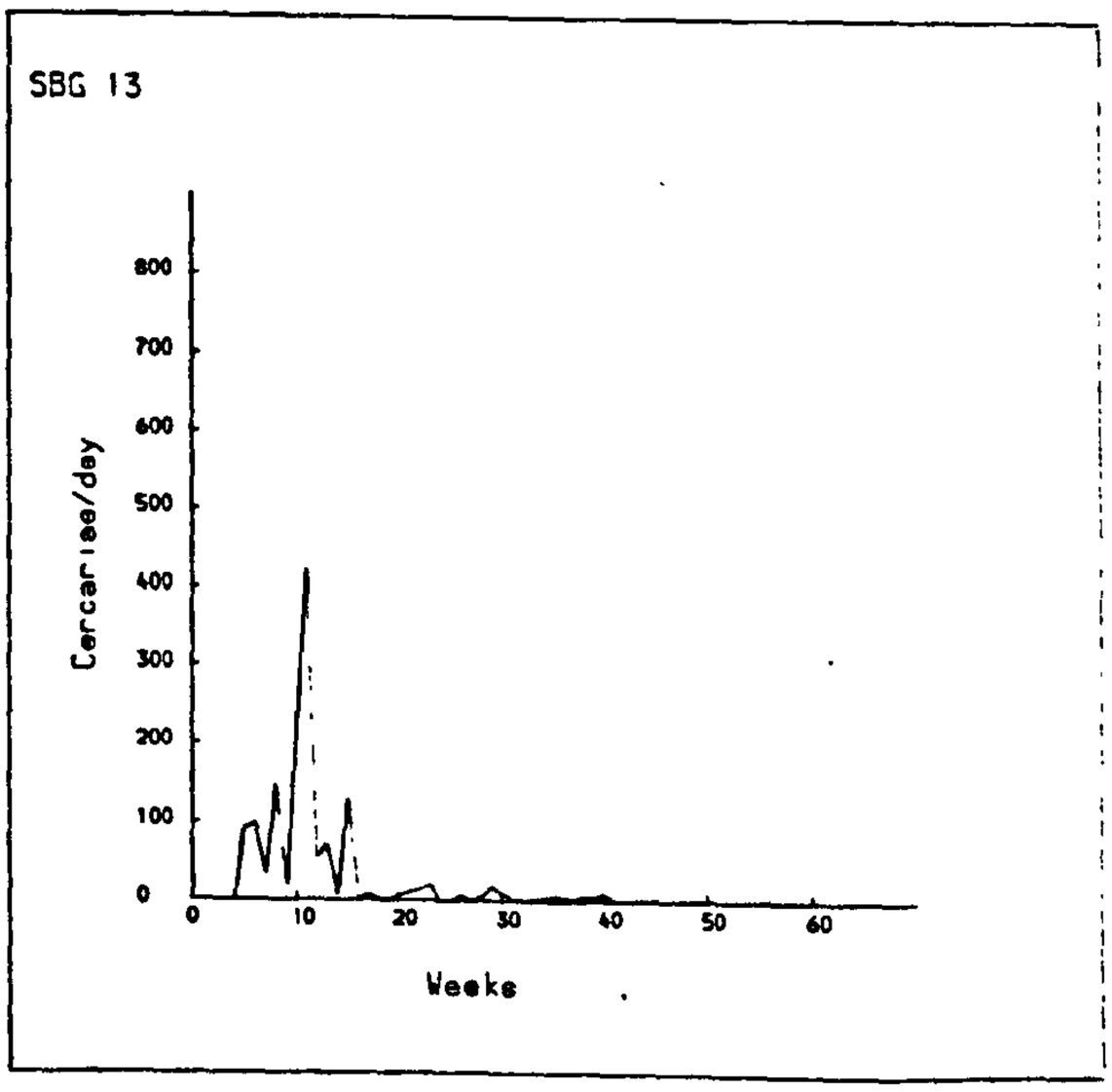


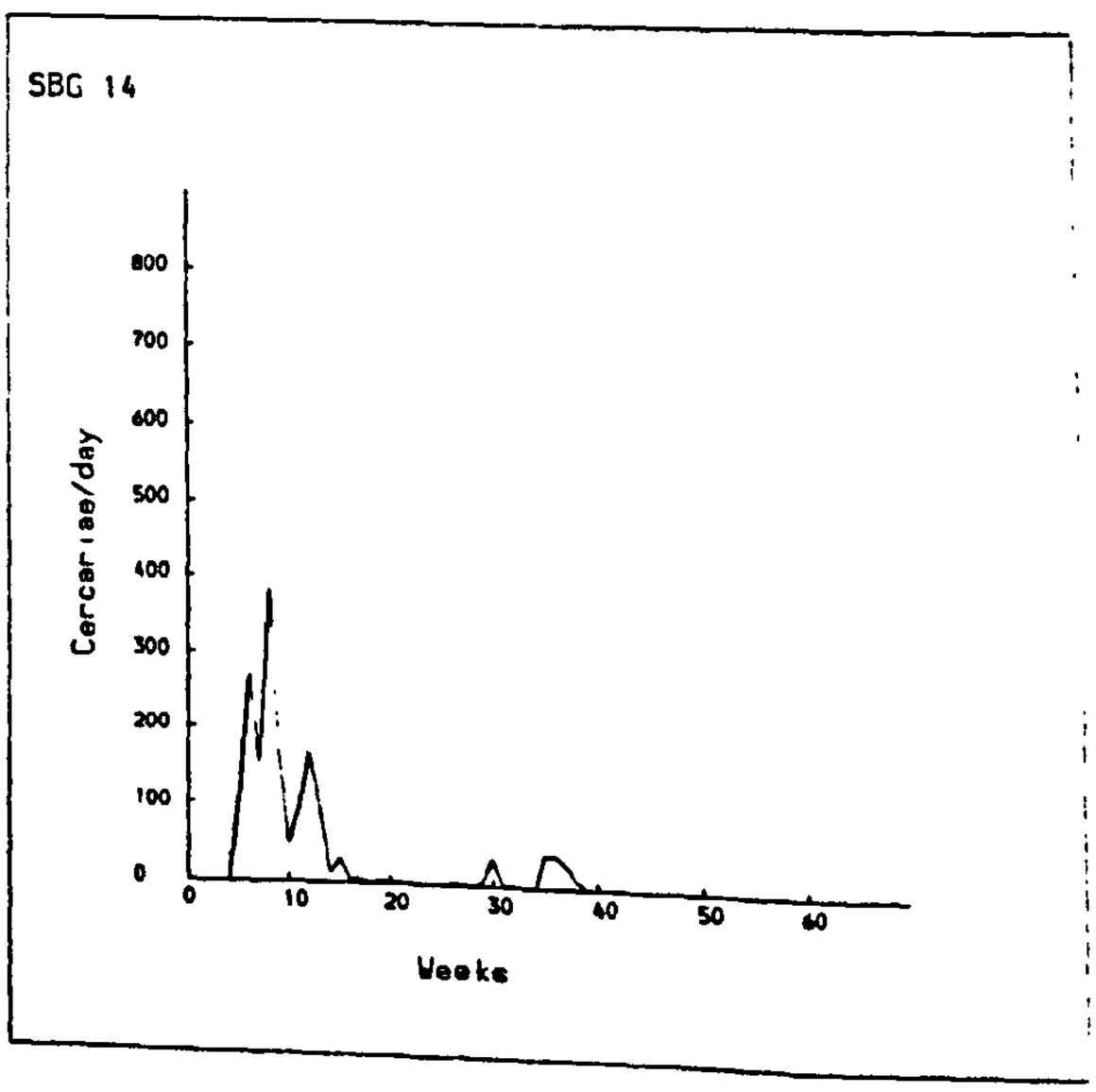

SBG 10

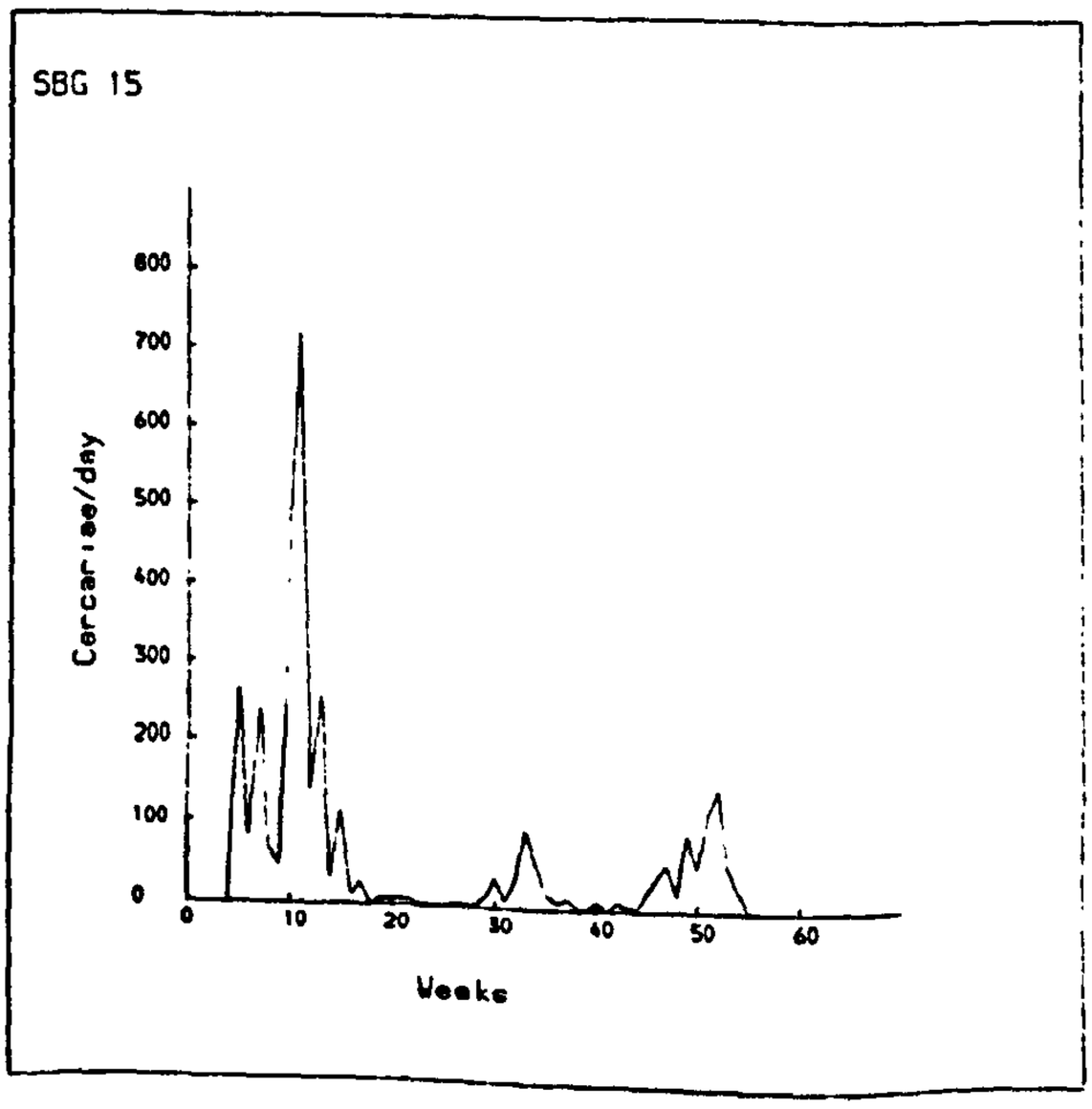


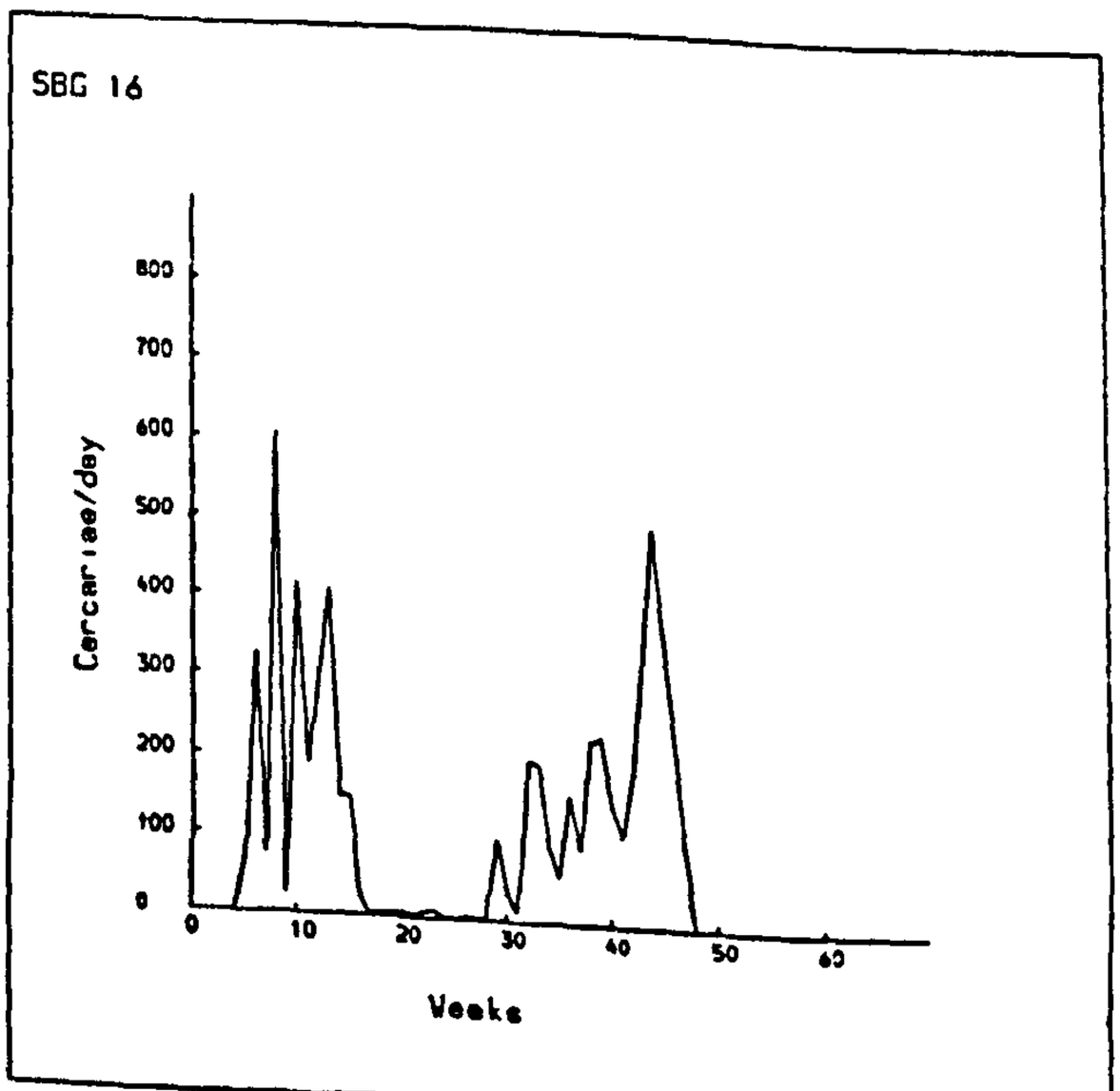

SBG 11
Cercariae/day
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Weeks


SBG 12
Cercariae/day
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Weeks

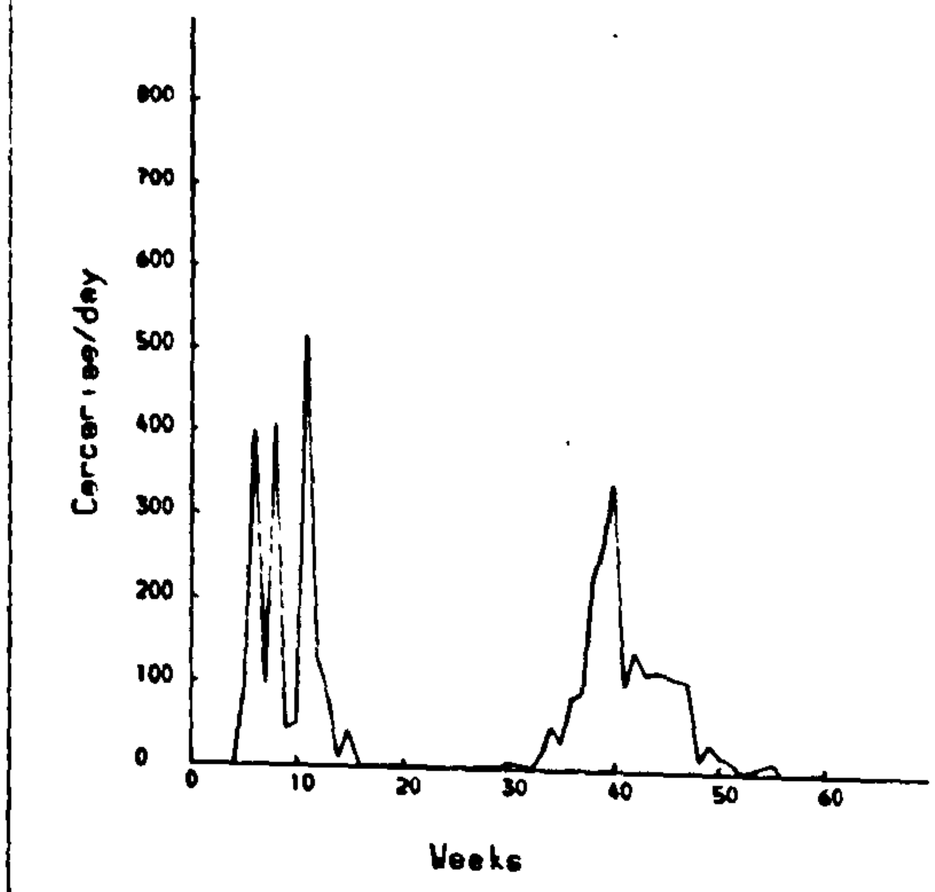

SBG 13
Cercariae/day
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Weeks


SBG 14
Cercariae/day
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Weeks

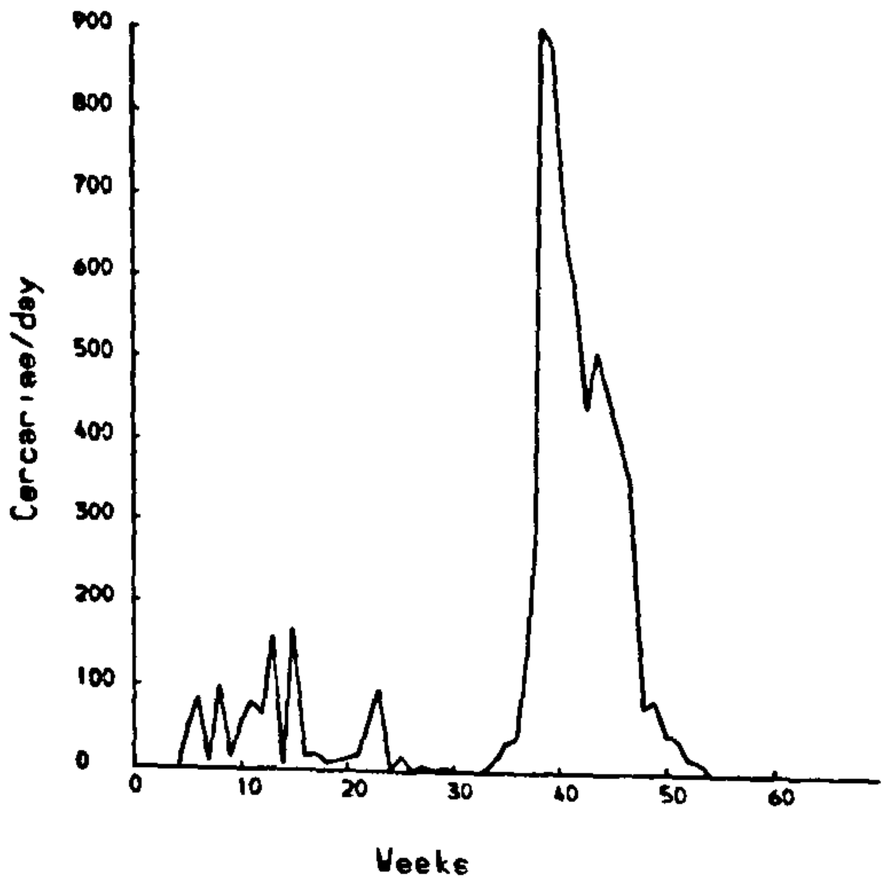

SBG 15
Cercariae/day
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Weeks


SBG 16
Cercariae/day
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Weeks

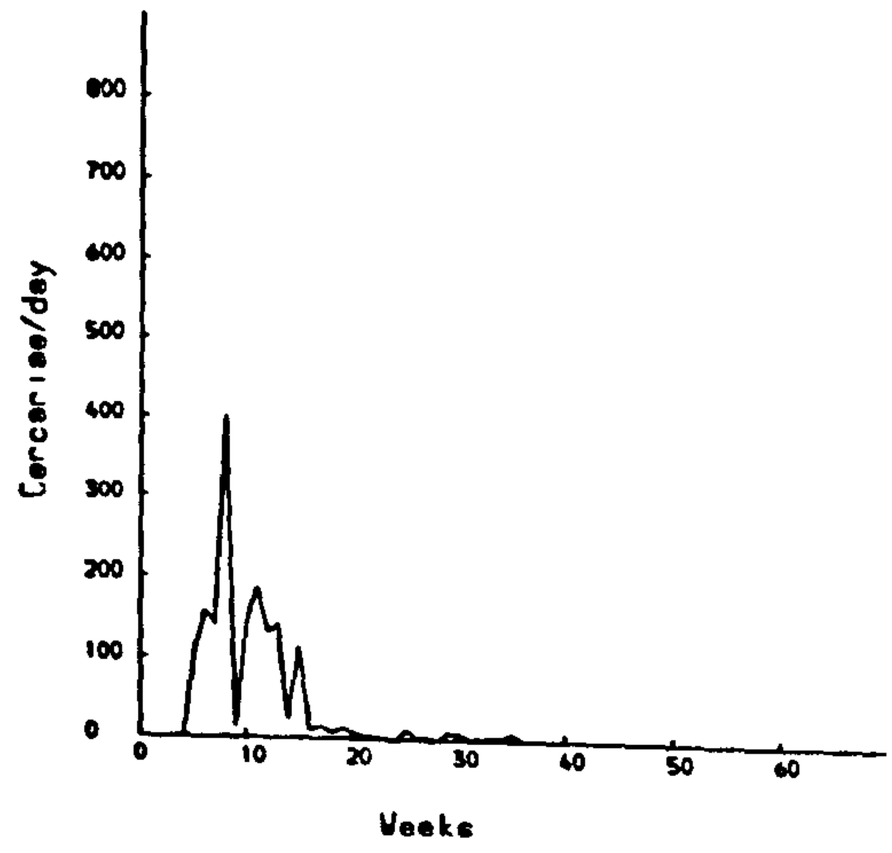
SBG 17



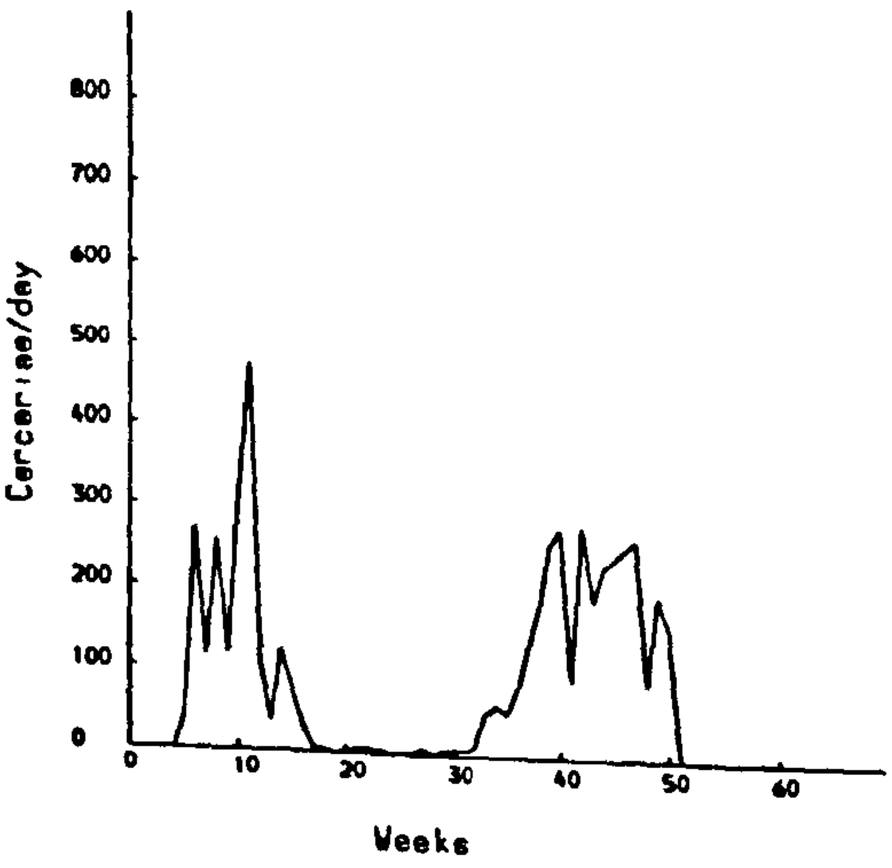

SBG 18



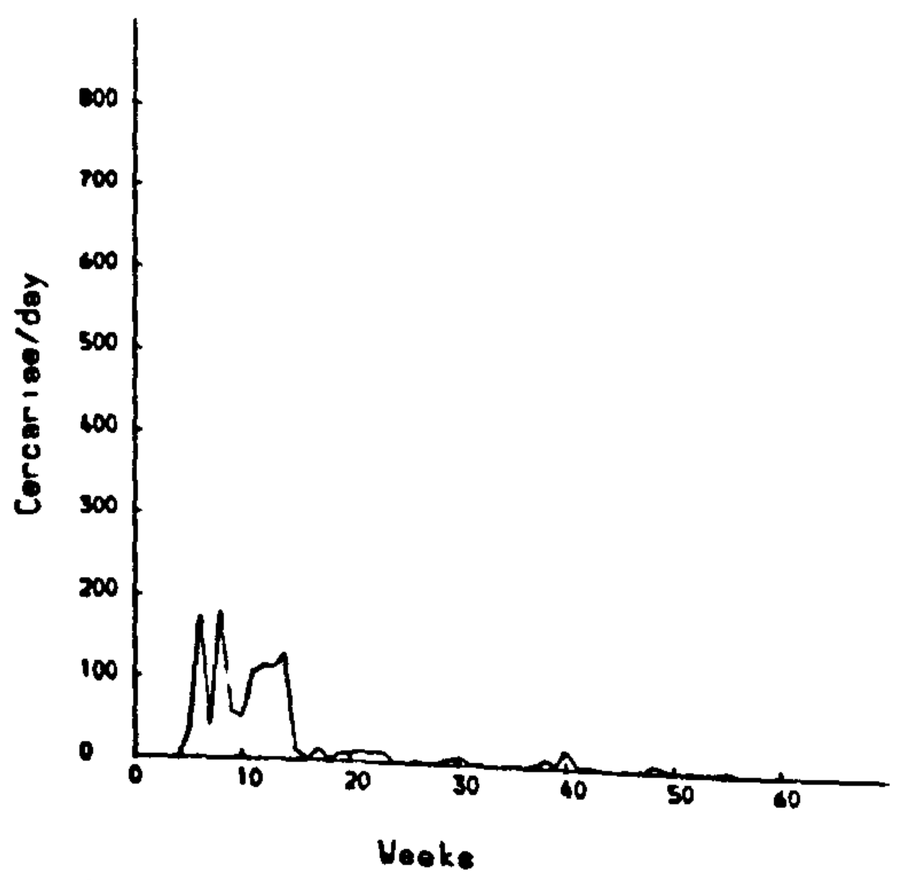

SBG 19



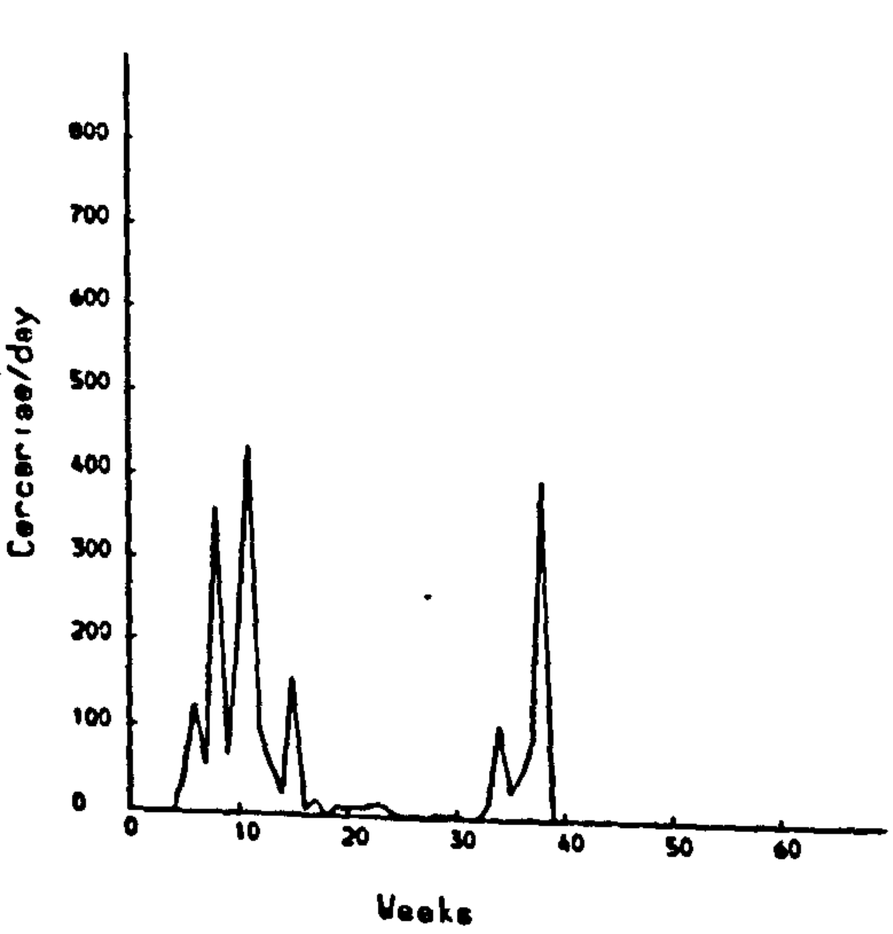

SBG 20



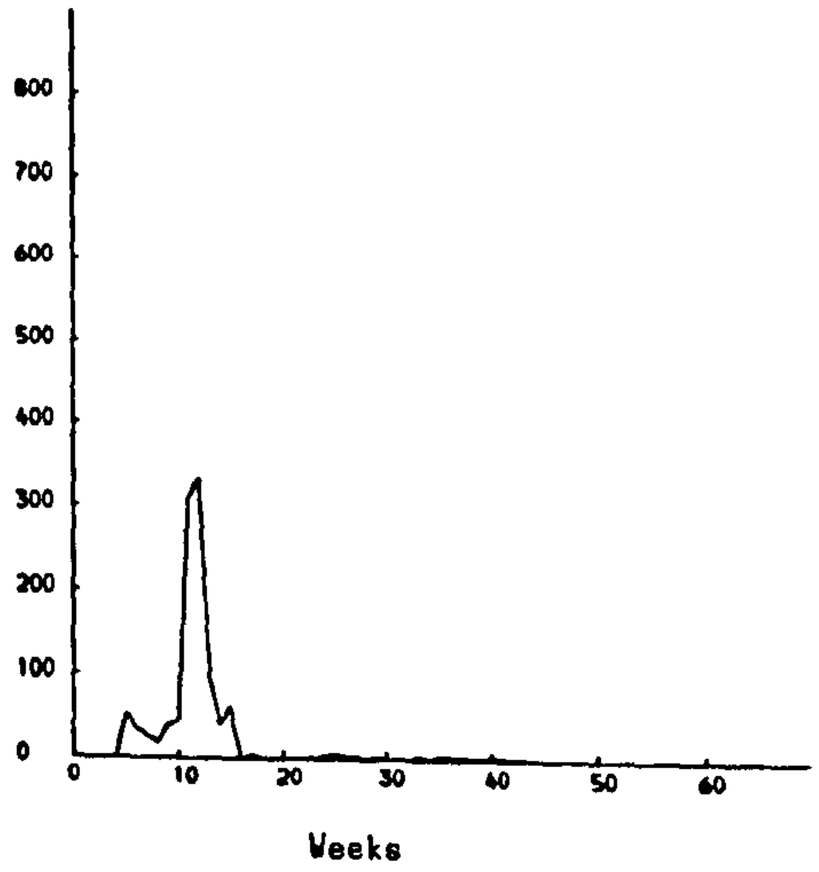

SBG 21



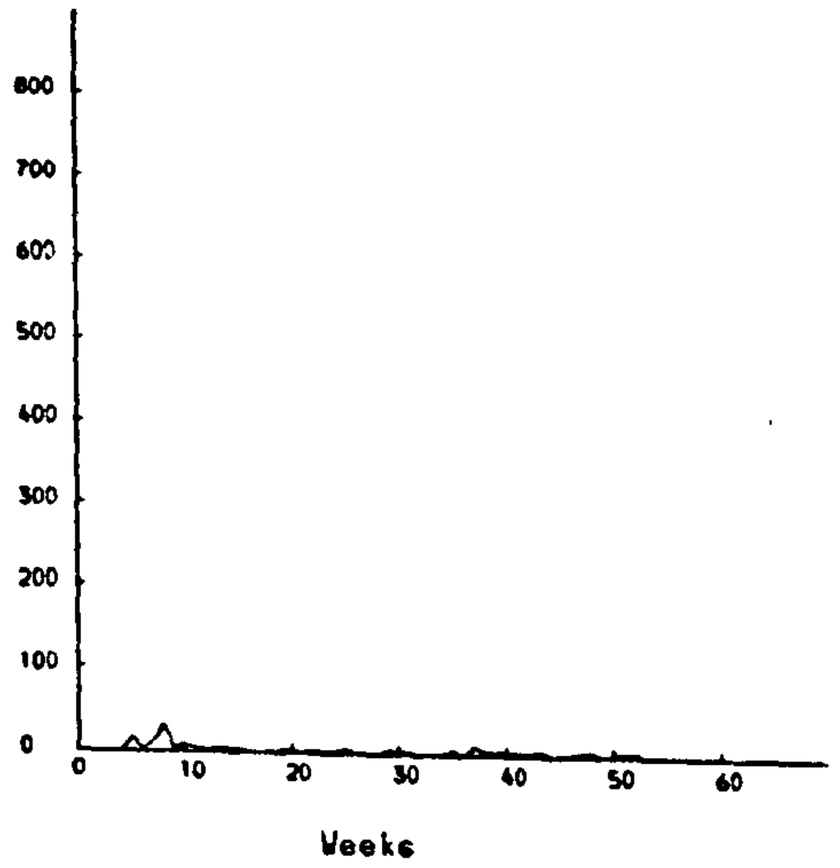

SBG 22



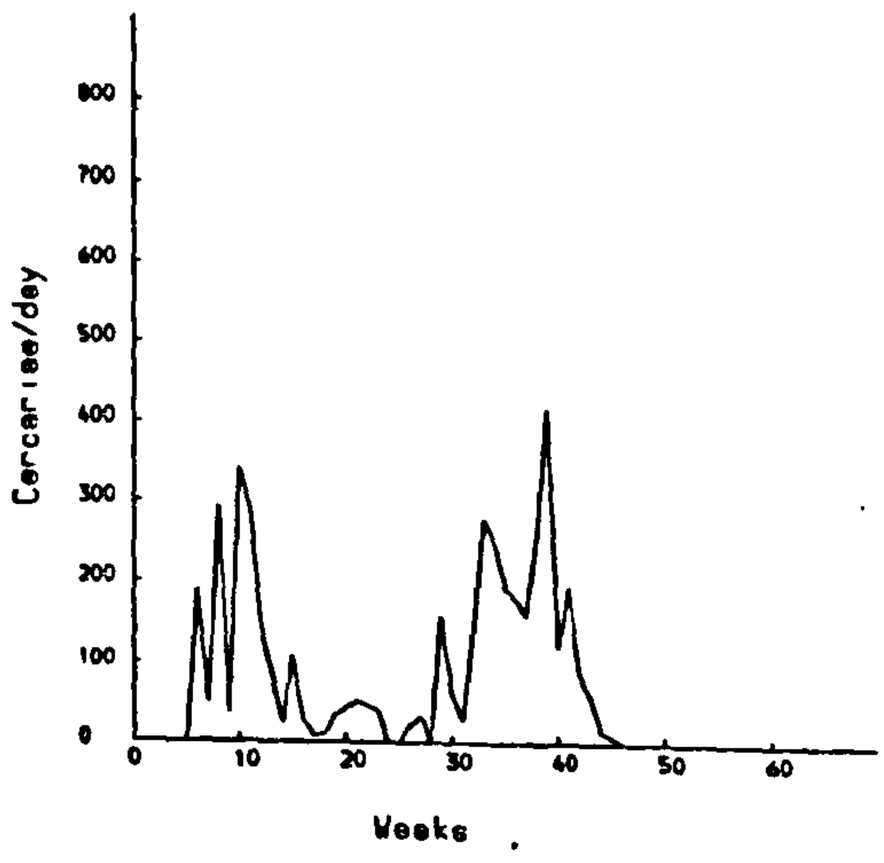
SBG 23



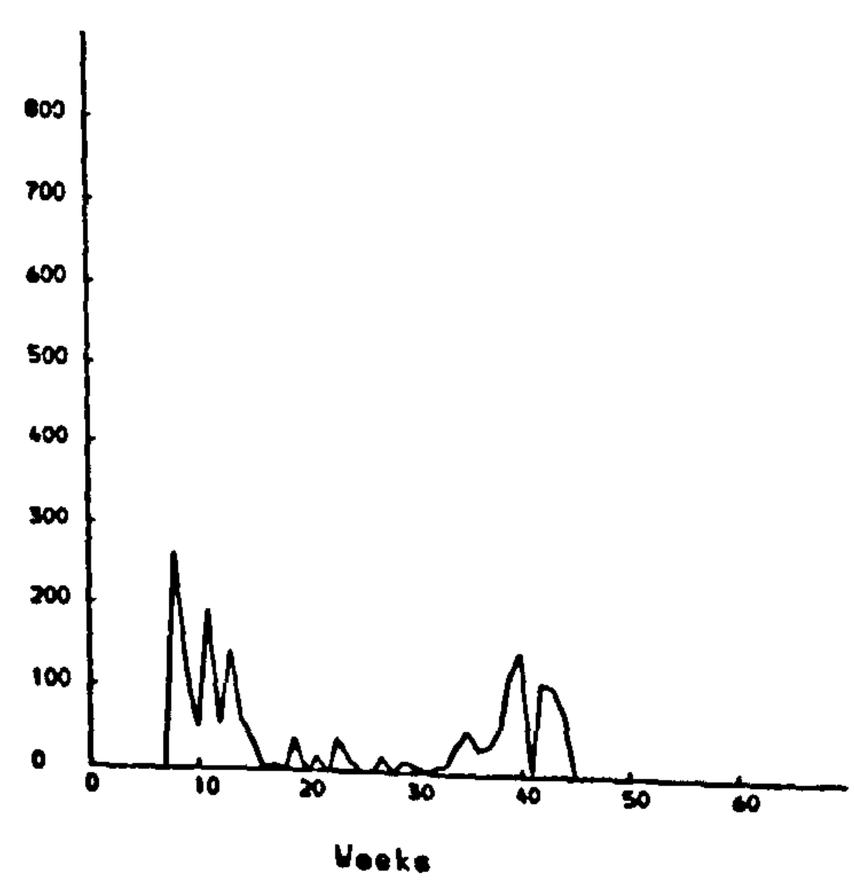

SBG 24



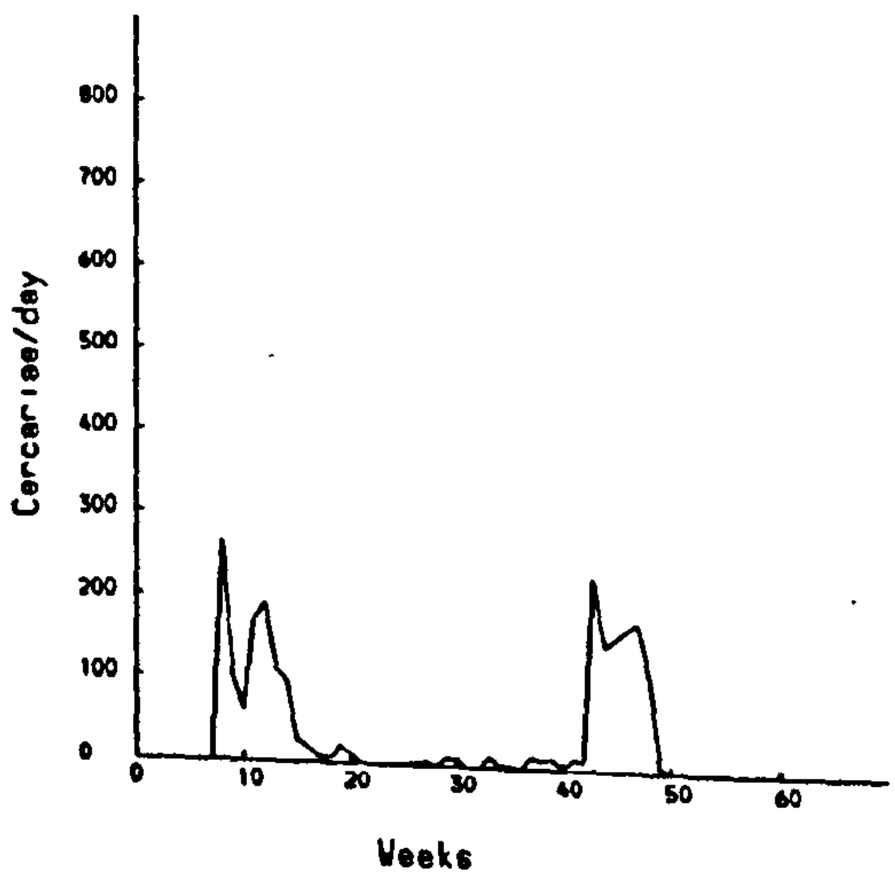

SBG 25



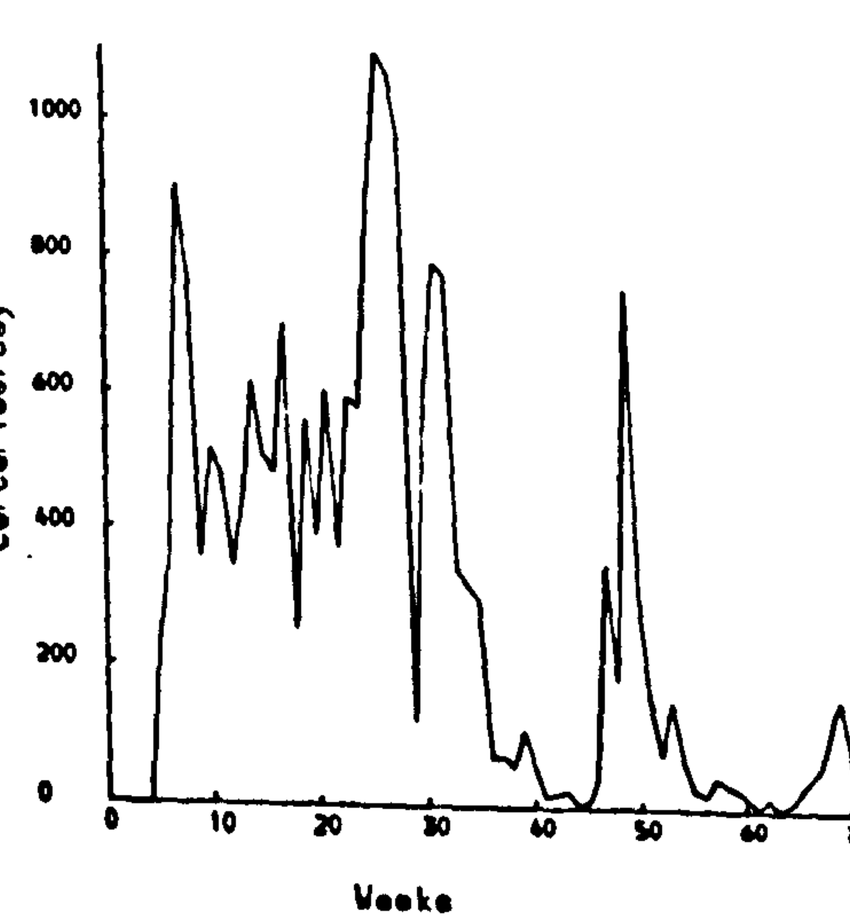

SBG 26

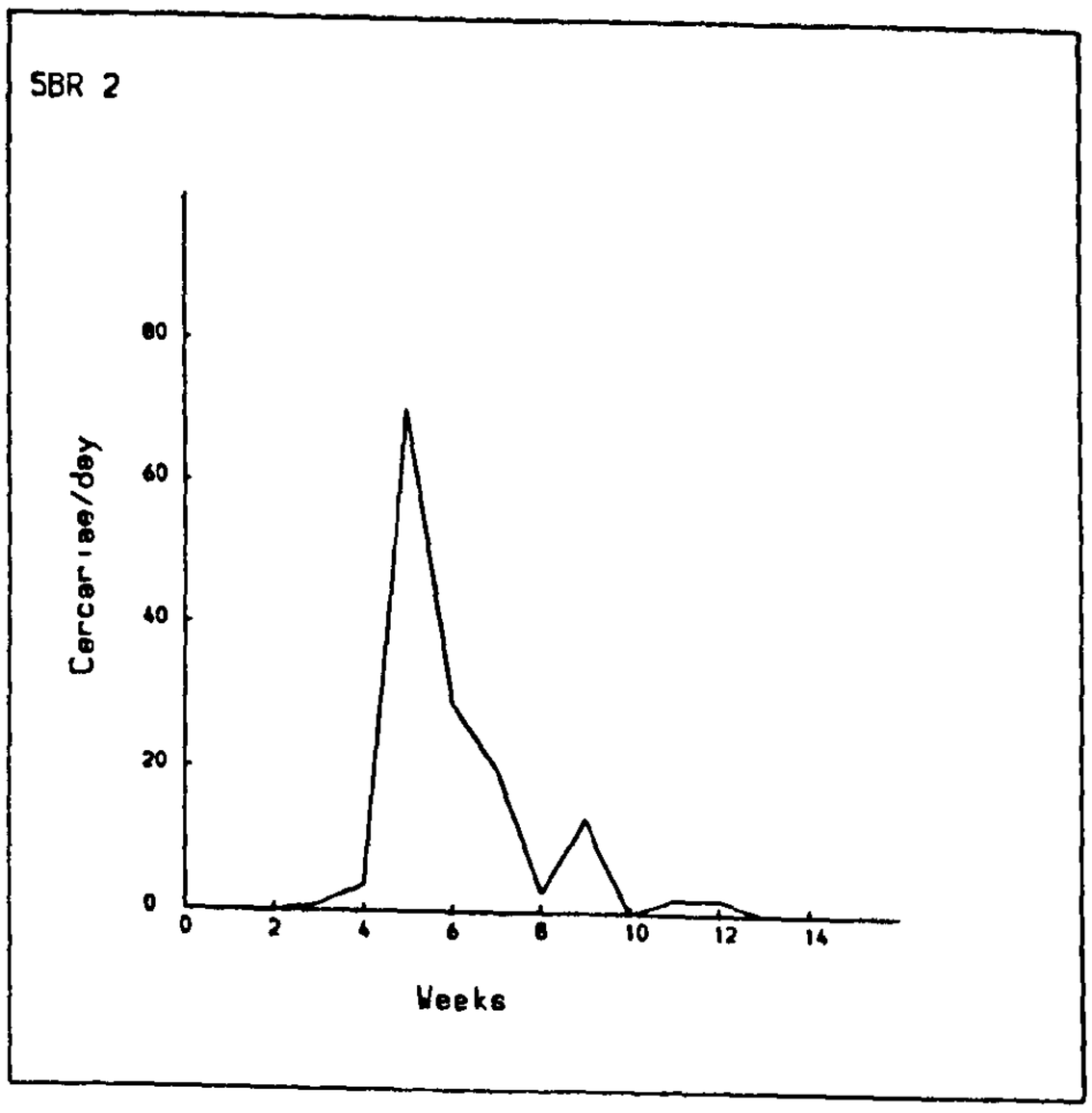


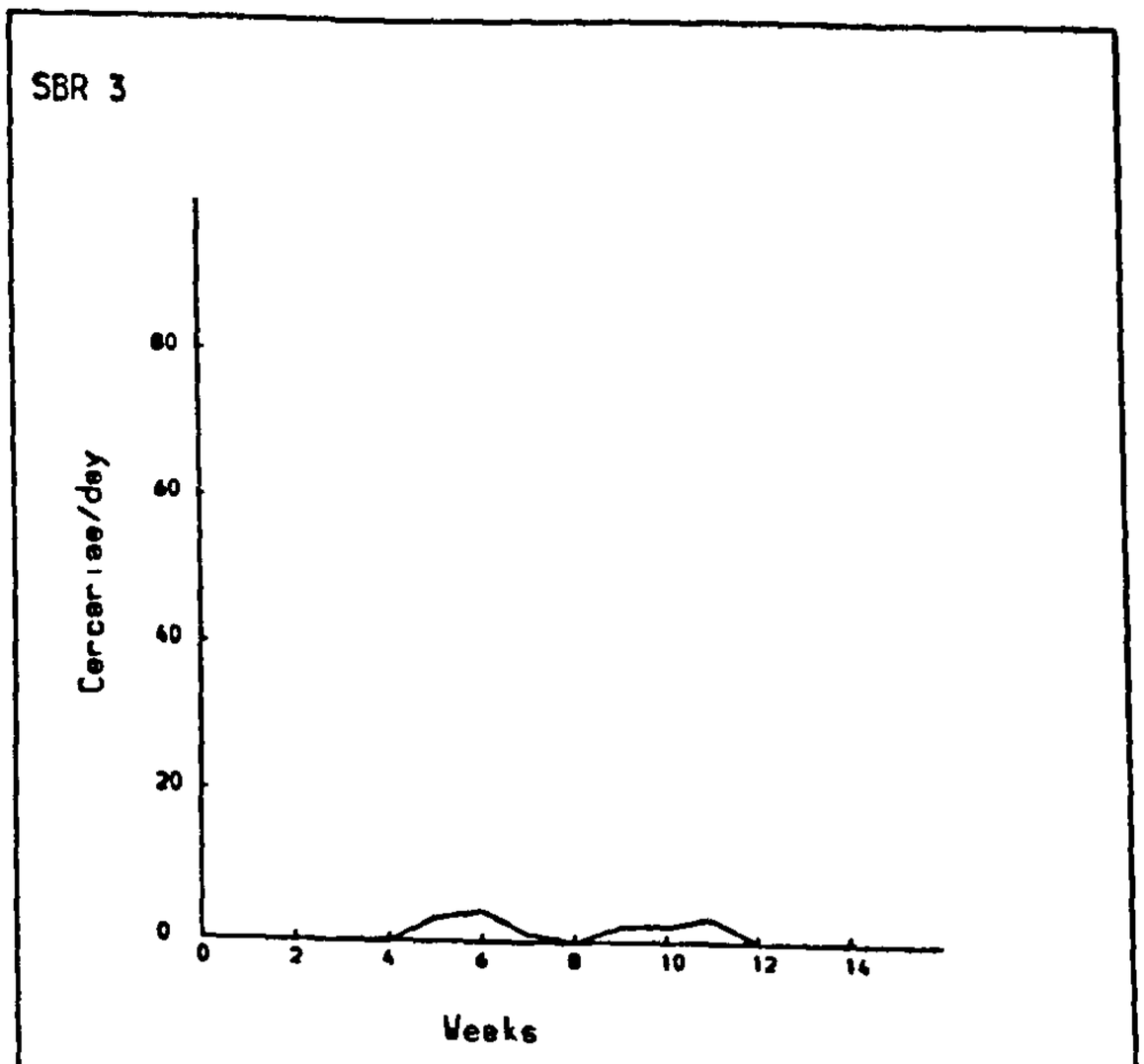

SBG 27

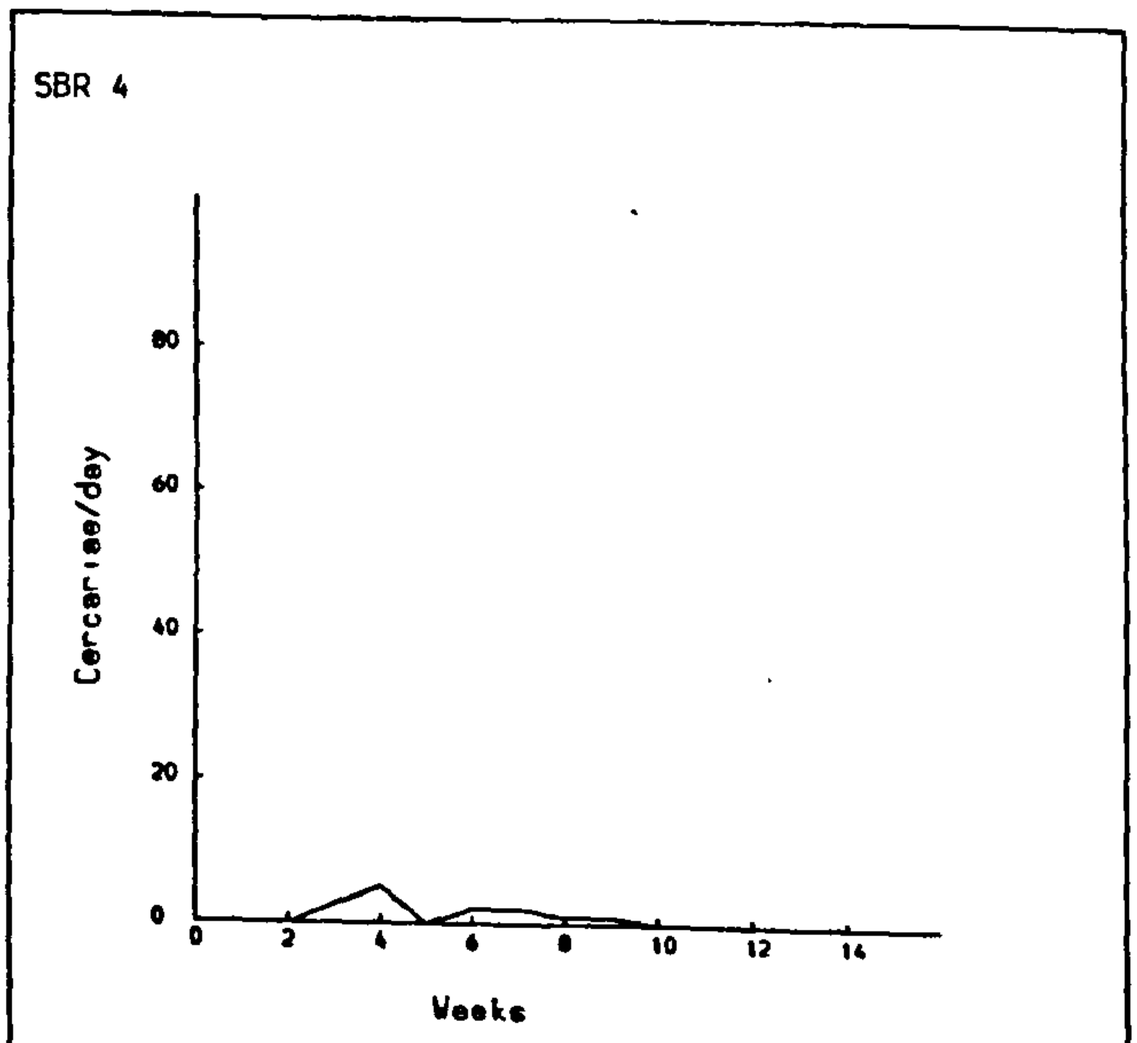


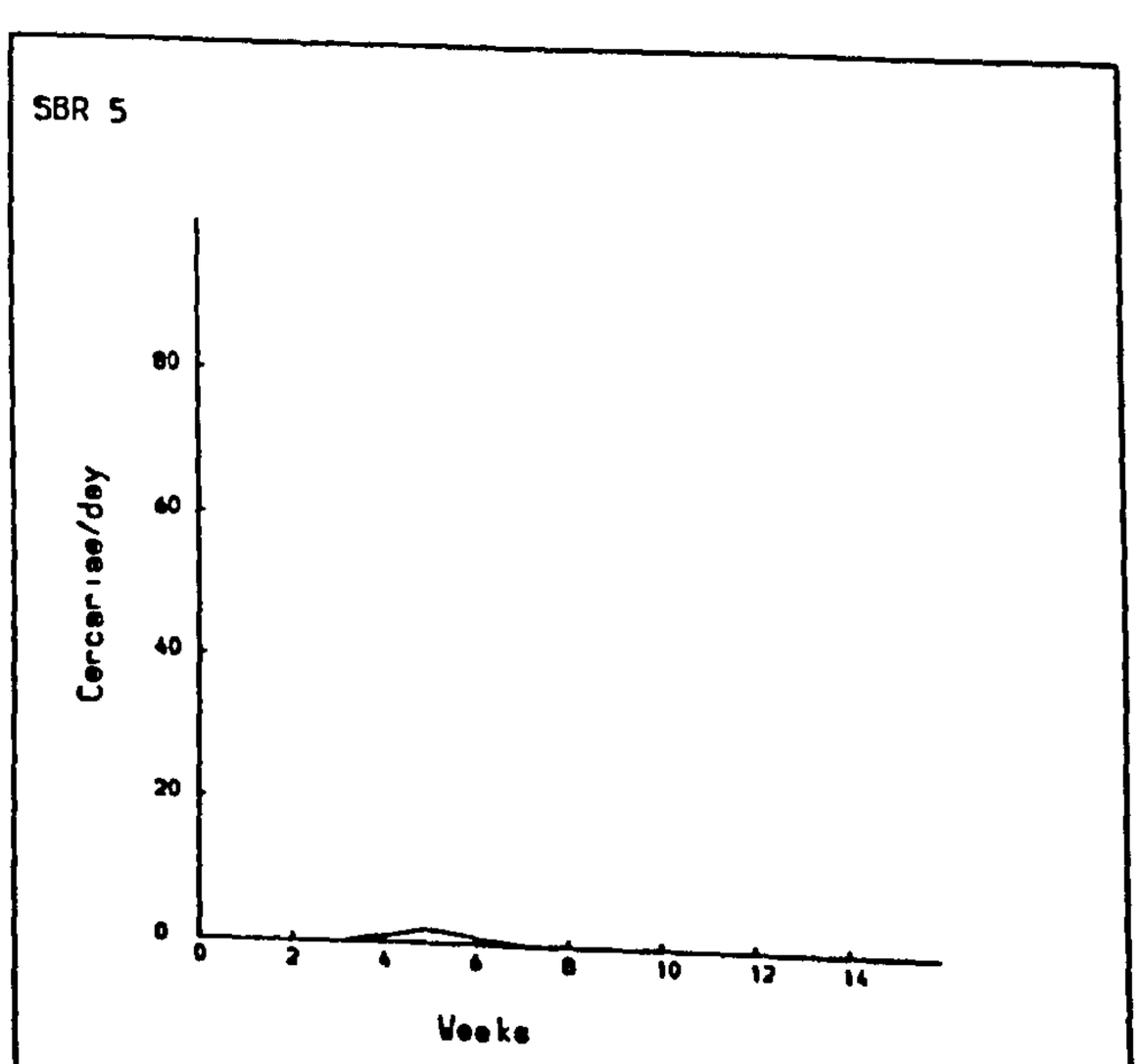

SBG 2

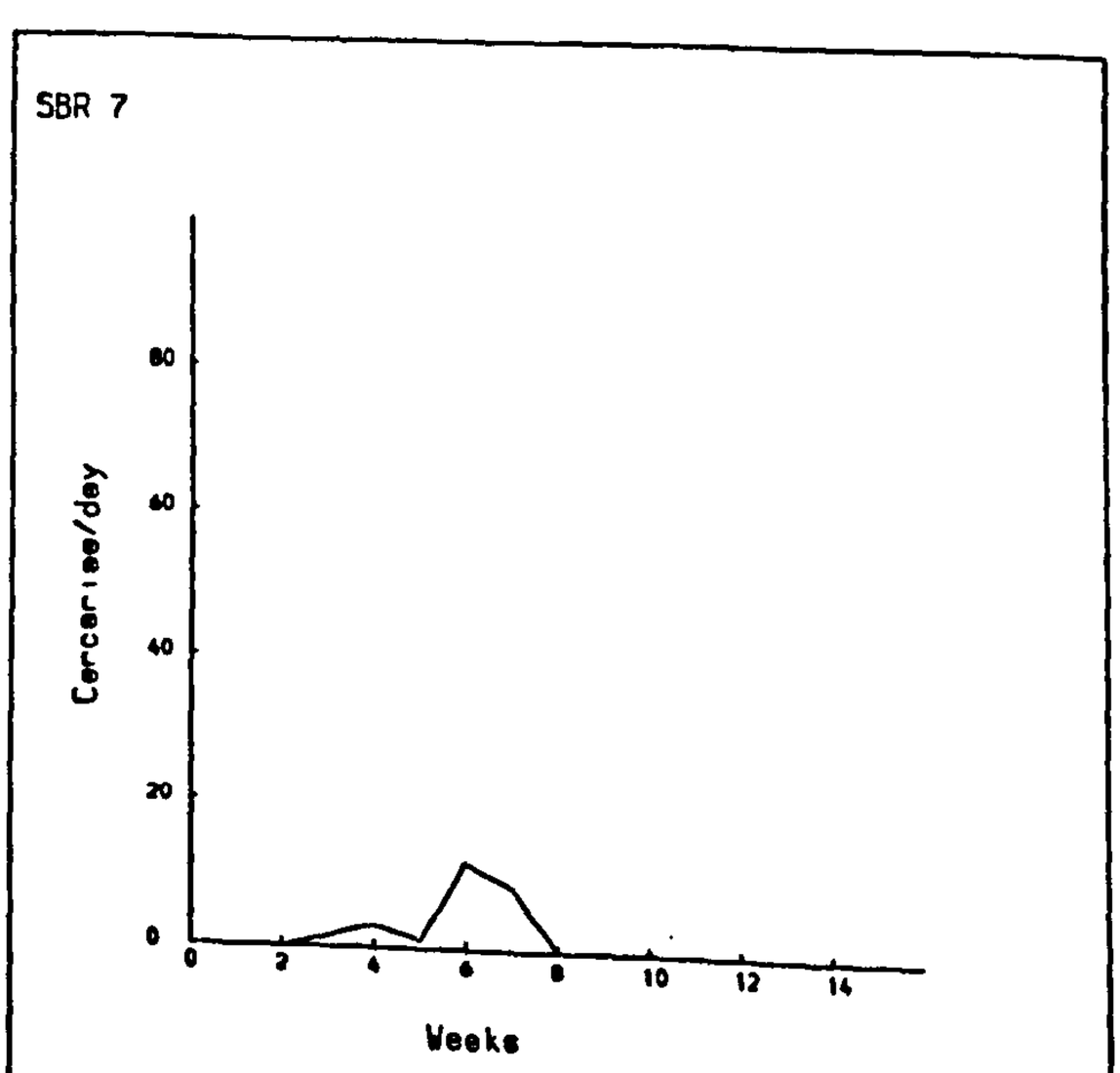


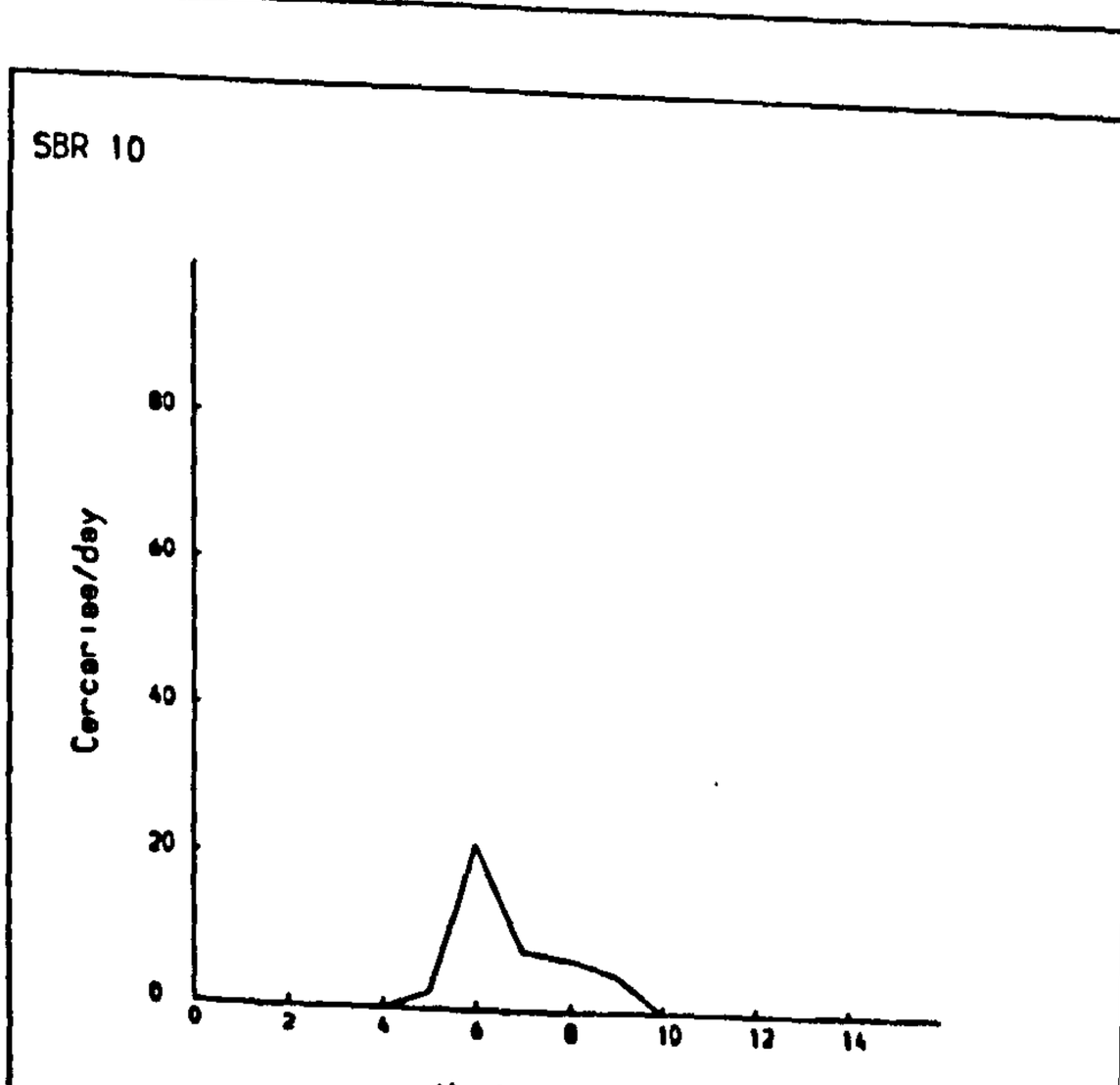

SBR 2
Cercariae/day
0
20
40
60
80
0
2
4
6
8
10
12
14
Weeks

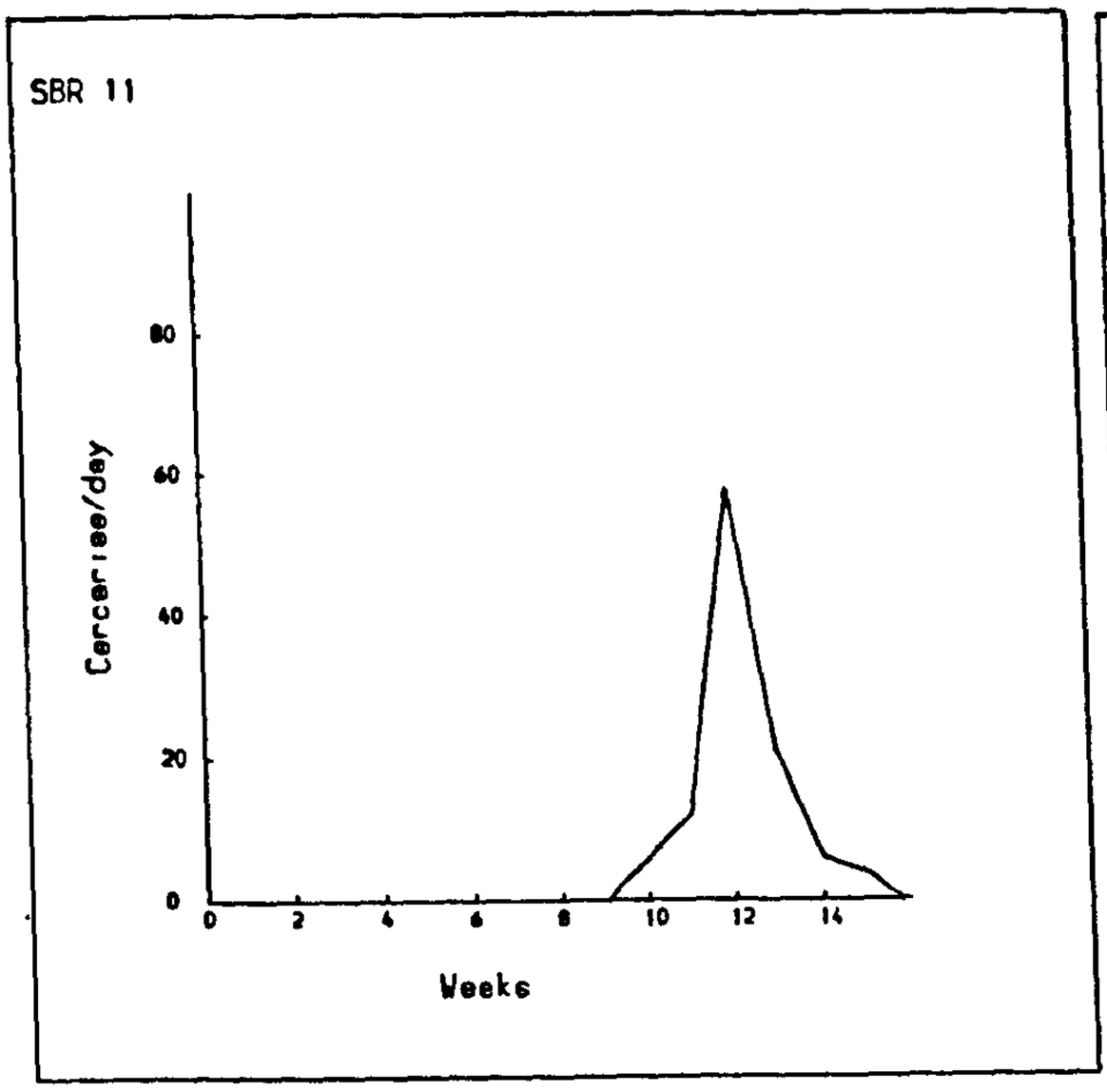

SBR 3
Cercariae/day
0
20
40
60
80
0
2
4
6
8
10
12
14
Weeks

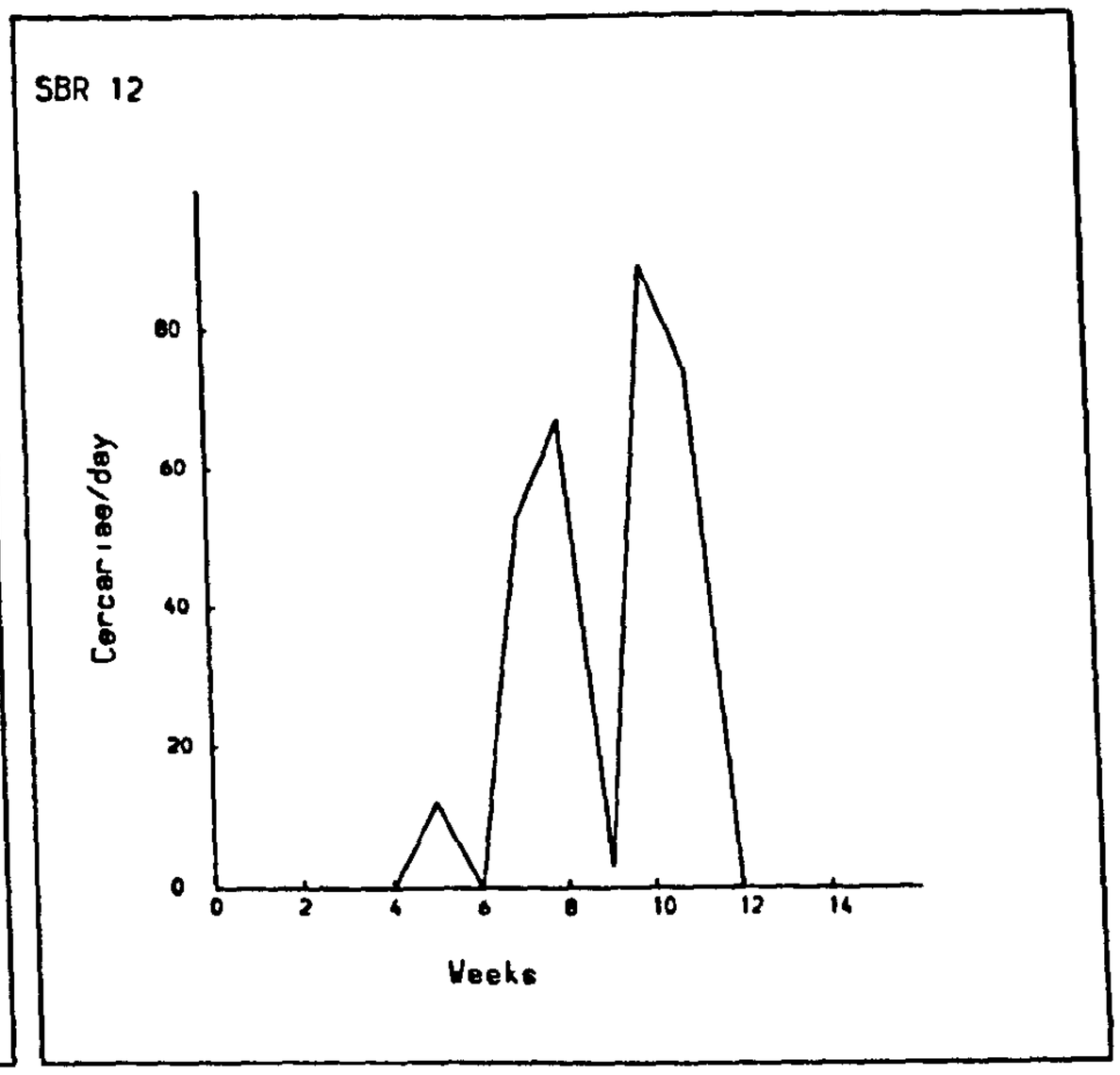

SBR 4
Cercariae/day
0
20
40
60
80
0
2
4
6
8
10
12
14
Weeks

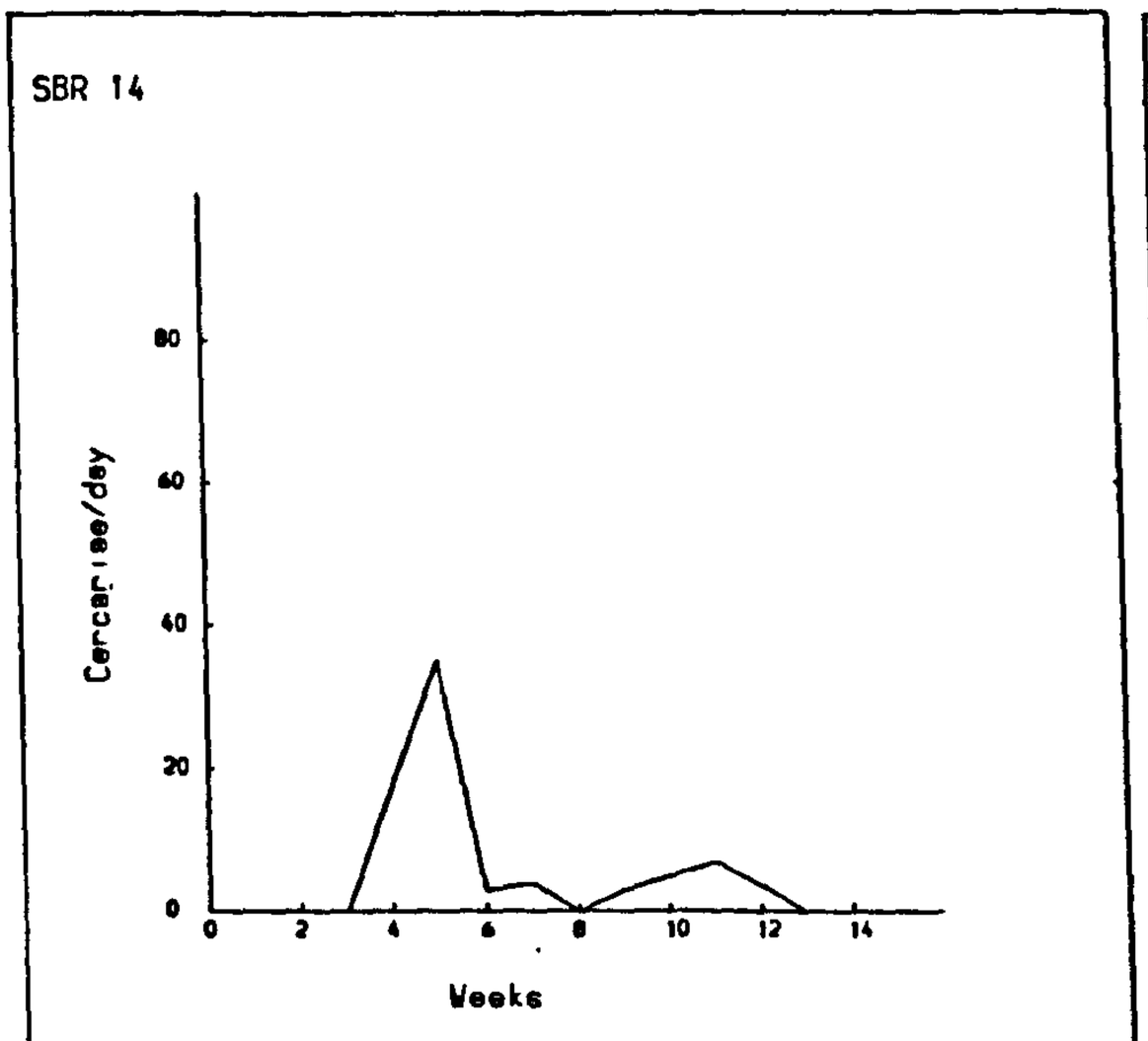

SBR 5
Cercariae/day
0
20
40
60
80
0
2
4
6
8
10
12
14
Weeks

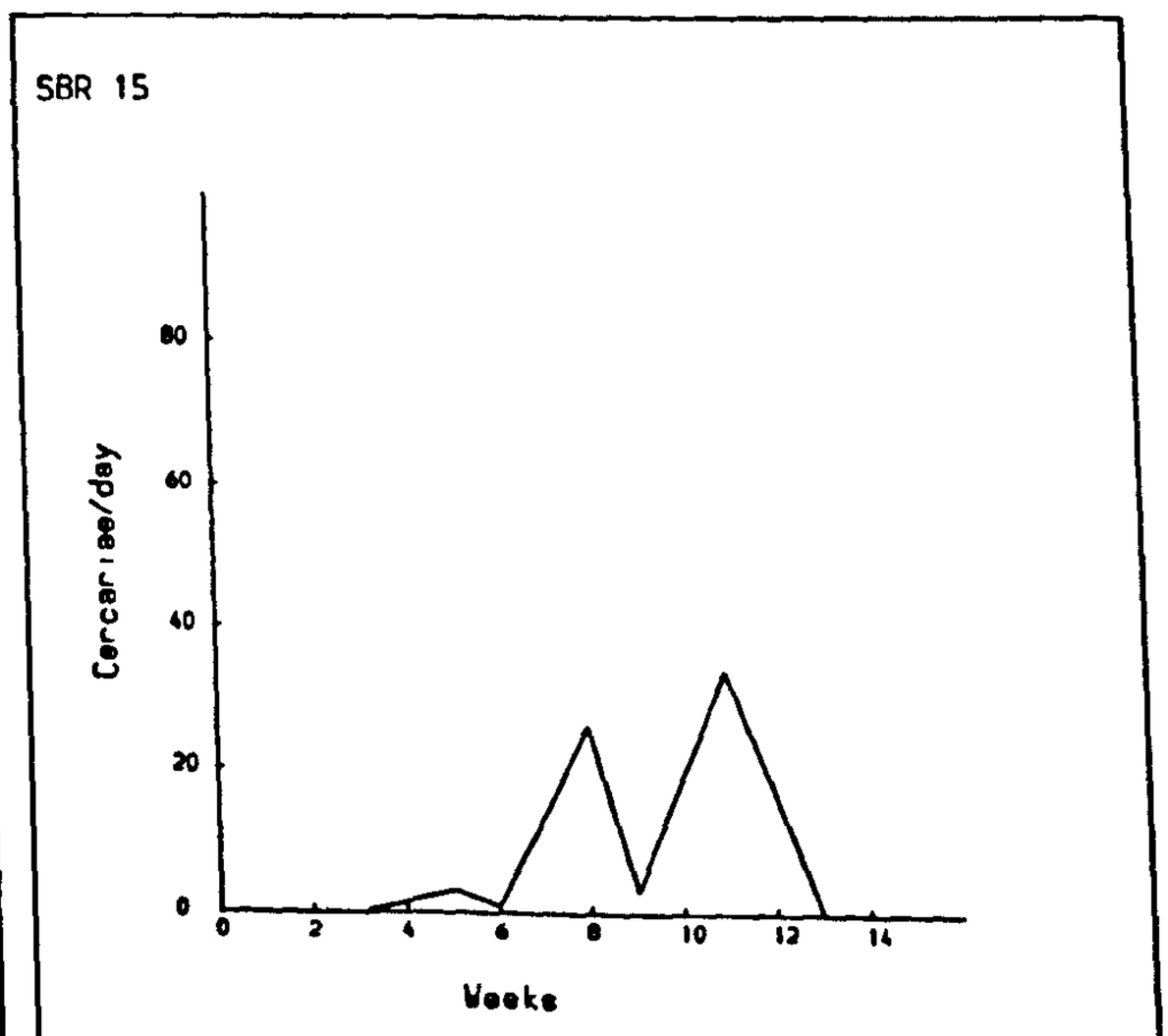

SBR 7
Cercariae/day
0
20
40
60
80
0
2
4
6
8
10
12
14
Weeks

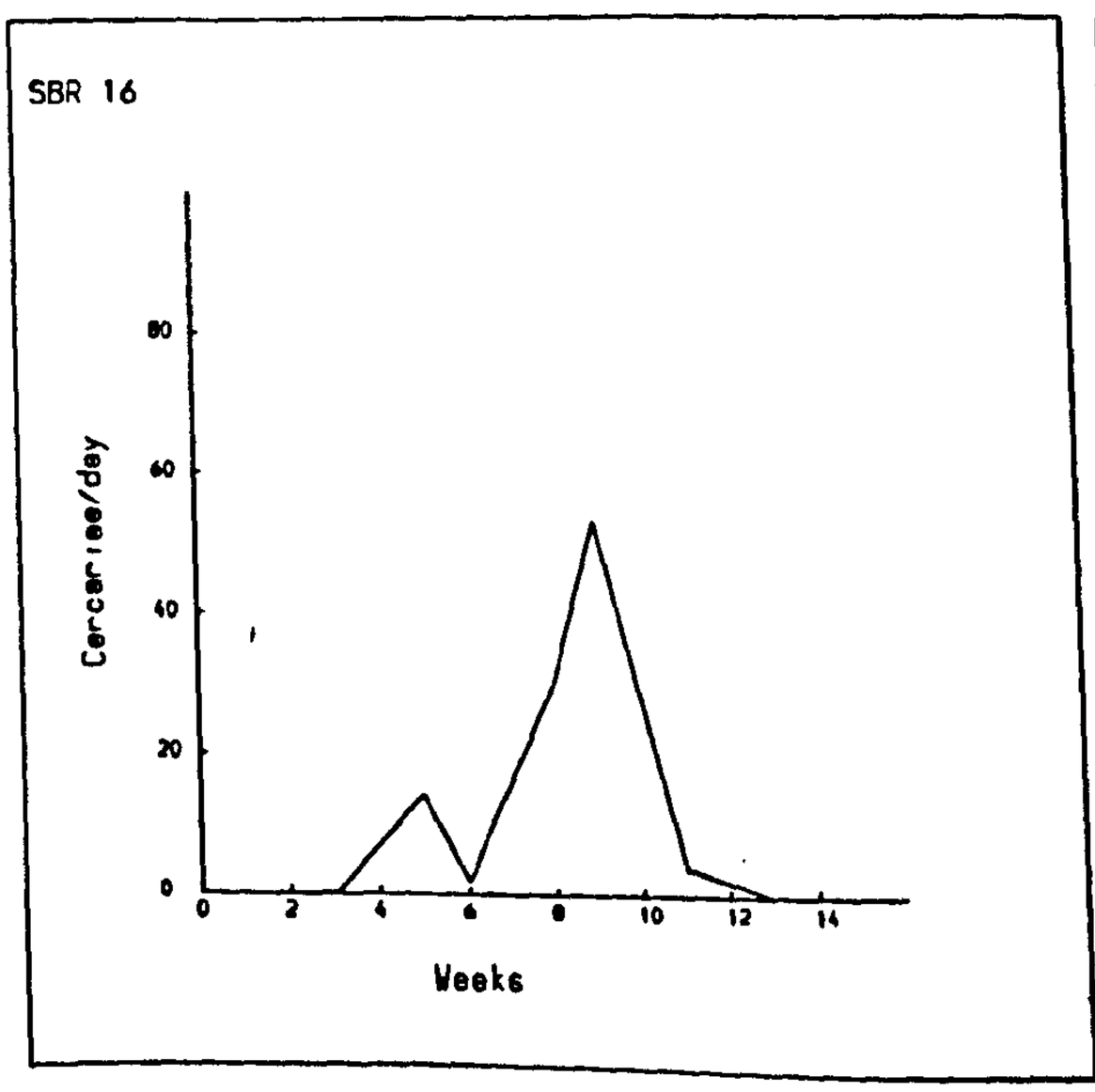

SBR 10
Cercariae/day
0
20
40
60
80
0
2
4
6
8
10
12
14
Weeks

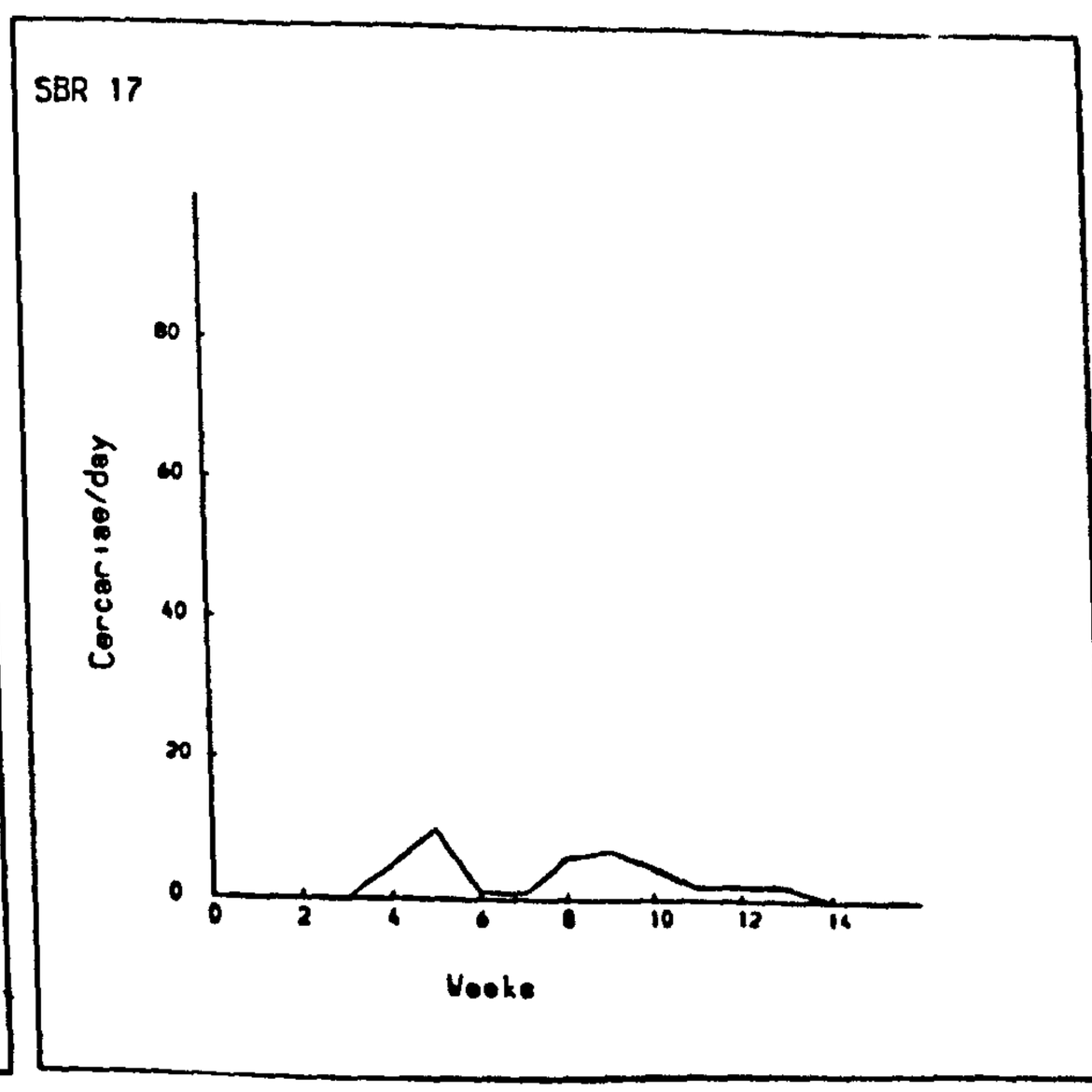

SBR 11
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

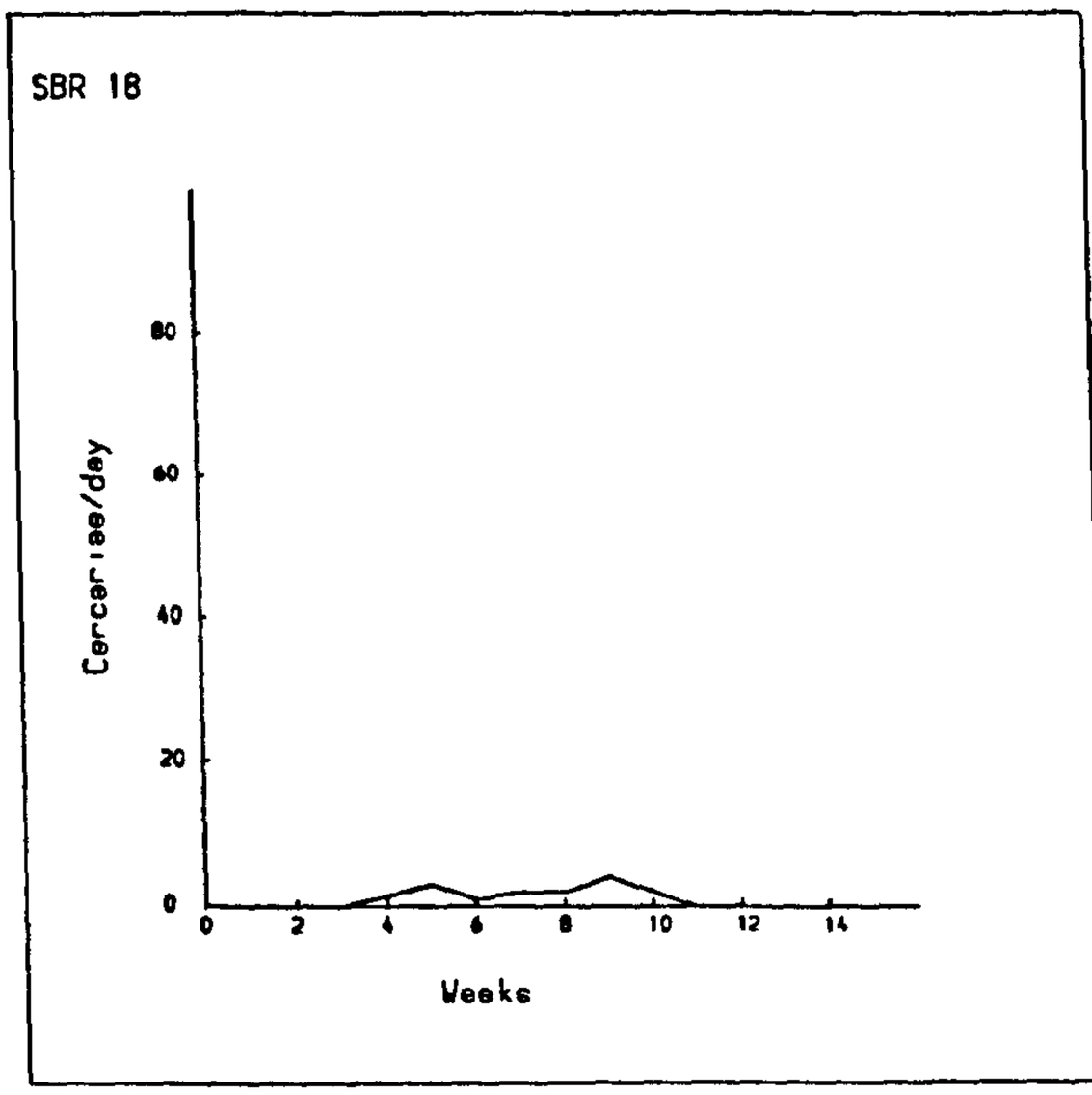

SBR 12
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

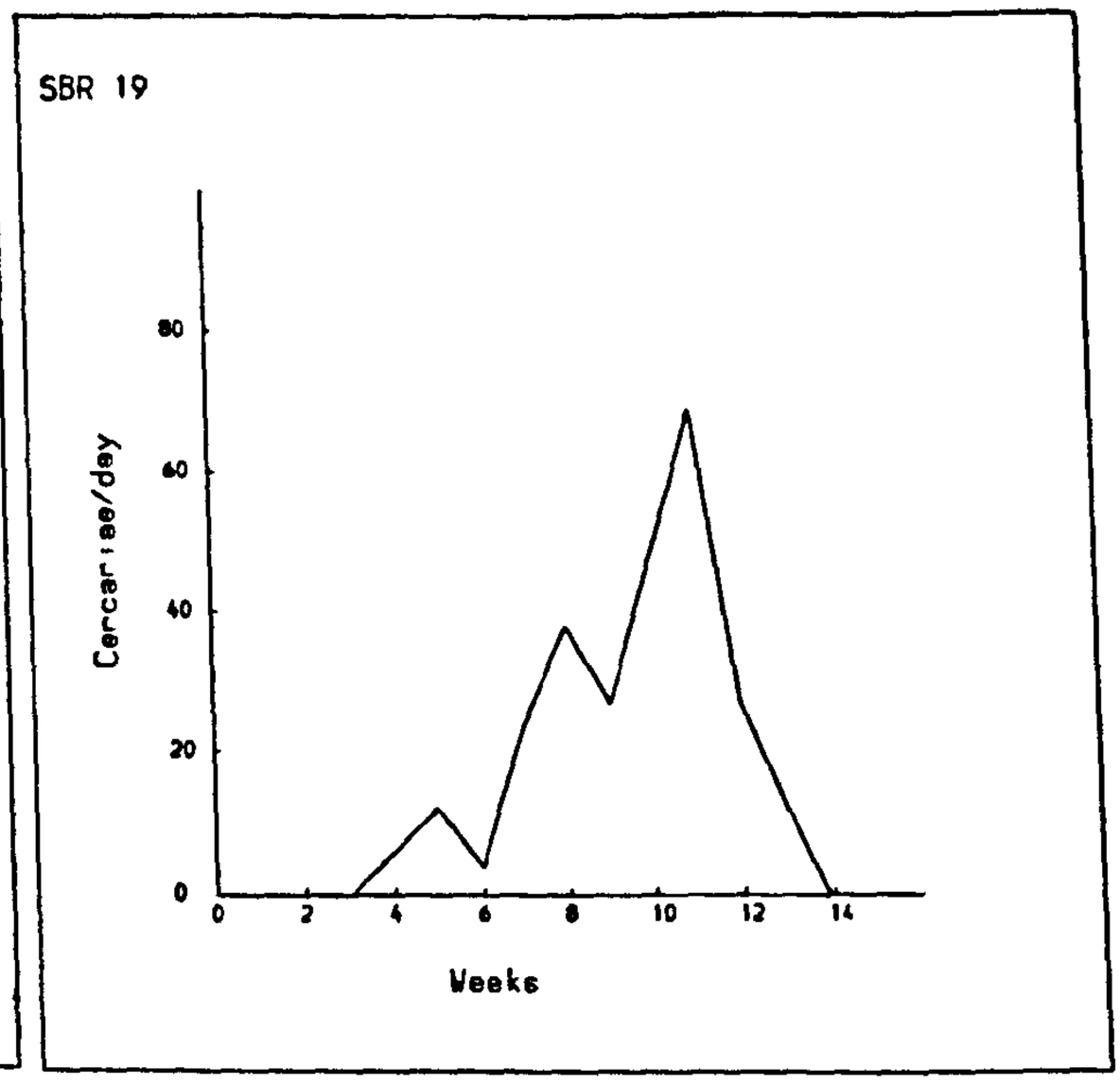

SBR 14
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

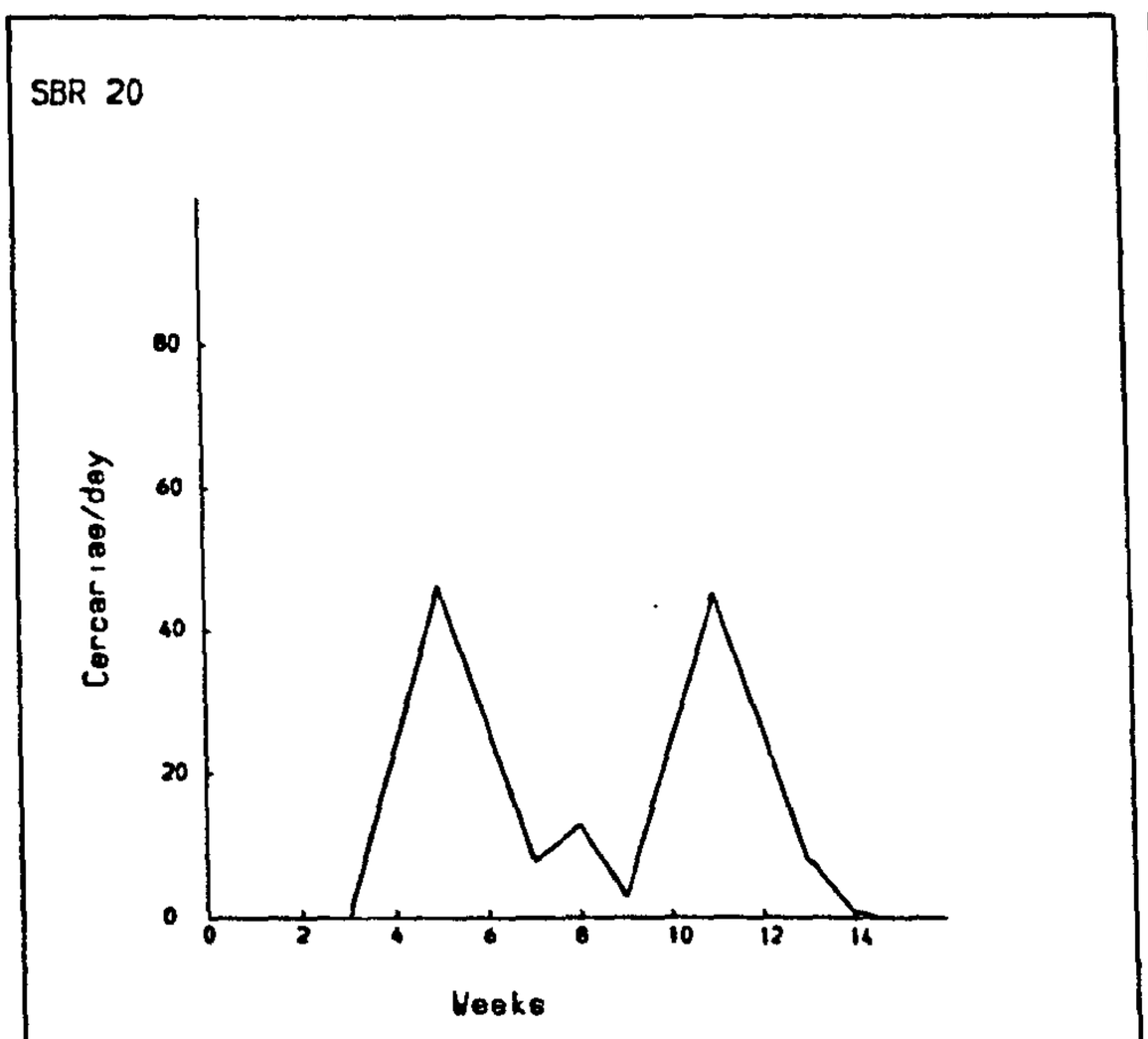

SBR 15
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

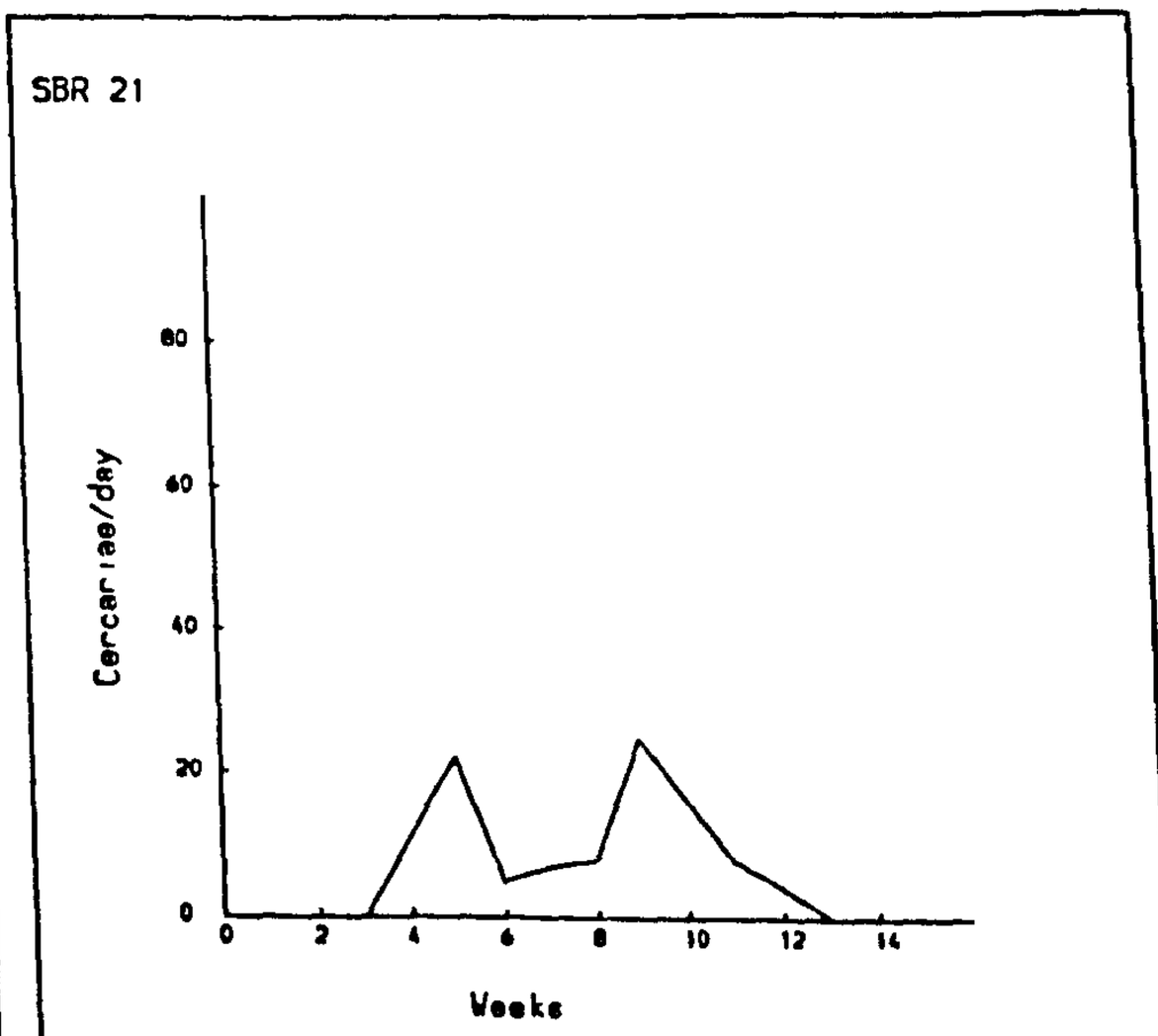

SBR 16
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

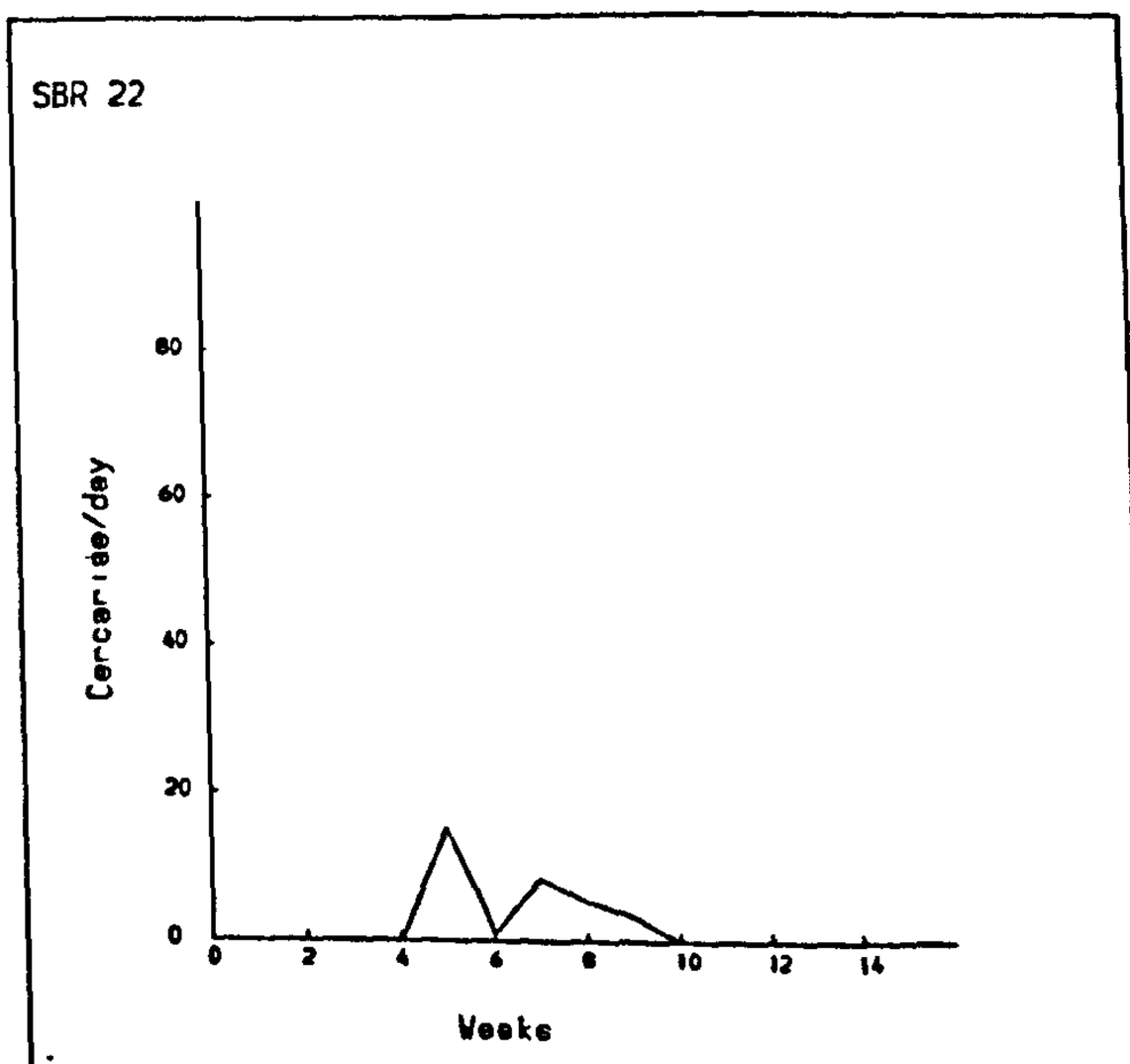

SBR 17
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

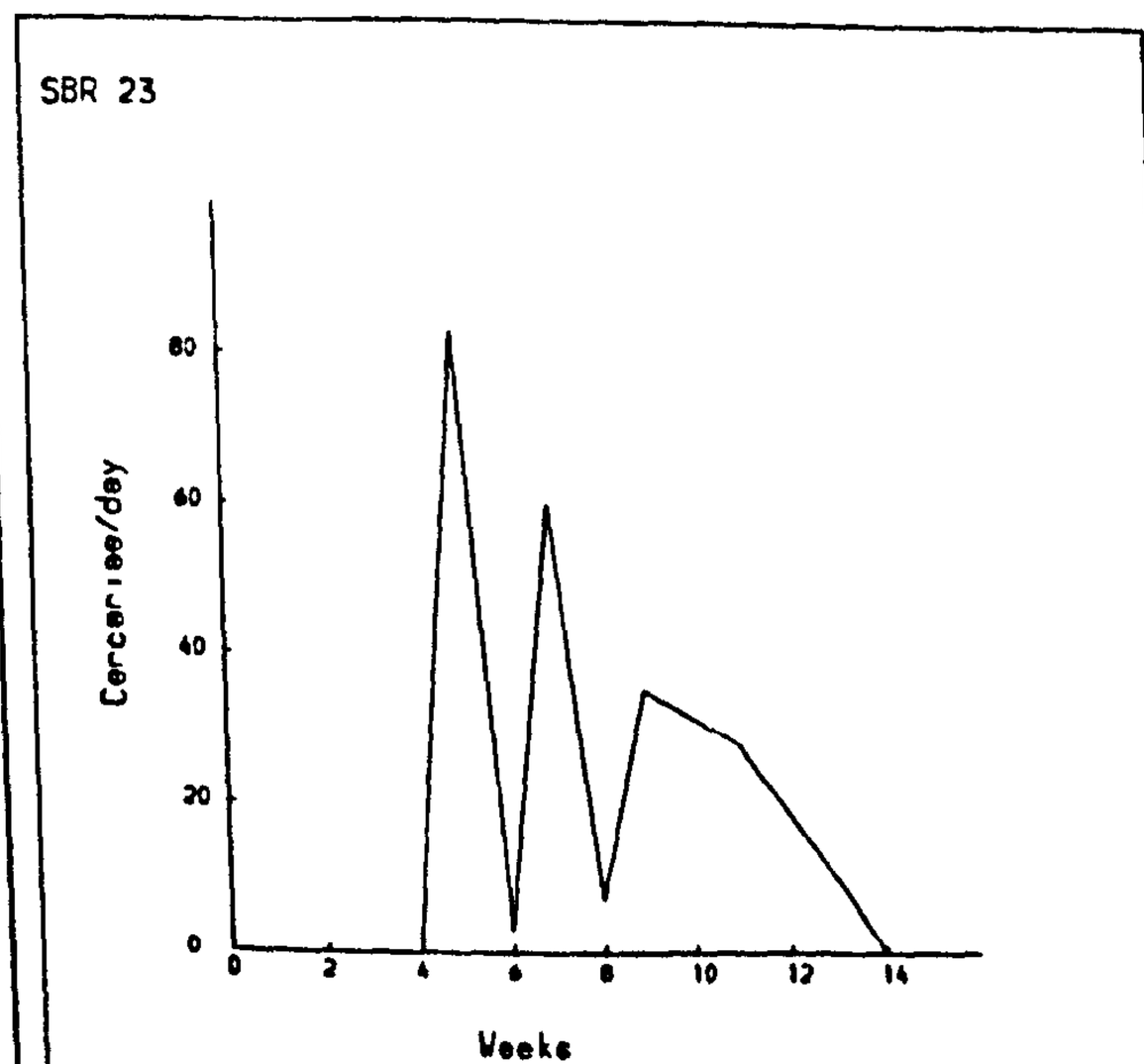

SBR 18
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

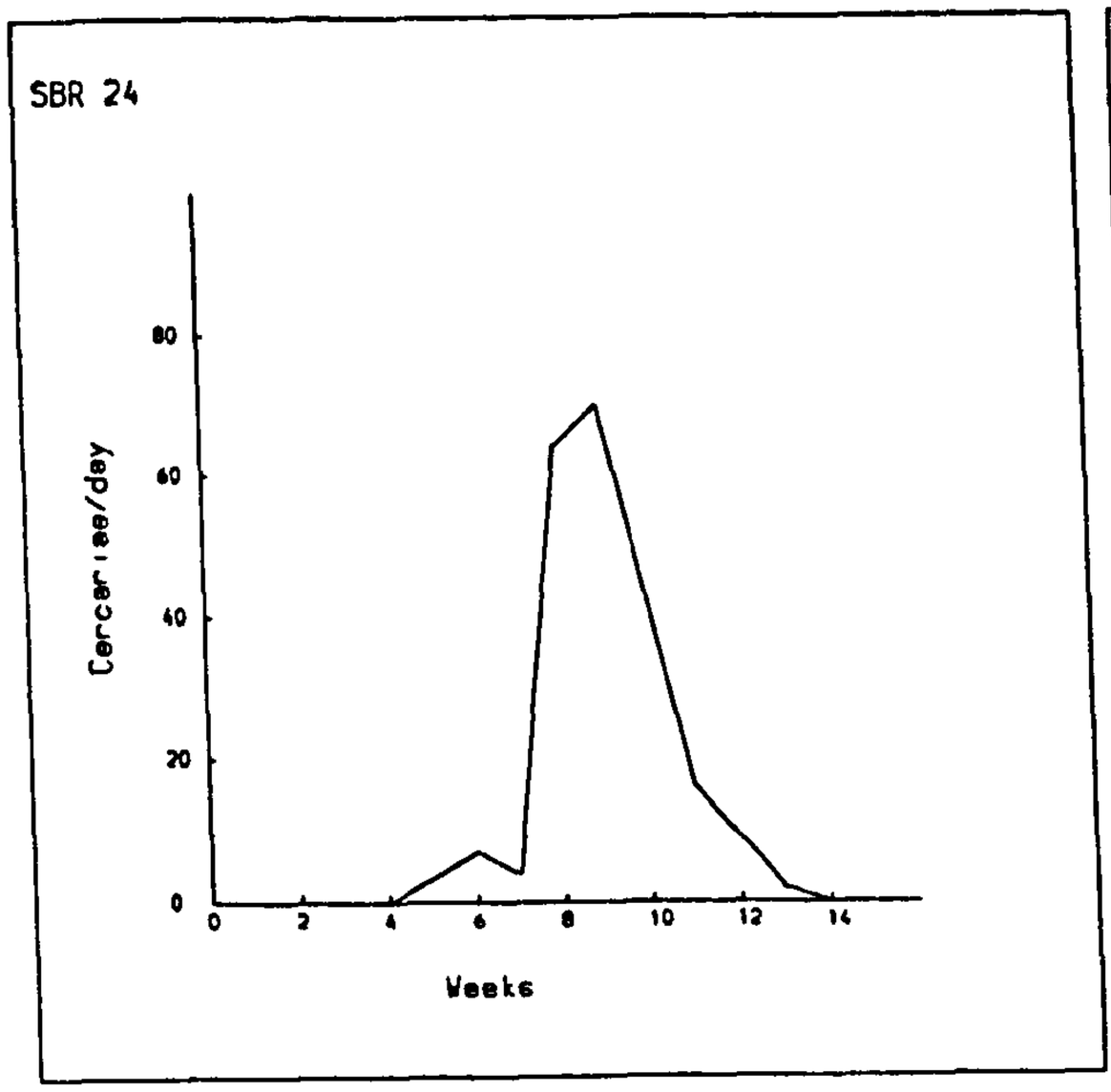

SBR 19
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

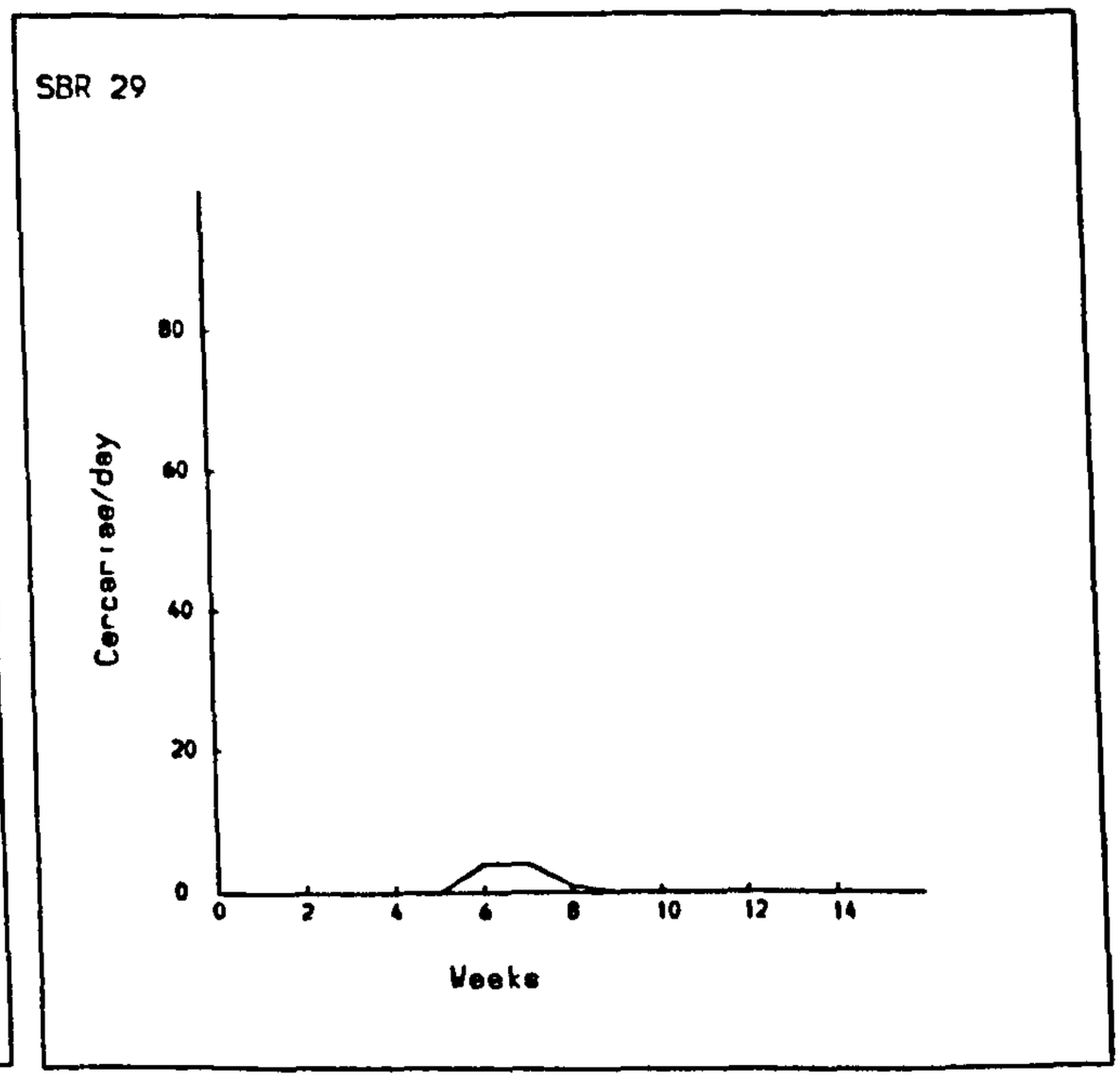

SBR 20
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

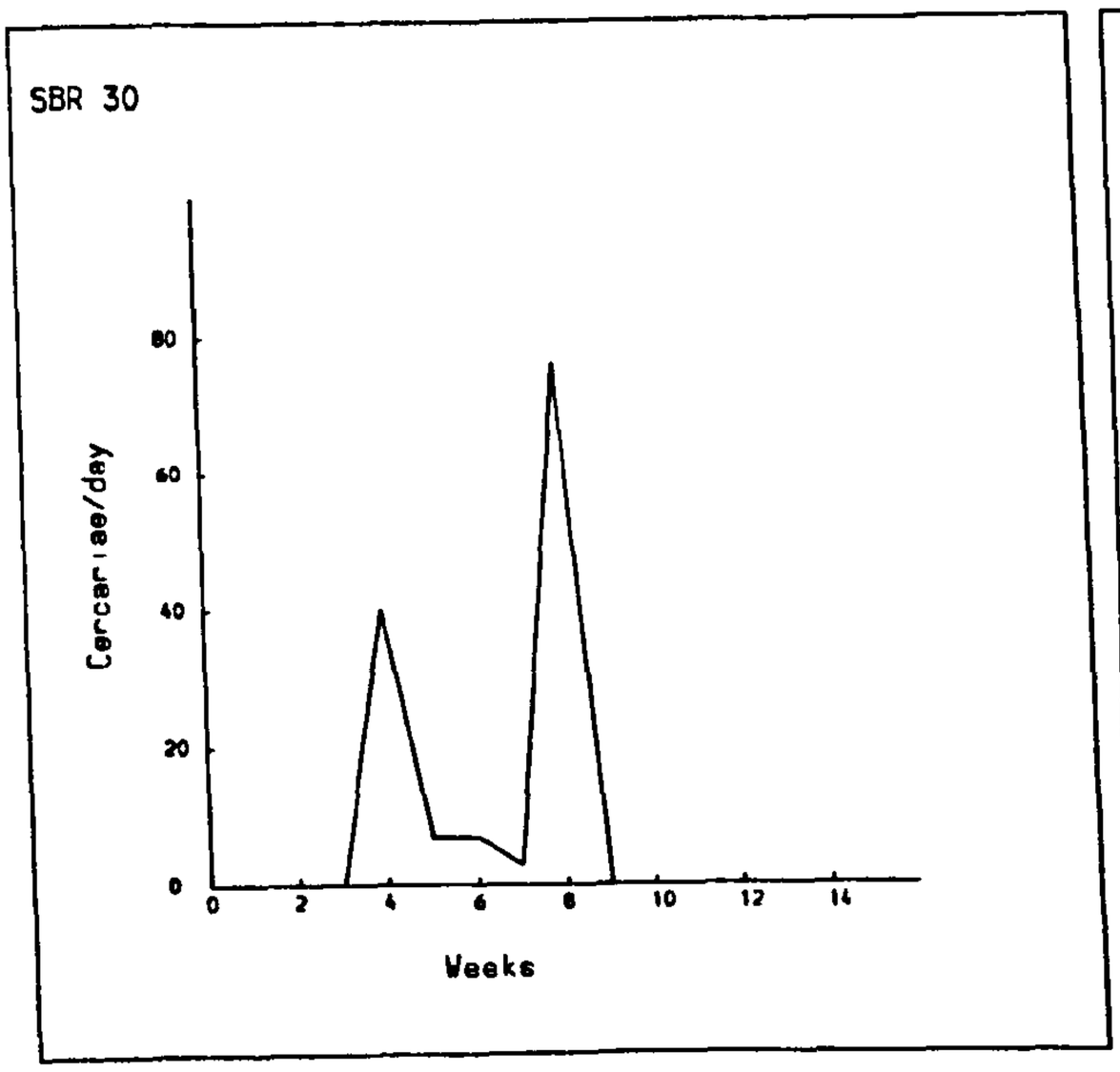

SBR 21
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

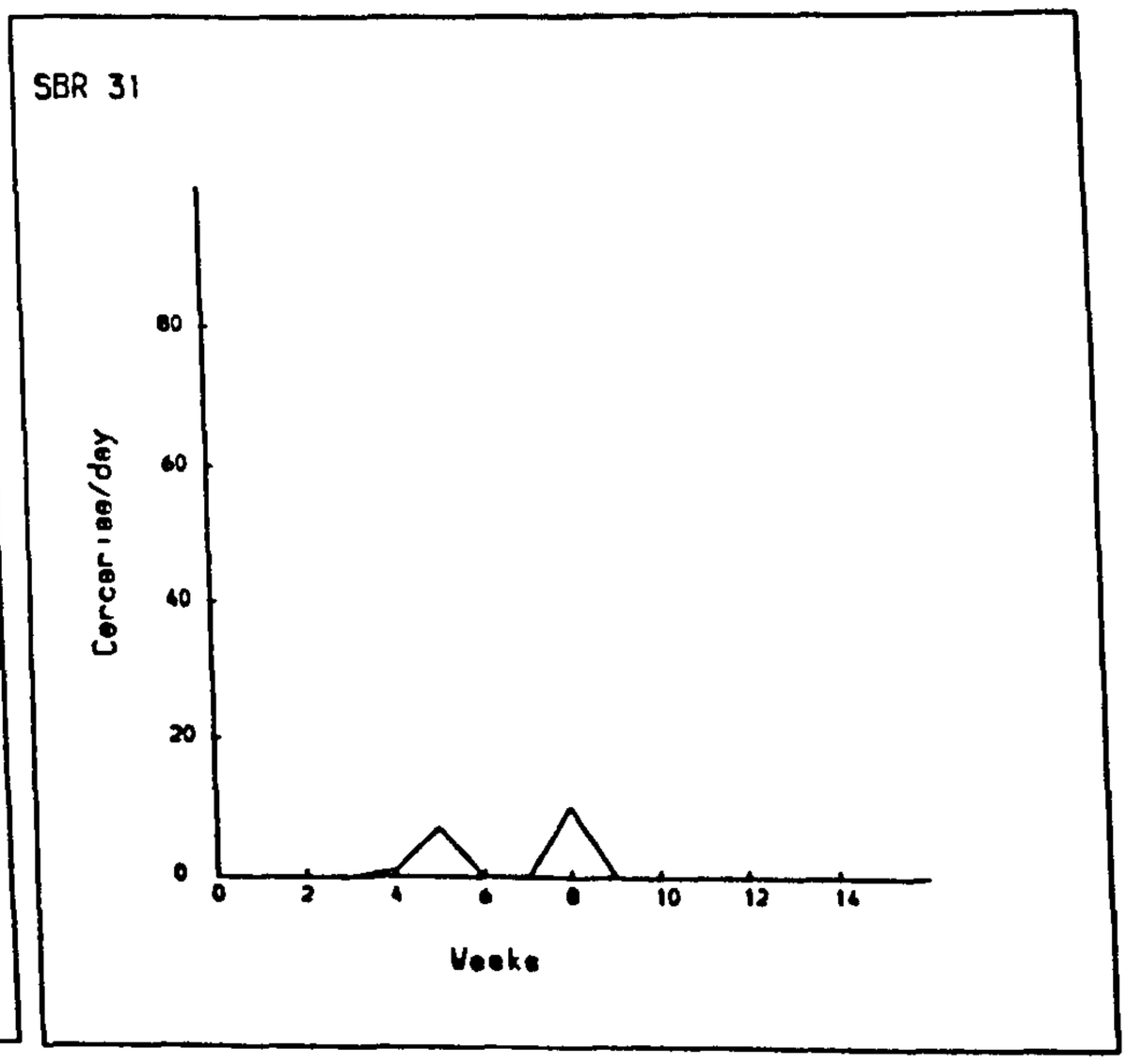

SBR 22
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

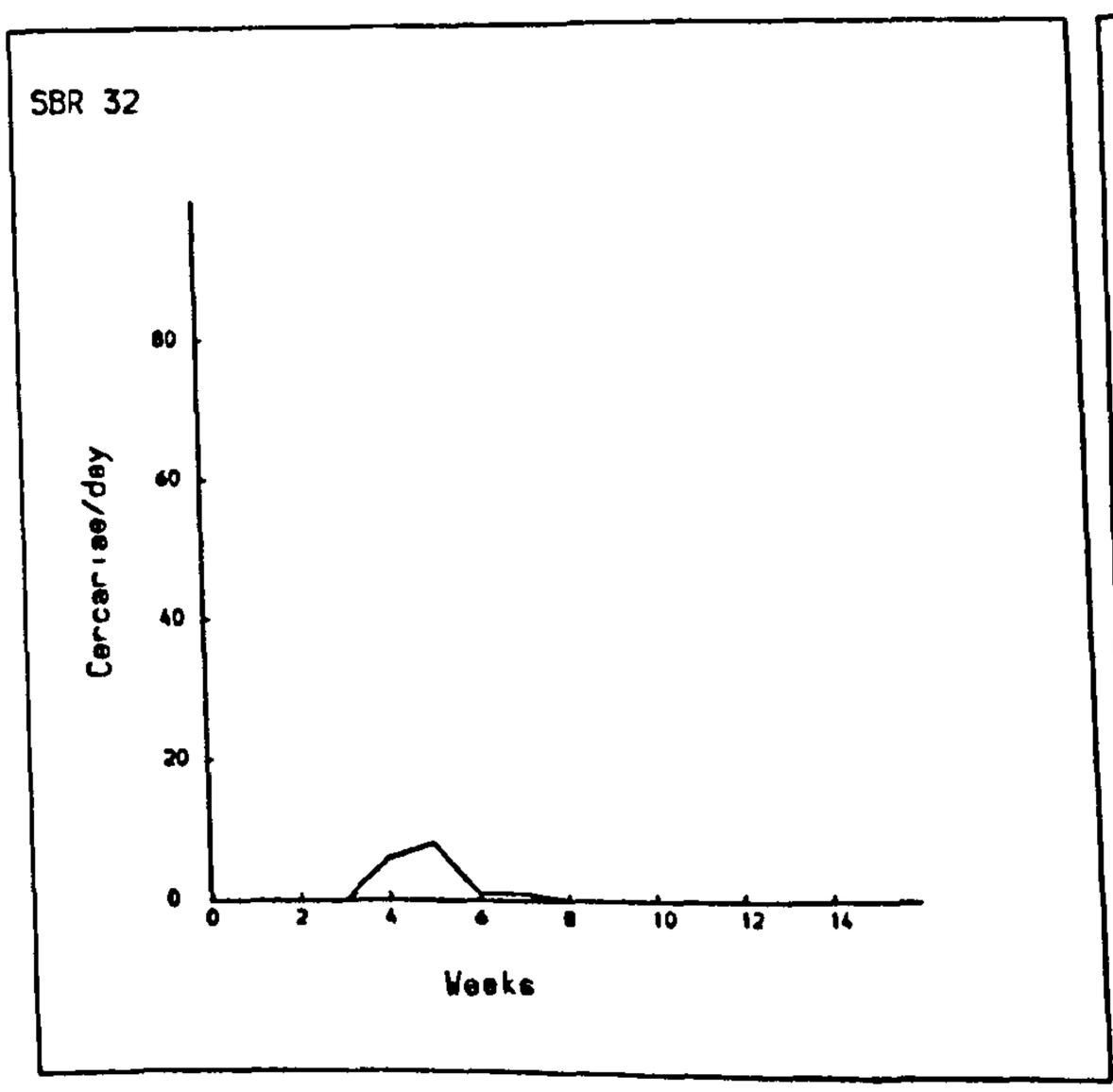

SBR 23
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

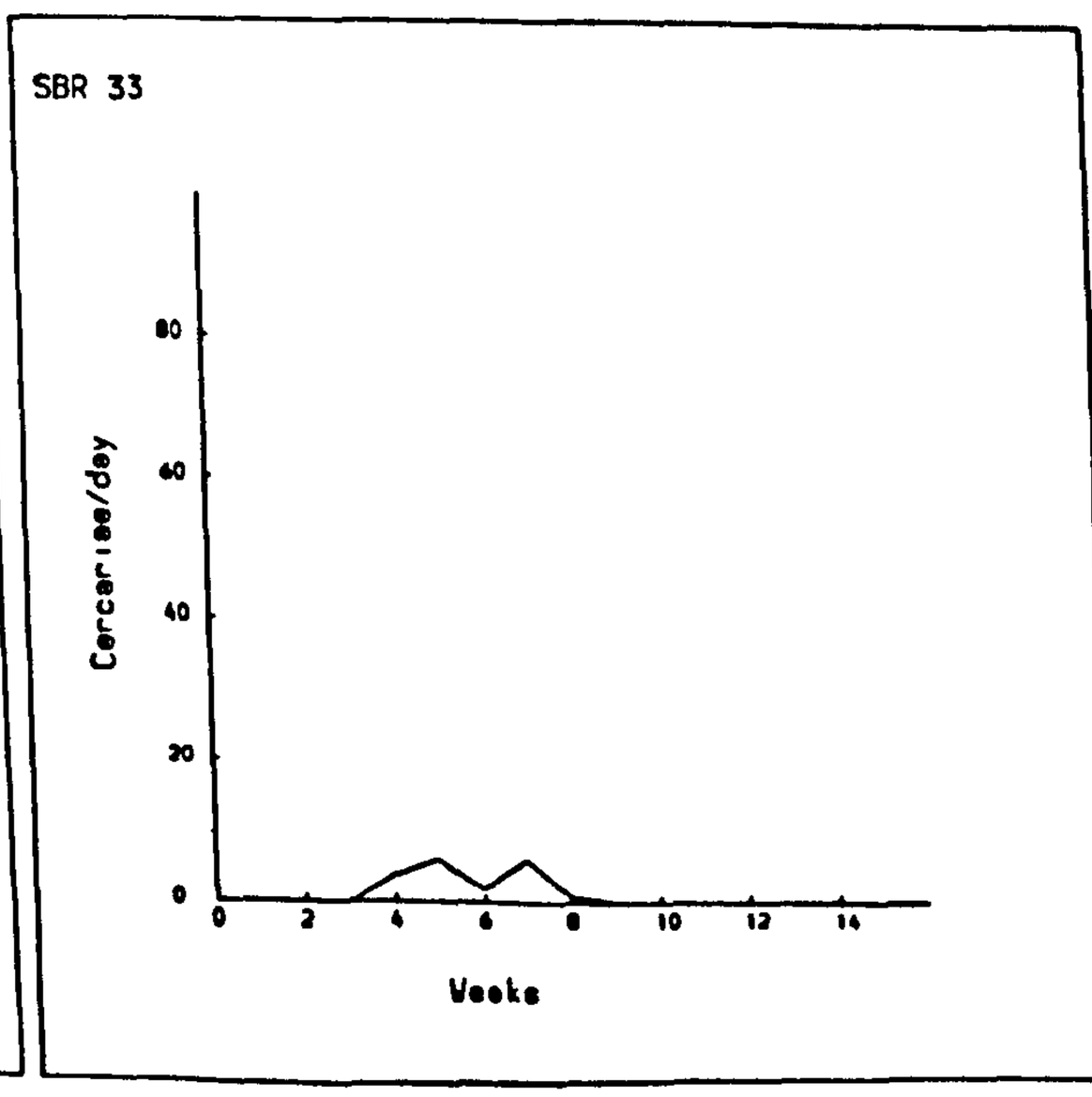

SBR 24
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

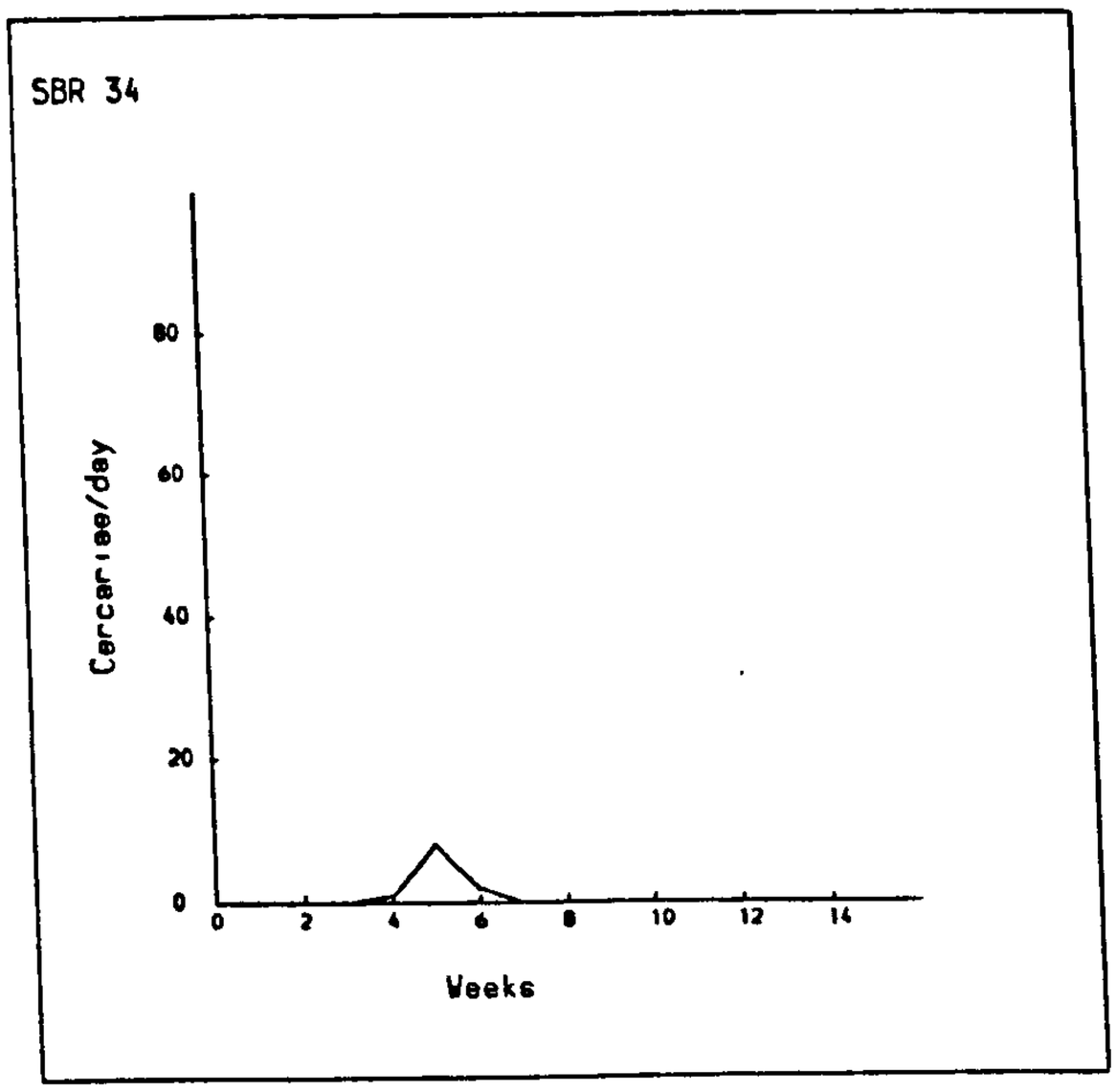

SBR 29
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

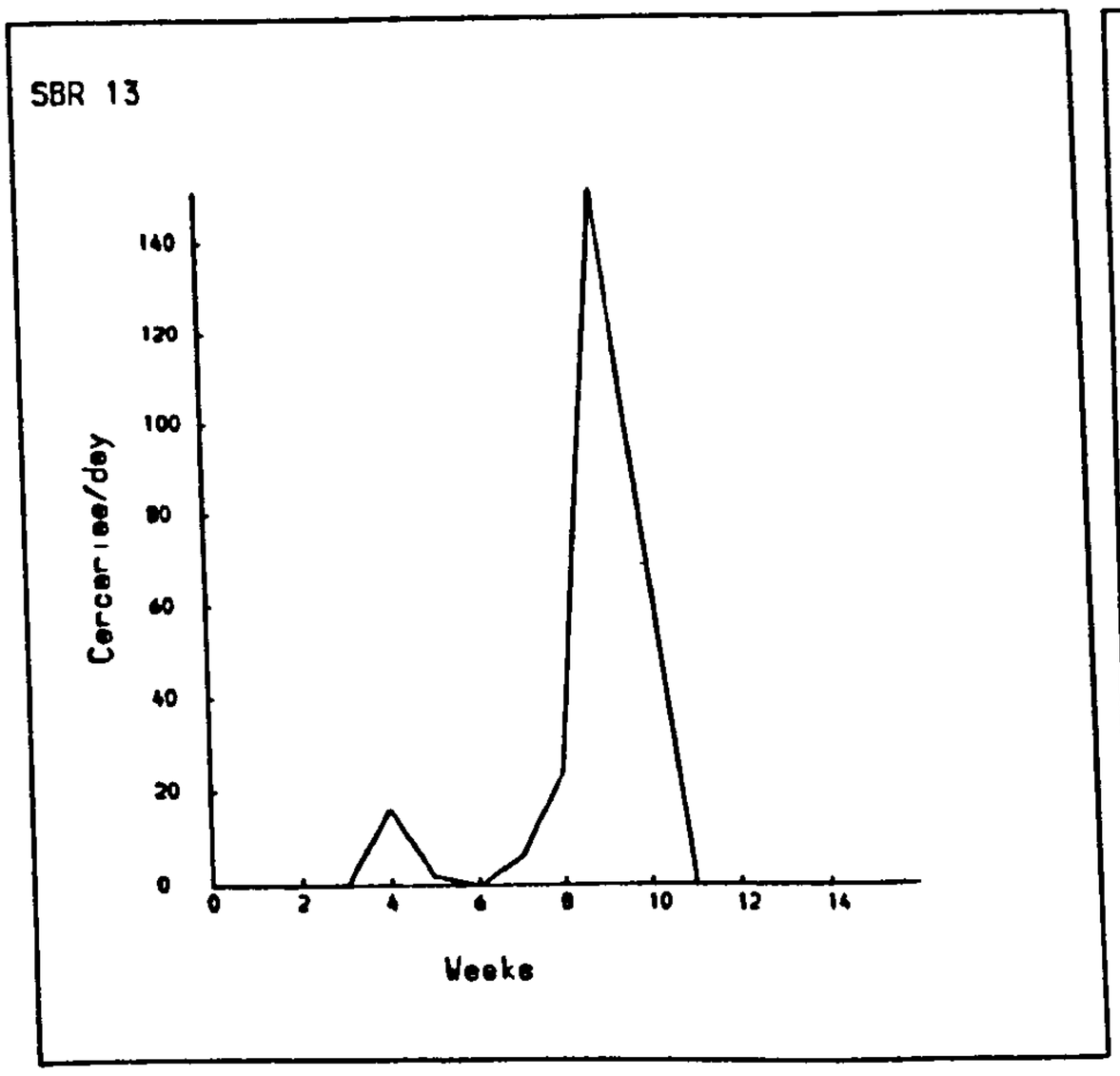

SBR 30
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

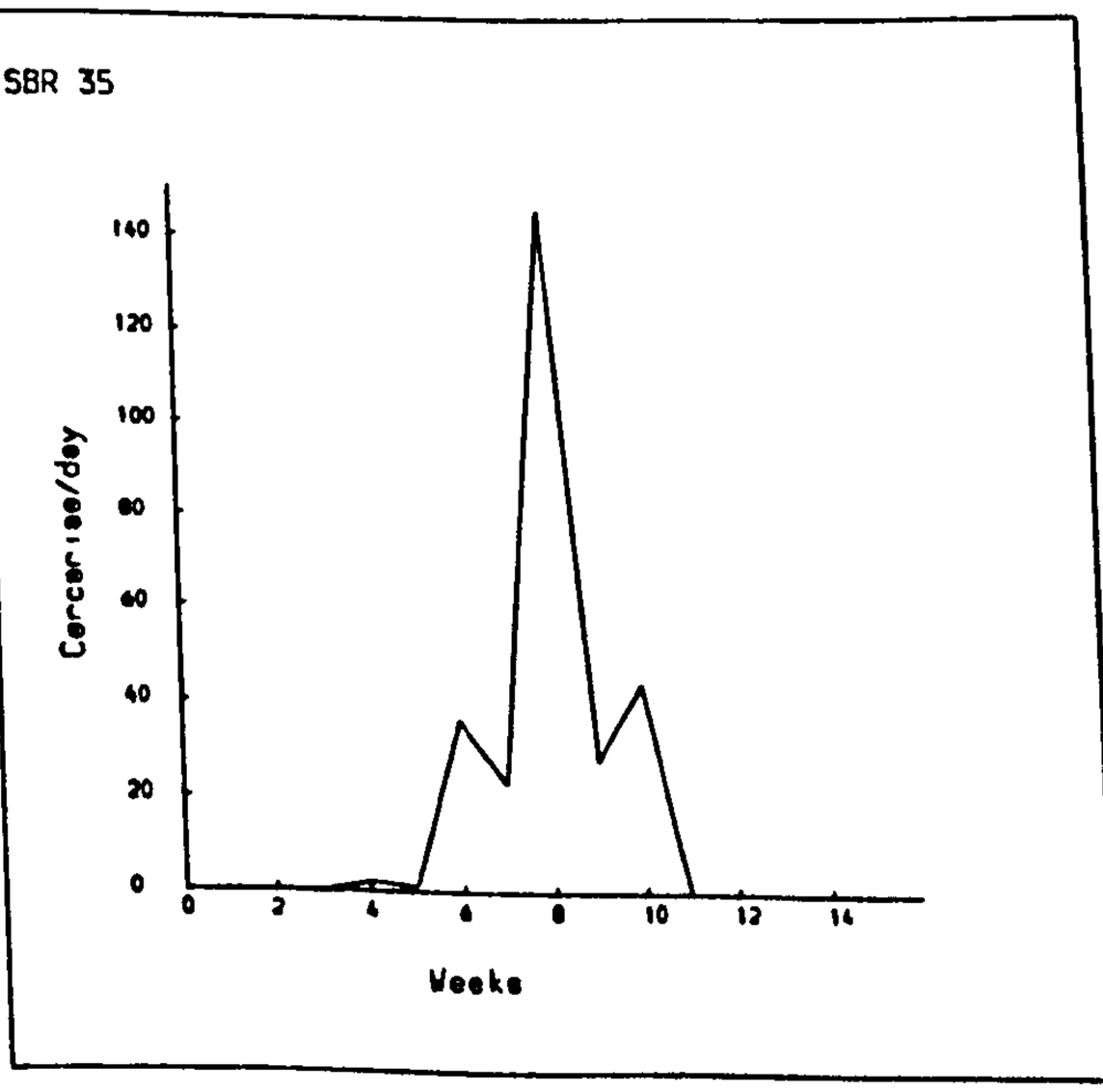

SBR 31
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

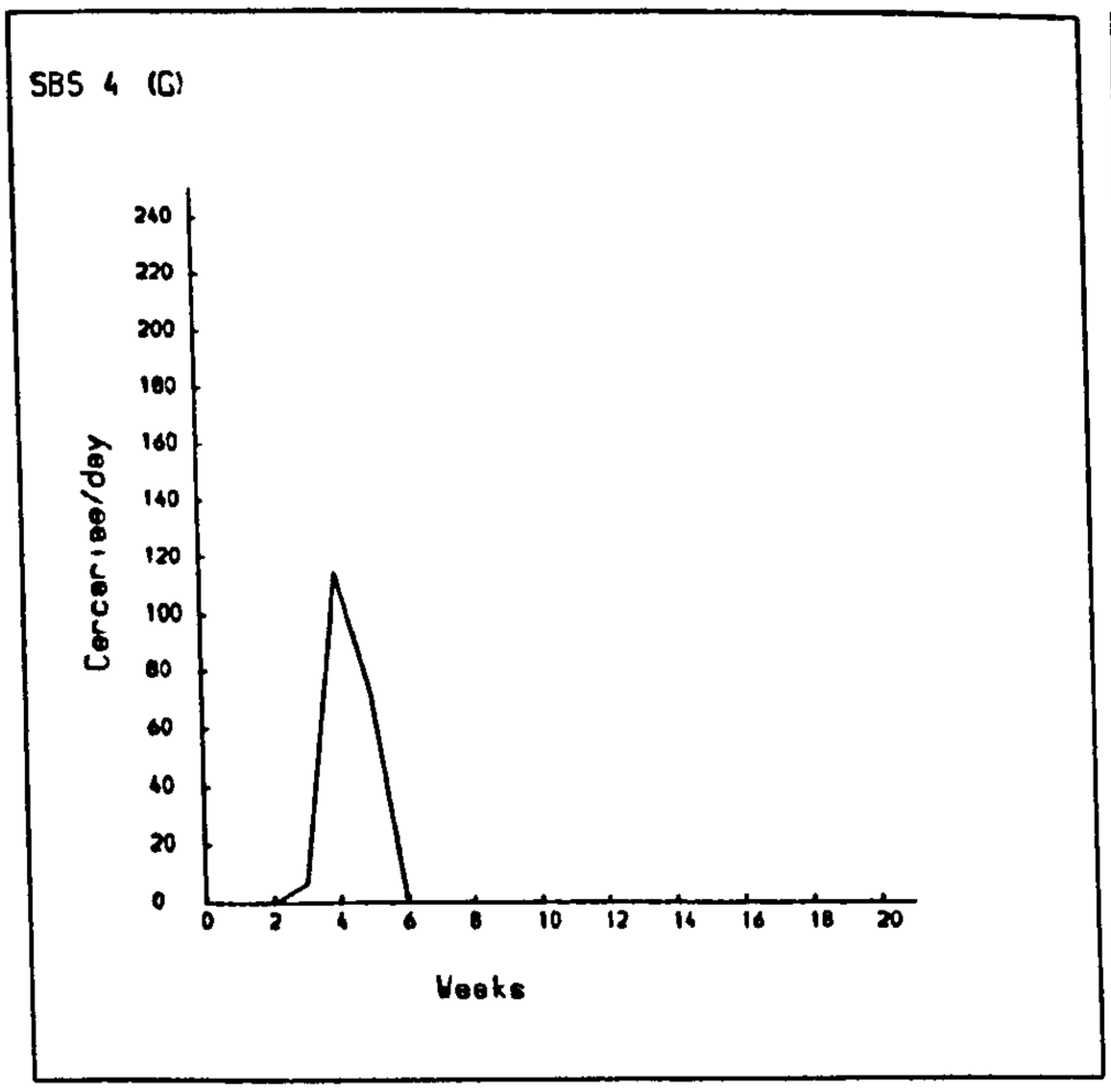

SBR 32
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

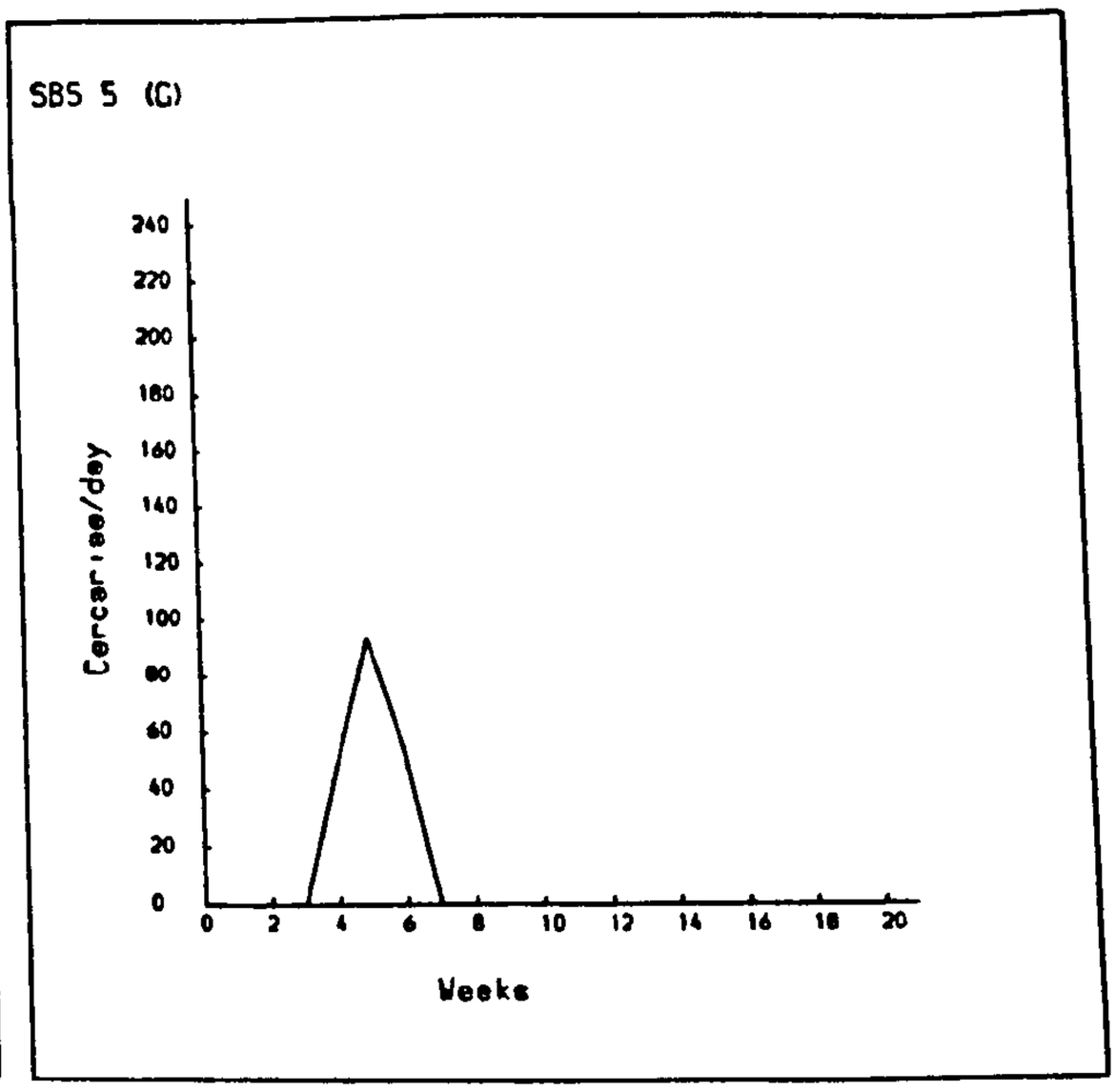

SBR 33
Cercariae/day
0
20
40
60
80
0 2 4 6 8 10 12 14
Weeks

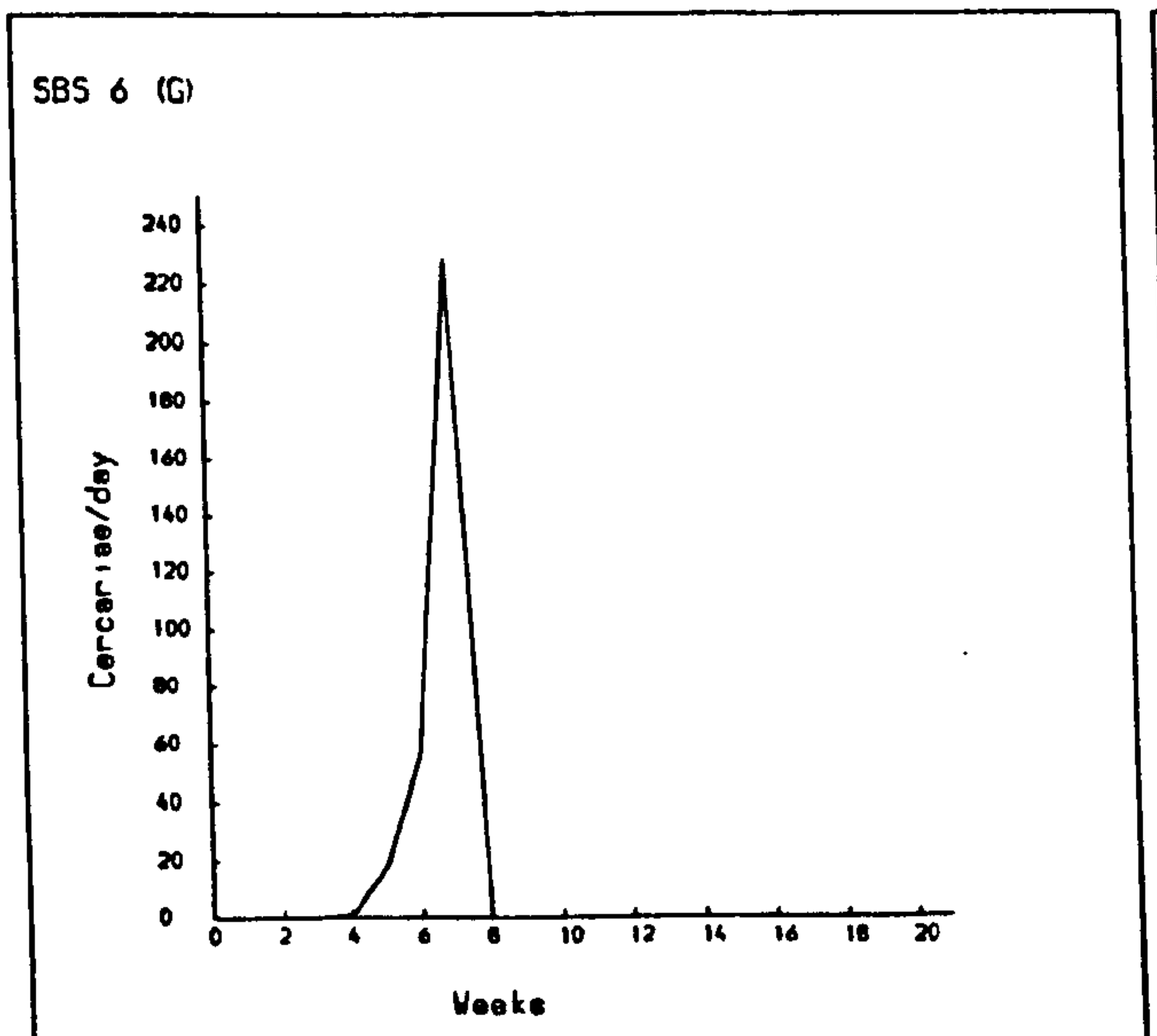

SBR 34
Cercariae/day
0
20
40
60
80
0
2
4
6
8
10
12
14
Weeks

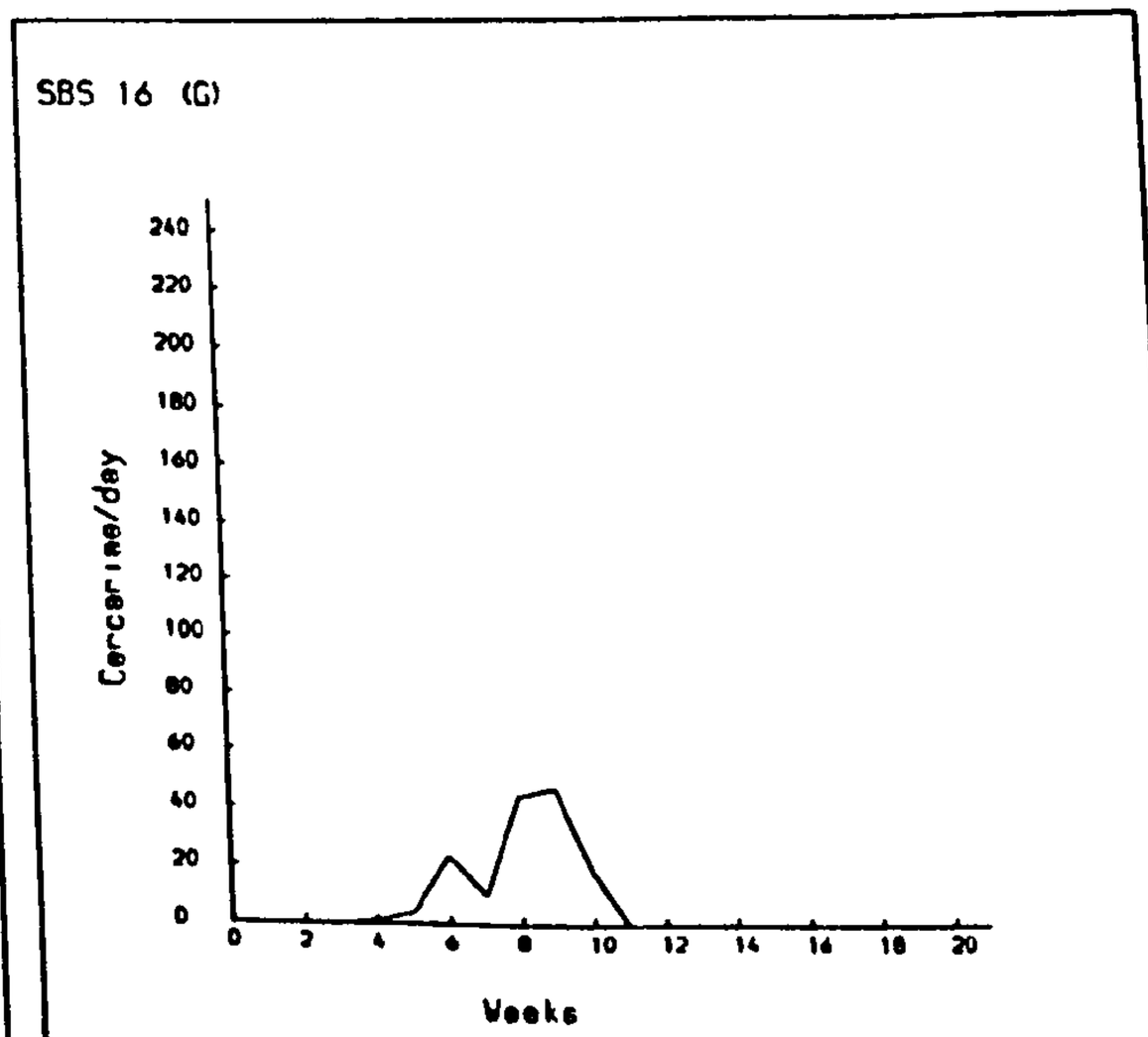

SBR 13
Cercariae/day
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
14
Weeks

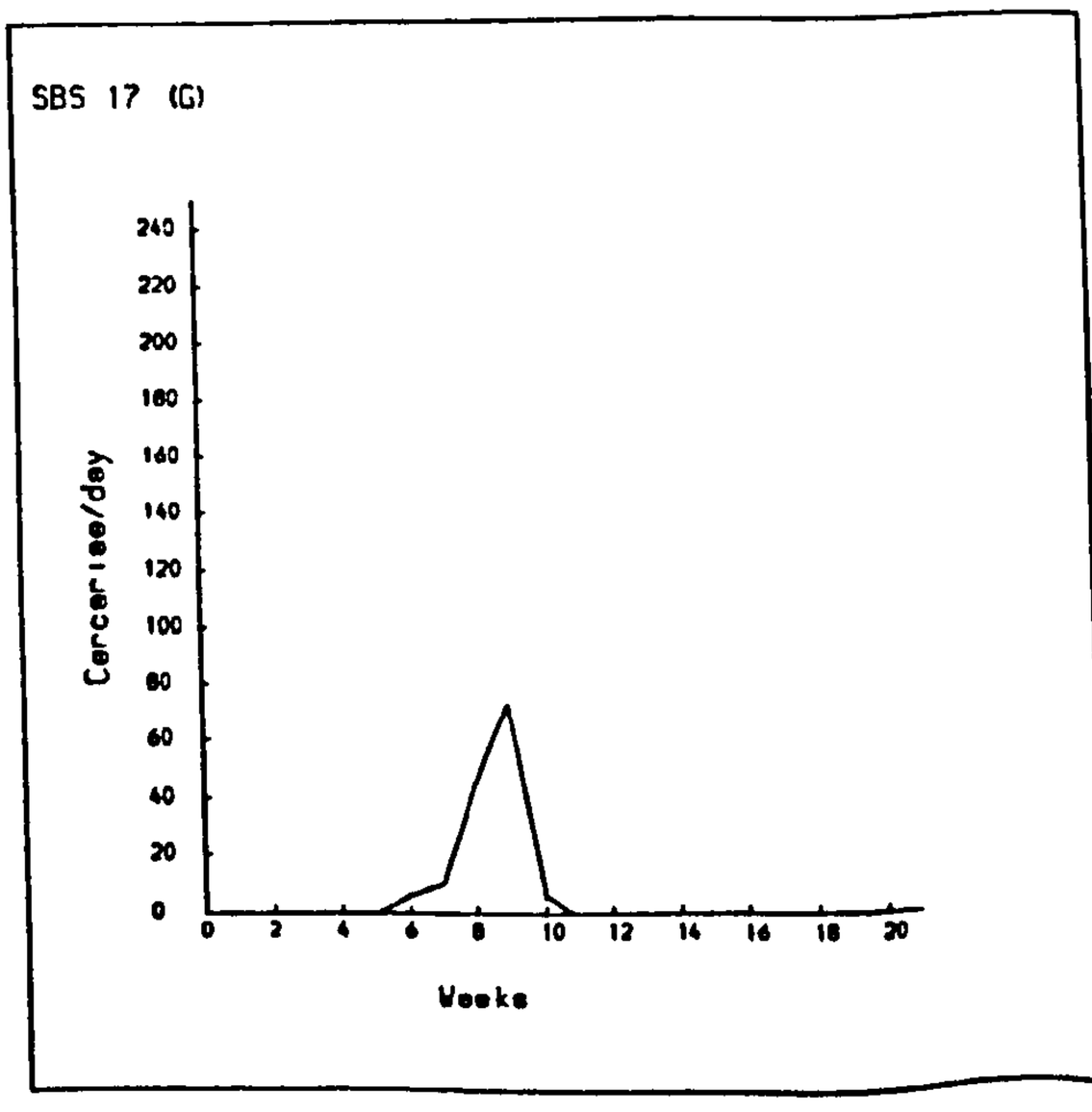

SBR 35
Cercariae/day
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
14
Weeks

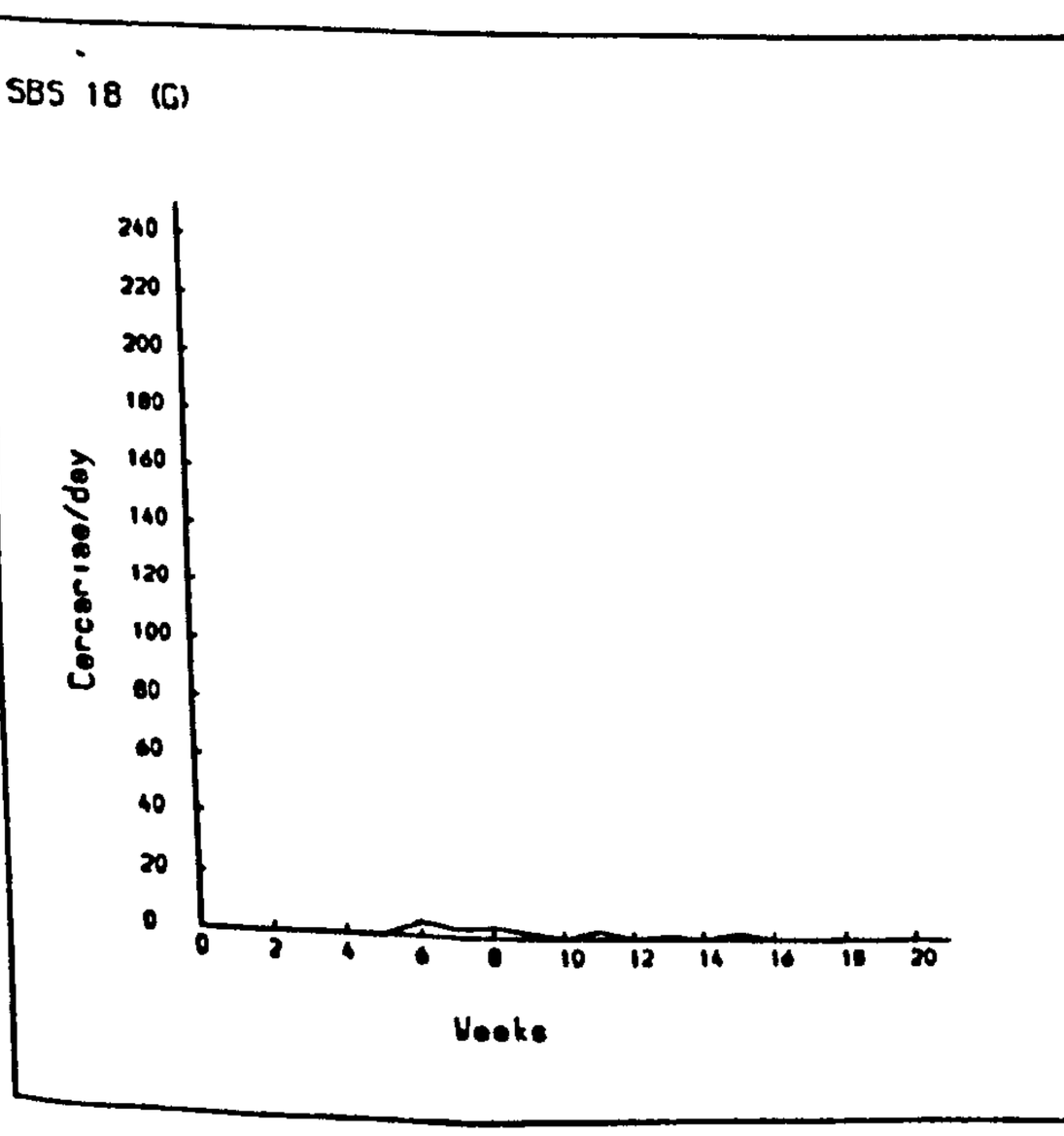

SBS 4 (G)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
200
220
240
0 2 4 6 8 10 12 14 16 18 20
Weeks


SBS 5 (G)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
200
220
240
0 2 4 6 8 10 12 14 16 18 20
Weeks

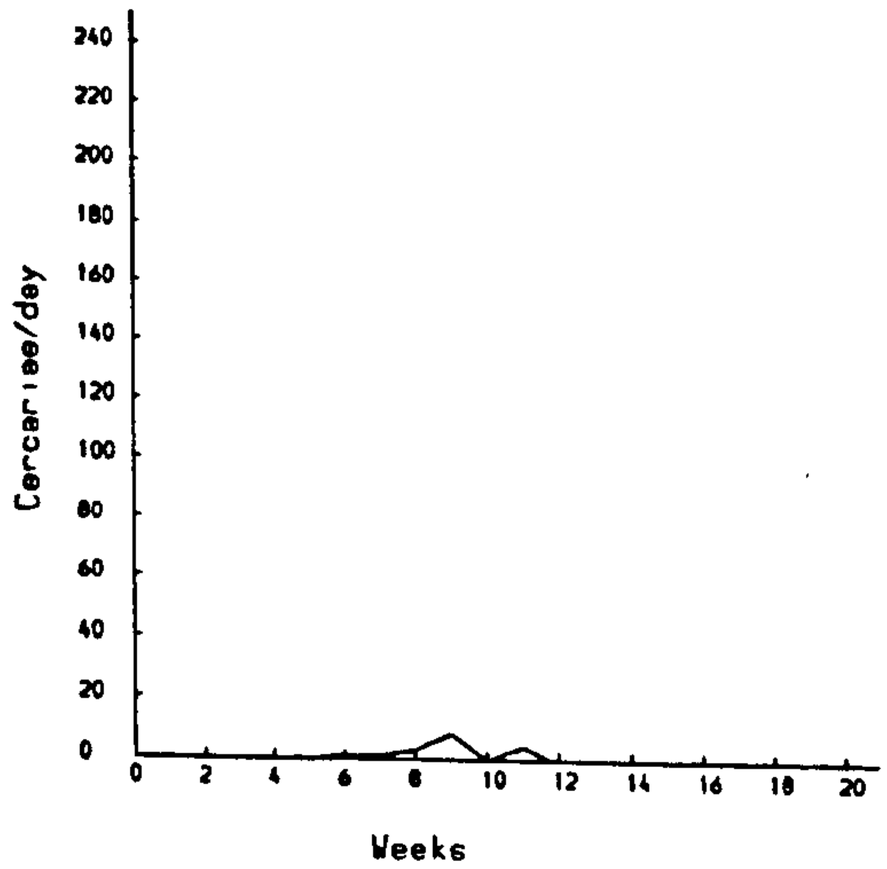

SBS 6 (G)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
200
220
240
0 2 4 6 8 10 12 14 16 18 20
Weeks


SBS 16 (G)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
200
220
240
0 2 4 6 8 10 12 14 16 18 20
Weeks

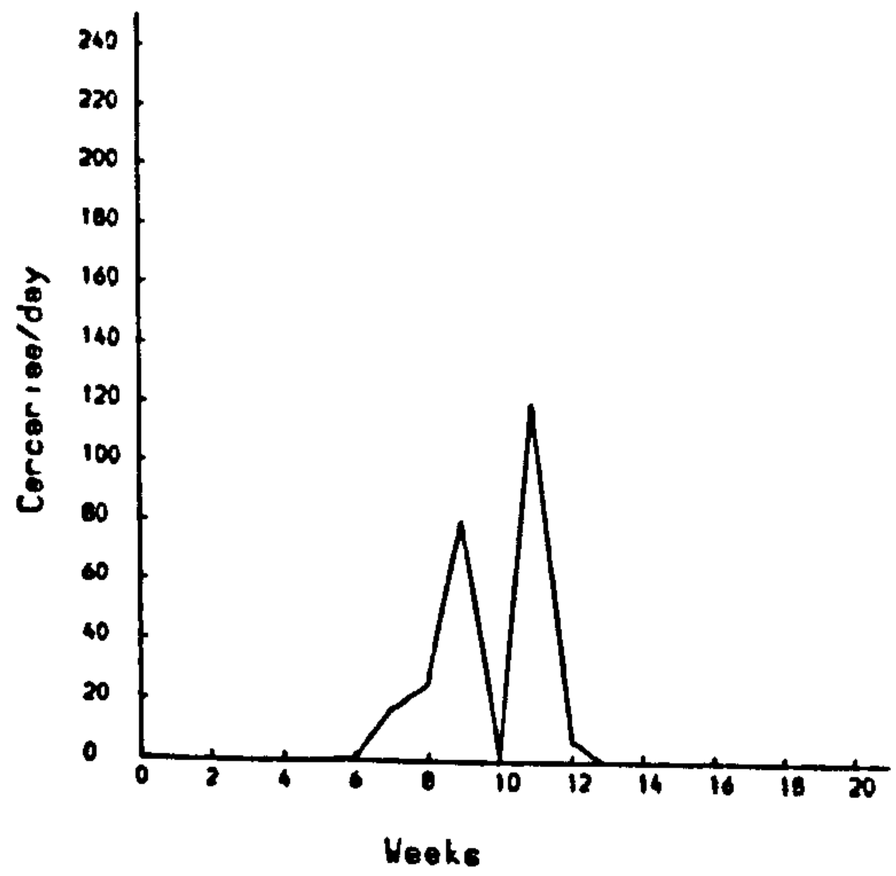

SBS 17 (G)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
200
220
240
0 2 4 6 8 10 12 14 16 18 20
Weeks


SBS 18 (G)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
200
220
240
0 2 4 6 8 10 12 14 16 18 20
Weeks

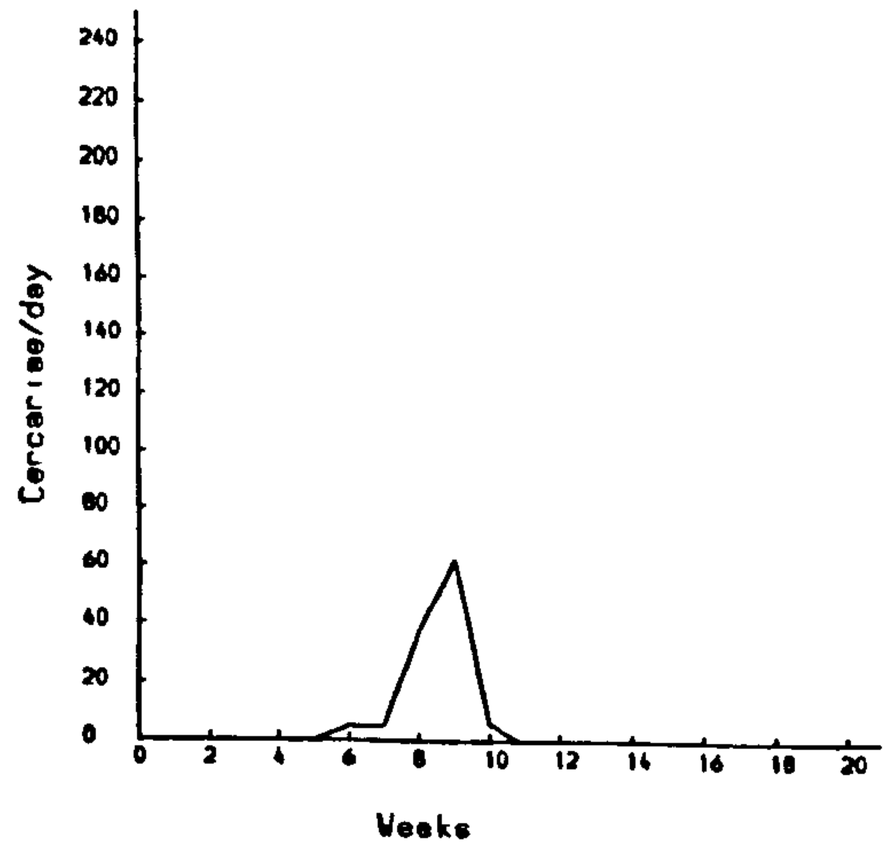
SBS 19 (G)



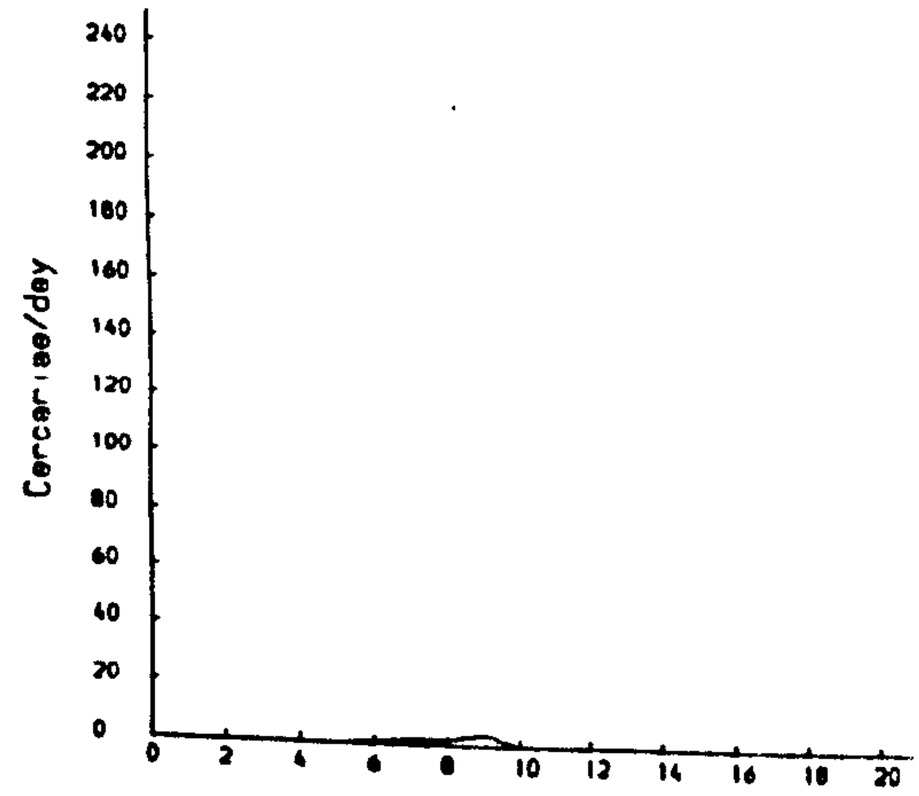

SBS 20 (G)



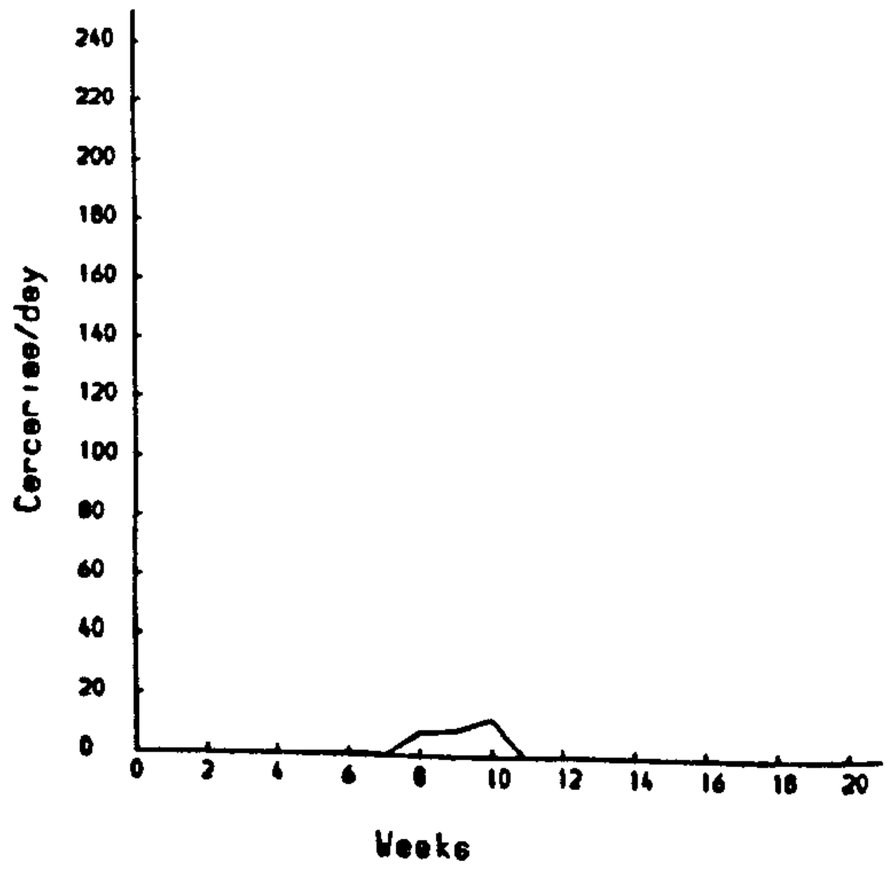

SBS 21 (G)



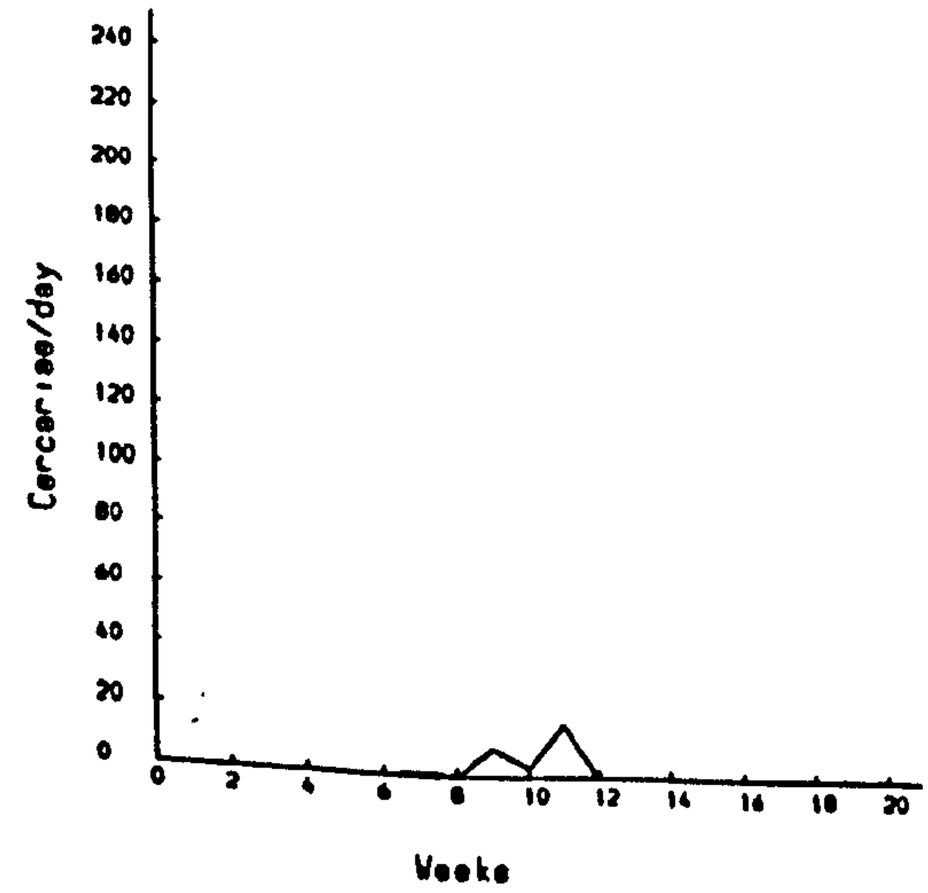

SBS 22 (G)

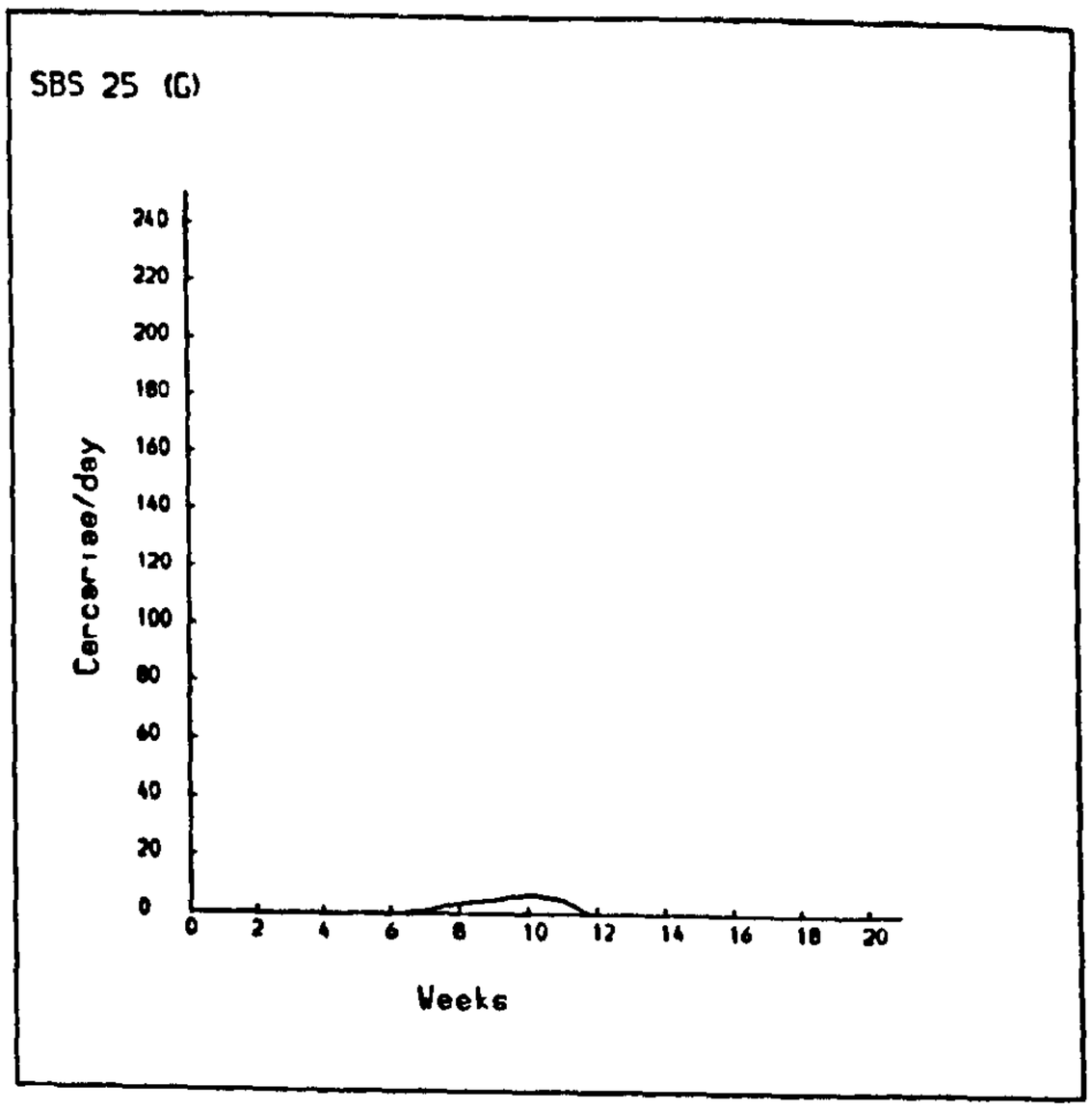


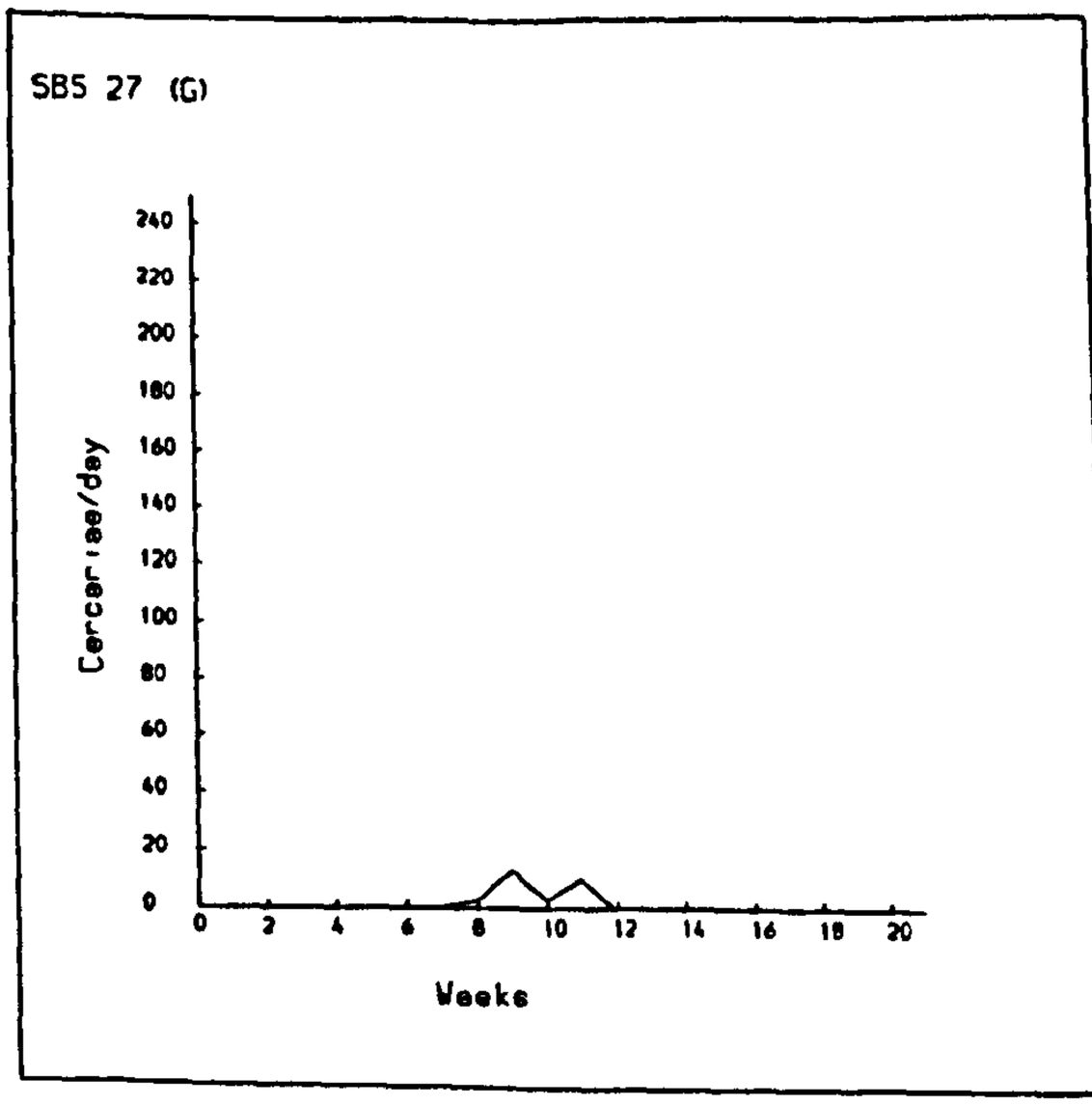

SBS 23 (G)

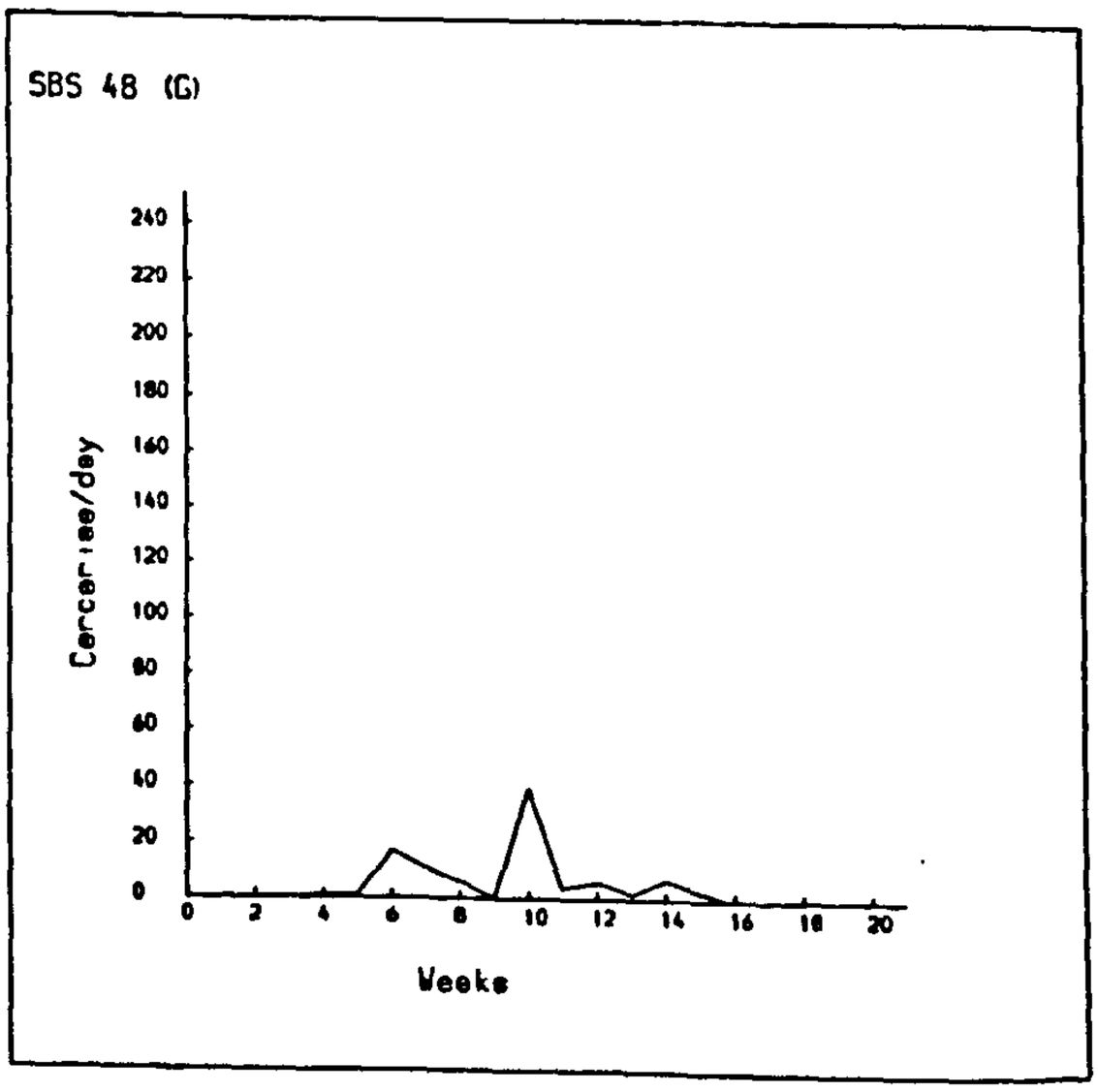


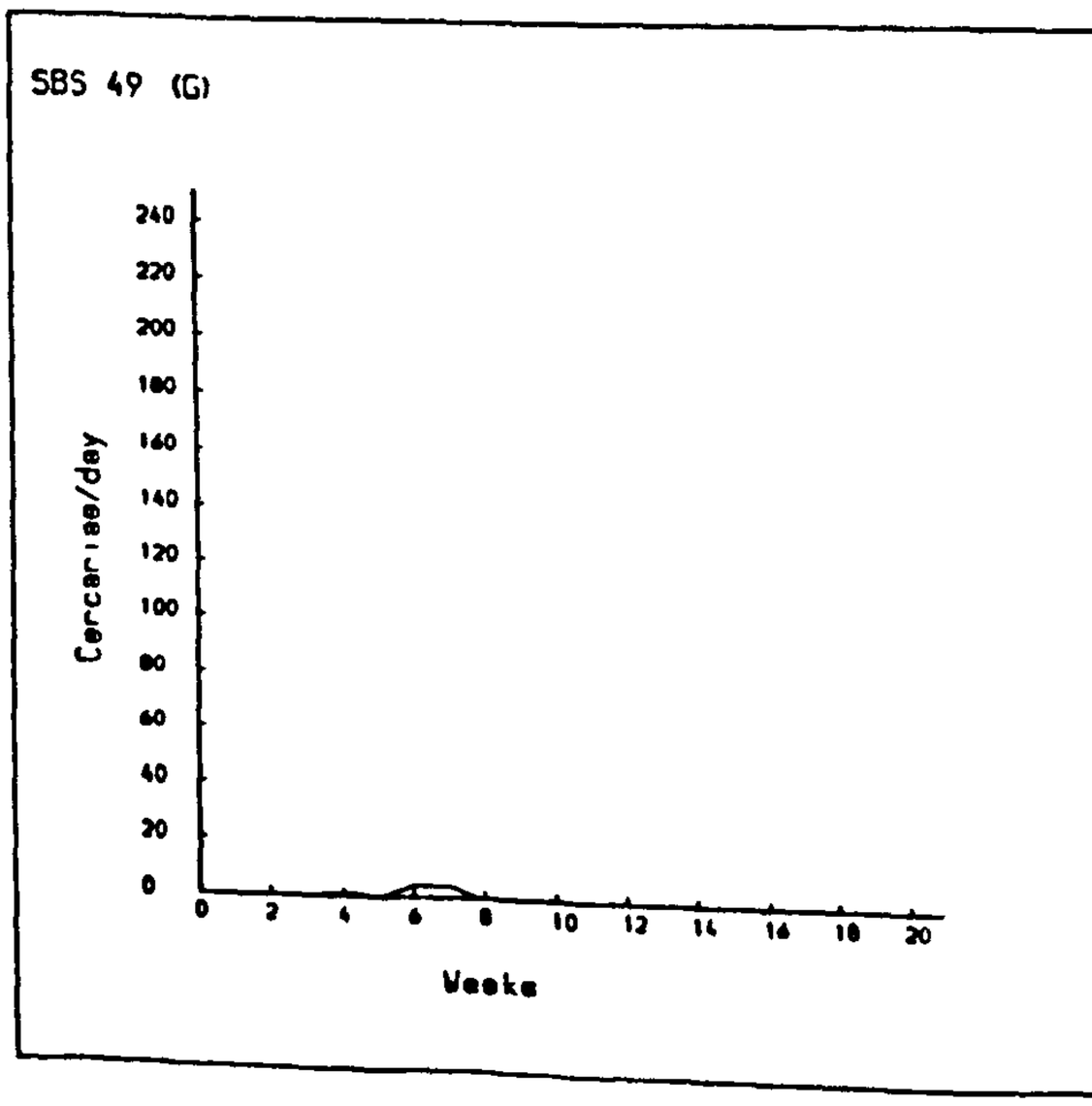

SBS 24 (G)

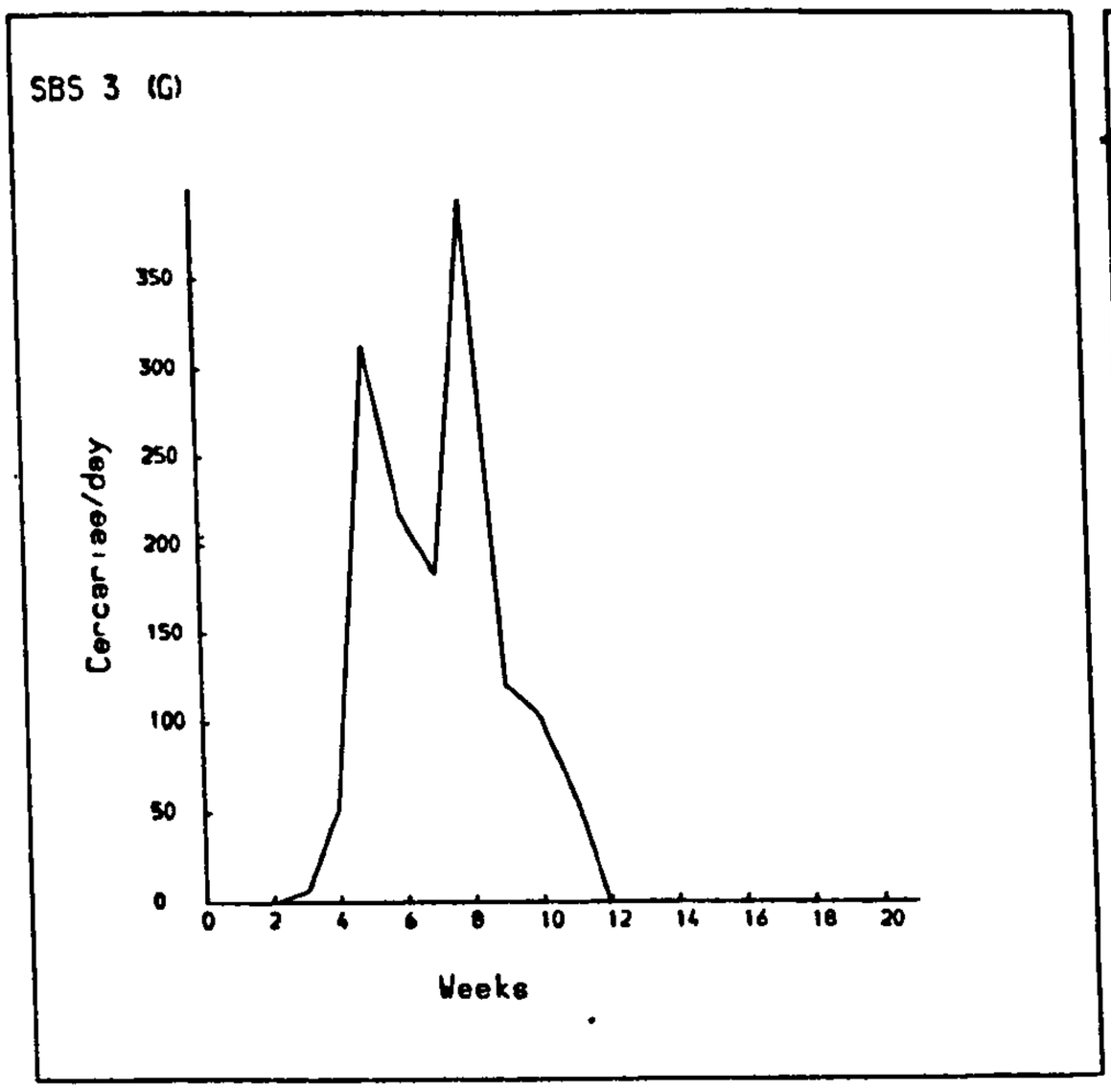


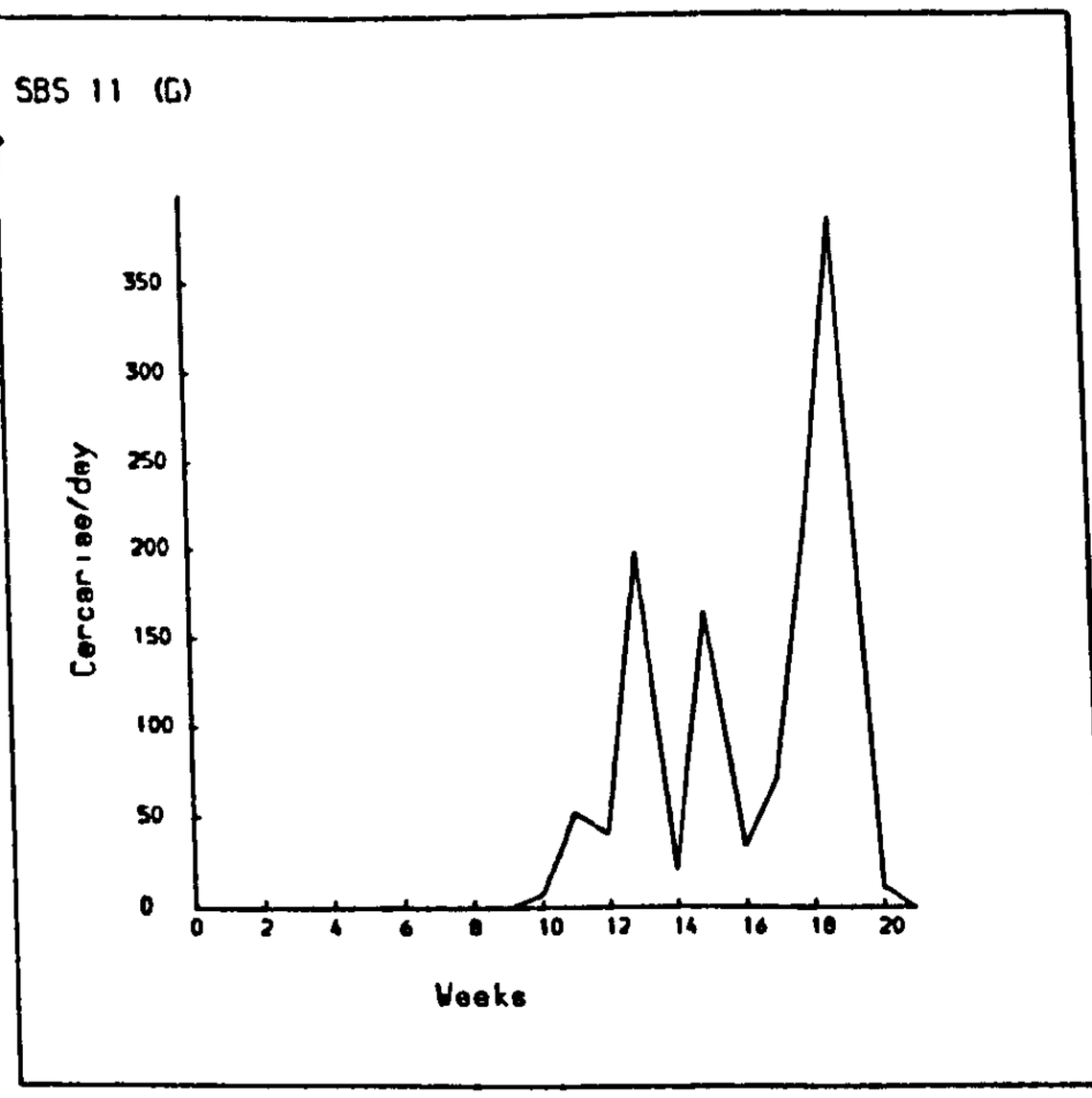

SBS 25 (G)
Cercariae/day
240
220
200
180
160
140
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20
Weeks

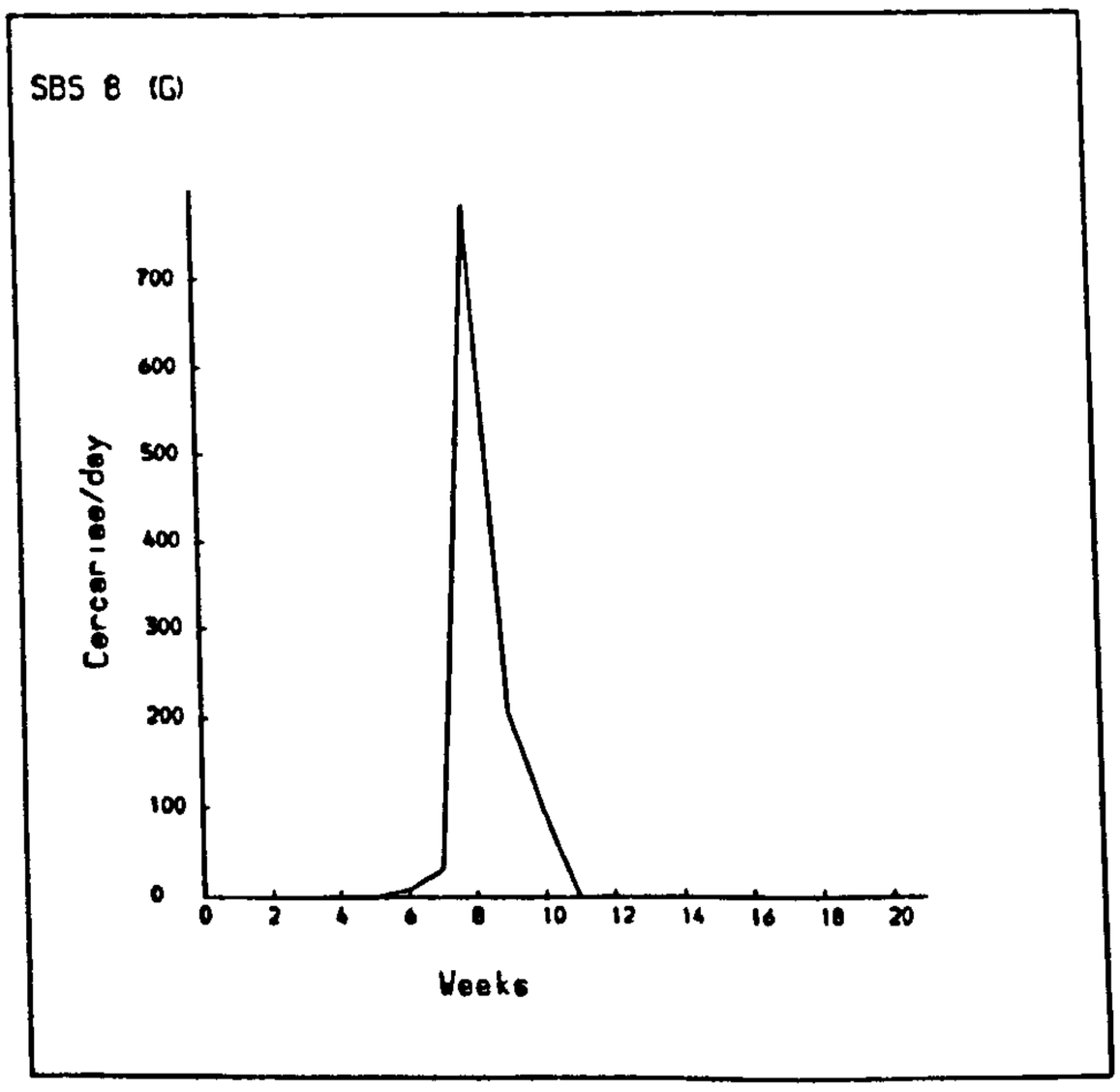

SBS 27 (G)
Cercariae/day
240
220
200
180
160
140
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20
Weeks

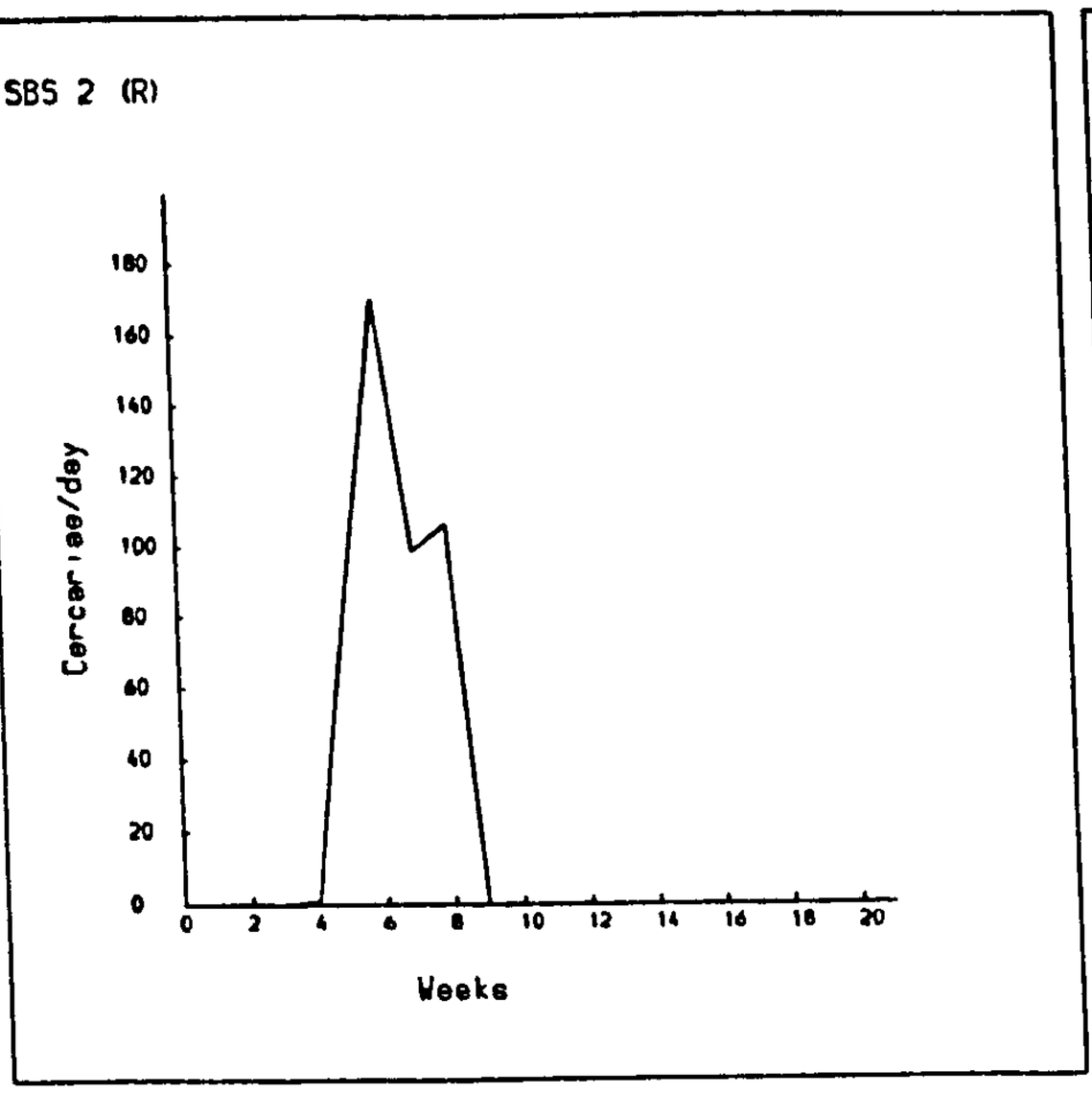

SBS 48 (G)
Cercariae/day
240
220
200
180
160
140
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20
Weeks

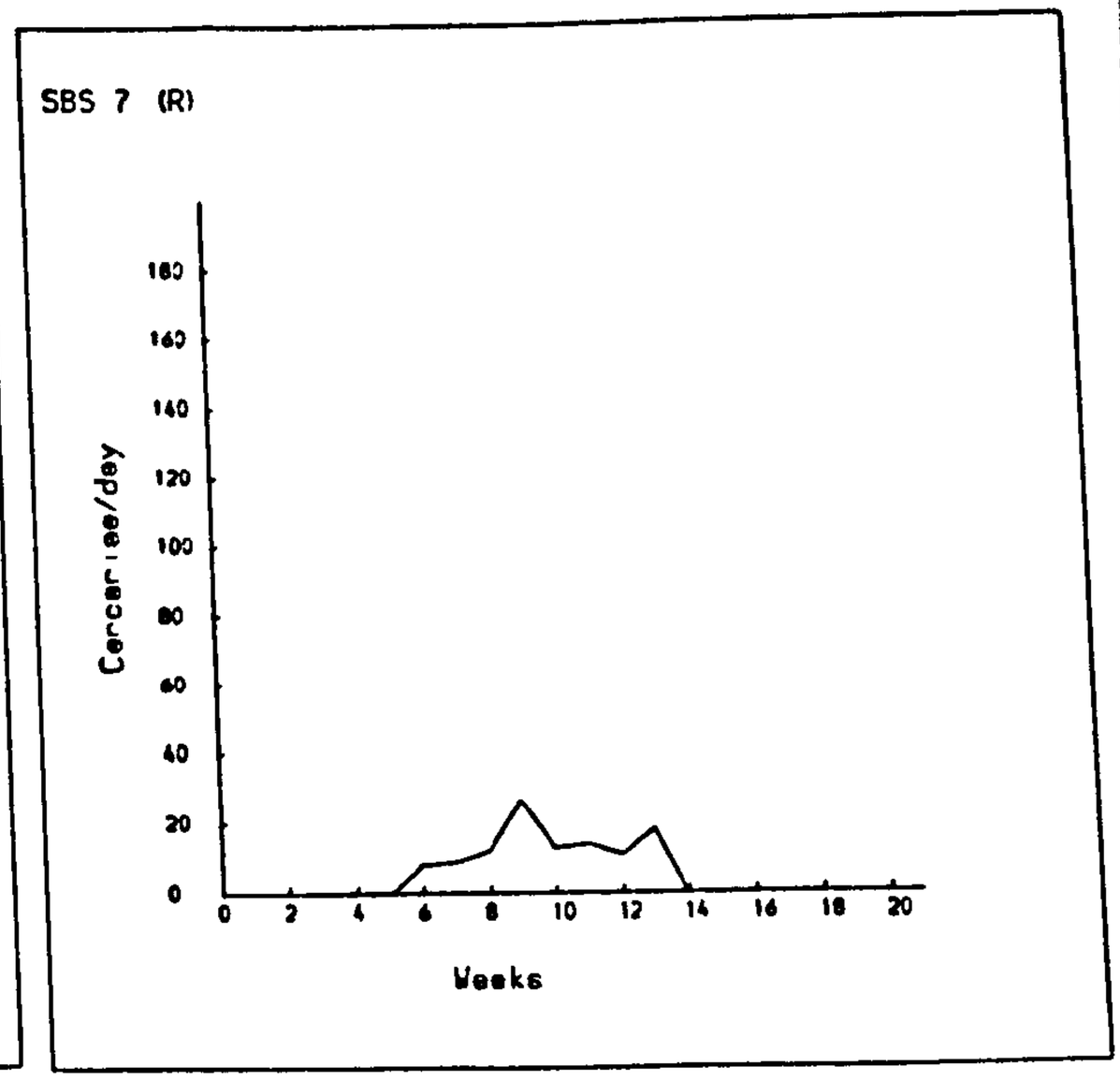

SBS 49 (G)
Cercariae/day
240
220
200
180
160
140
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20
Weeks

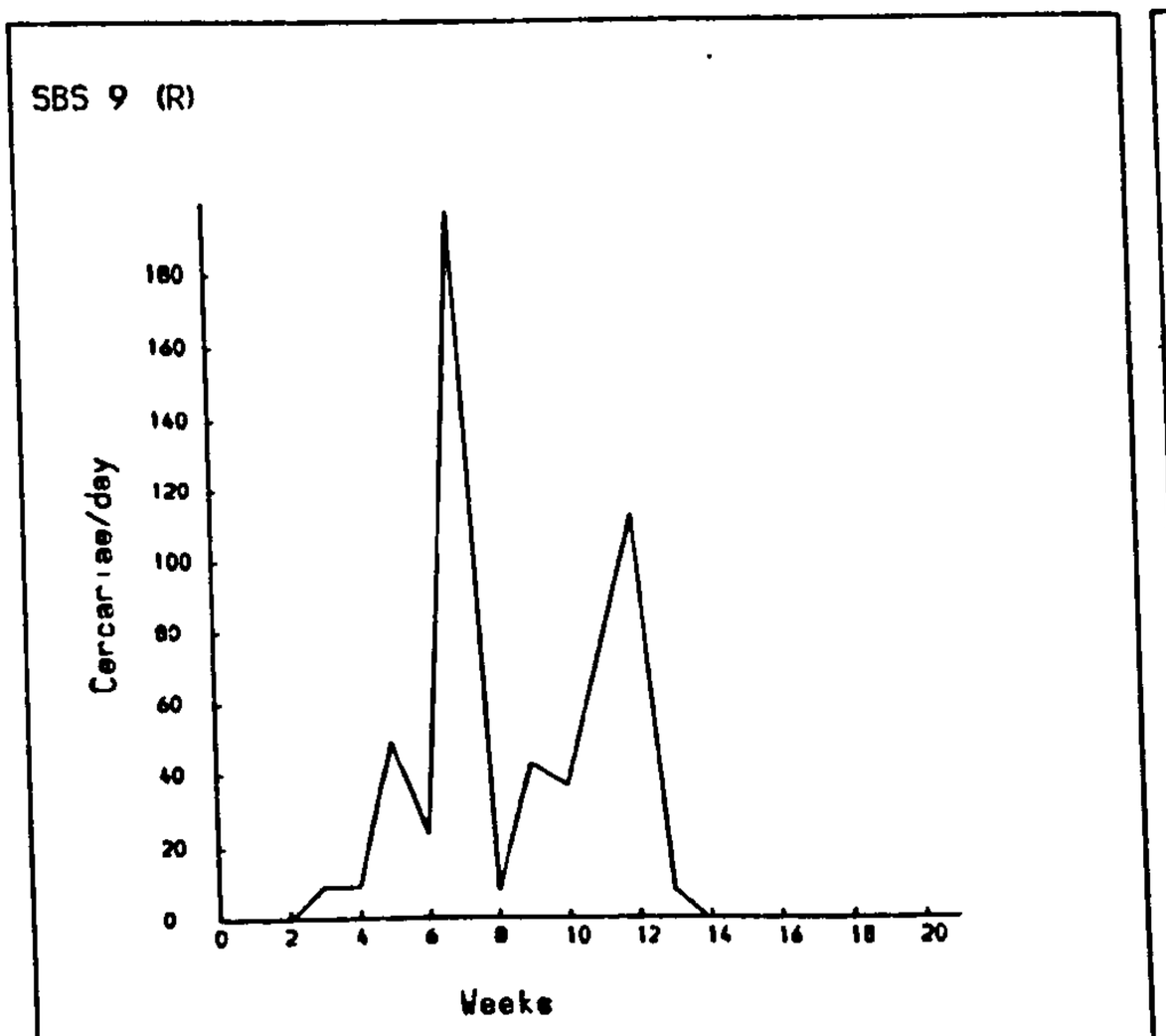

SBS 3 (G)
Cercariae/day
0
50
100
150
200
250
300
350
0 2 4 6 8 10 12 14 16 18 20
Weeks

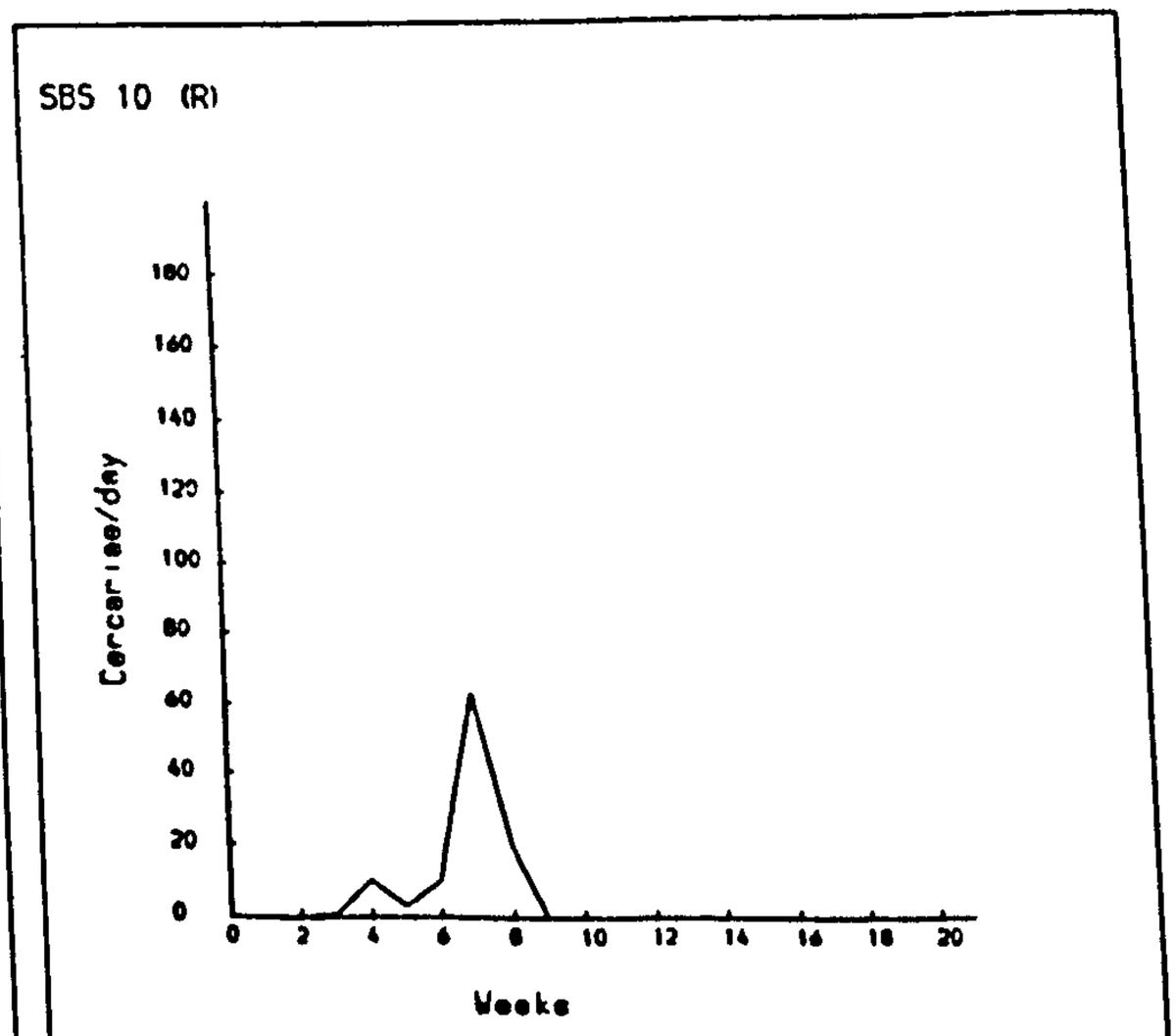

SBS 11 (G)
Cercariae/day
0
50
100
150
200
250
300
350
0 2 4 6 8 10 12 14 16 18 20
Weeks

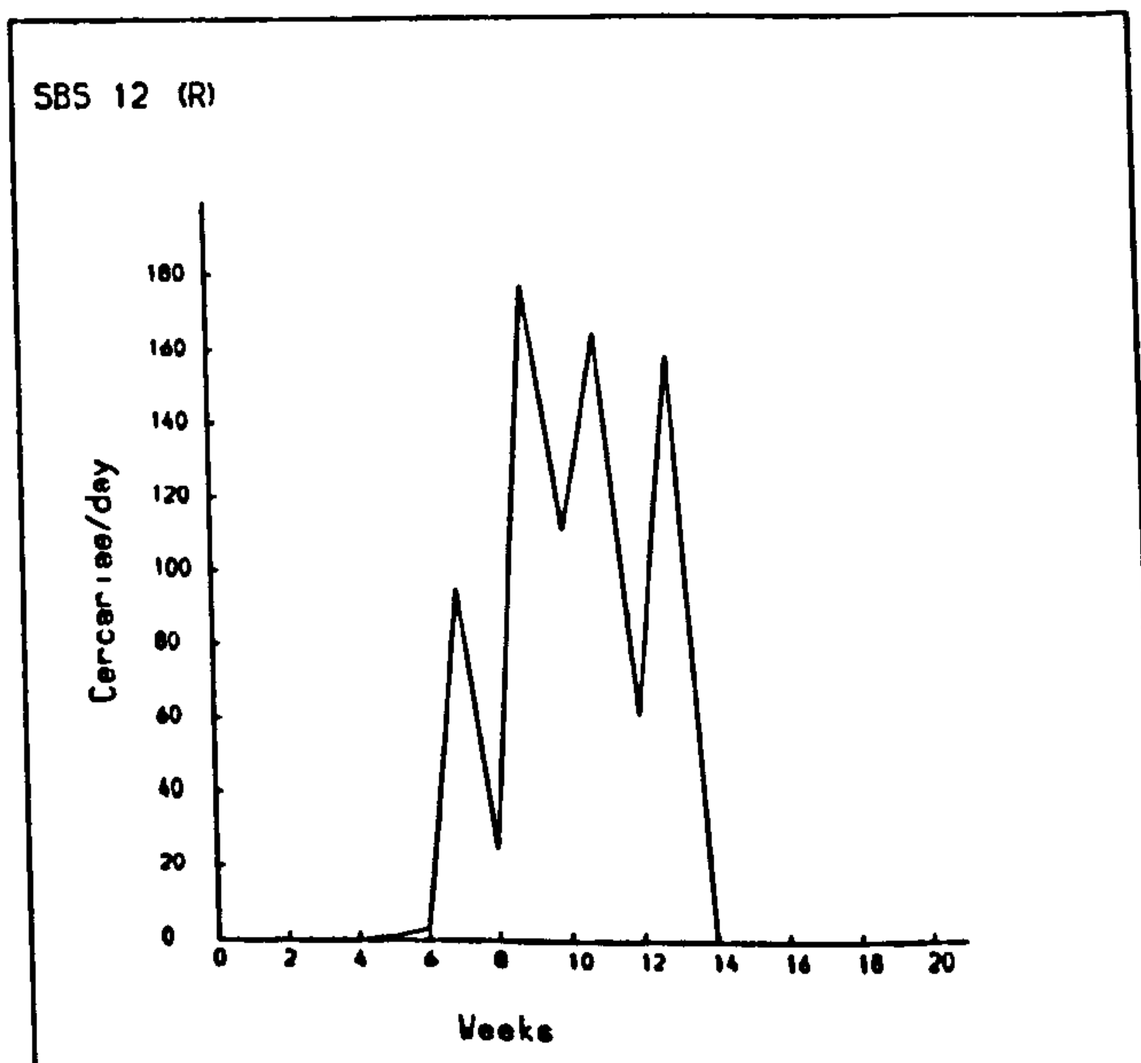

SBS 8 (G)
Cercariae/day
0
100
200
300
400
500
600
700
0 2 4 6 8 10 12 14 16 18 20
Weeks

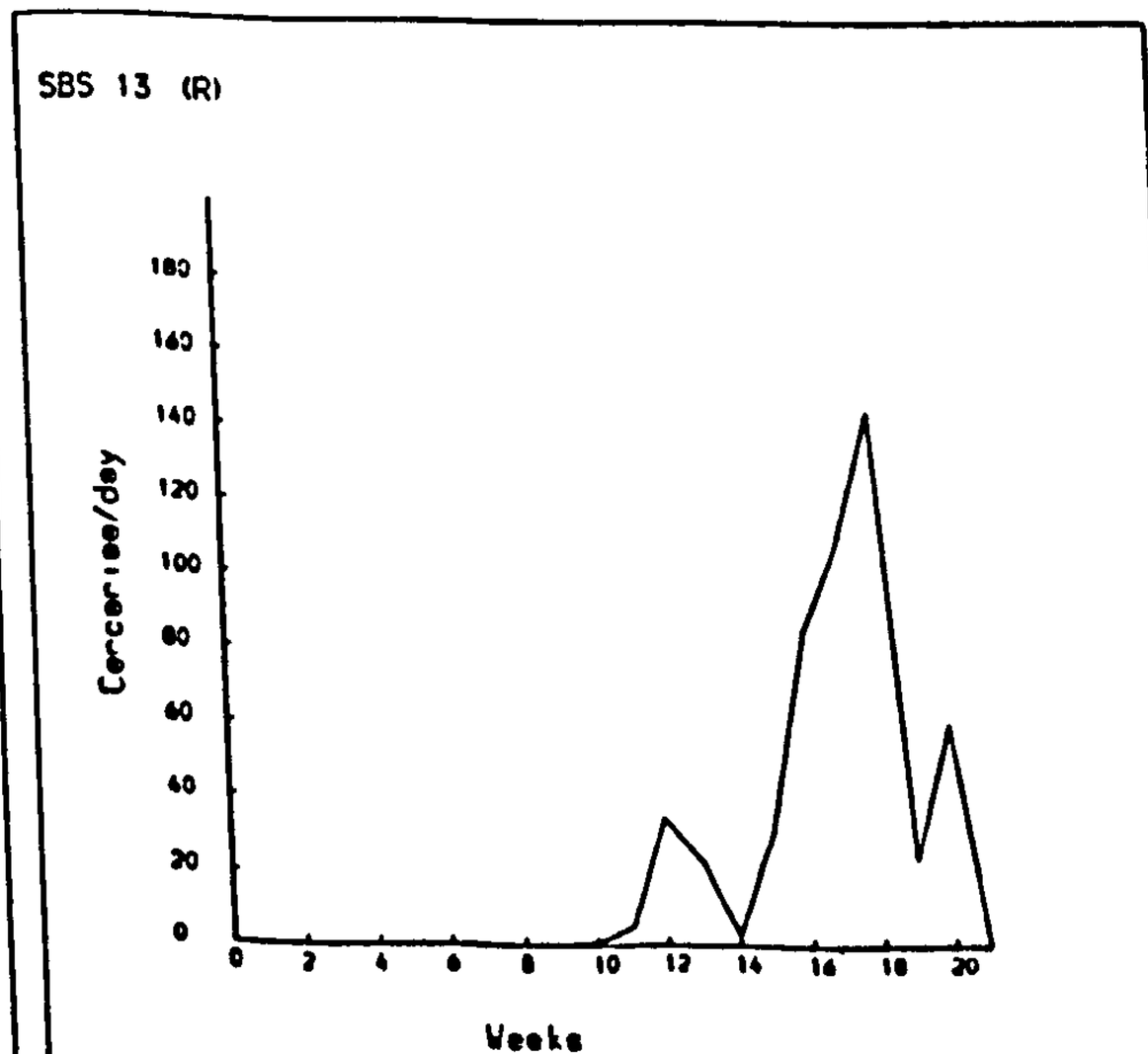

SBS 2 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks

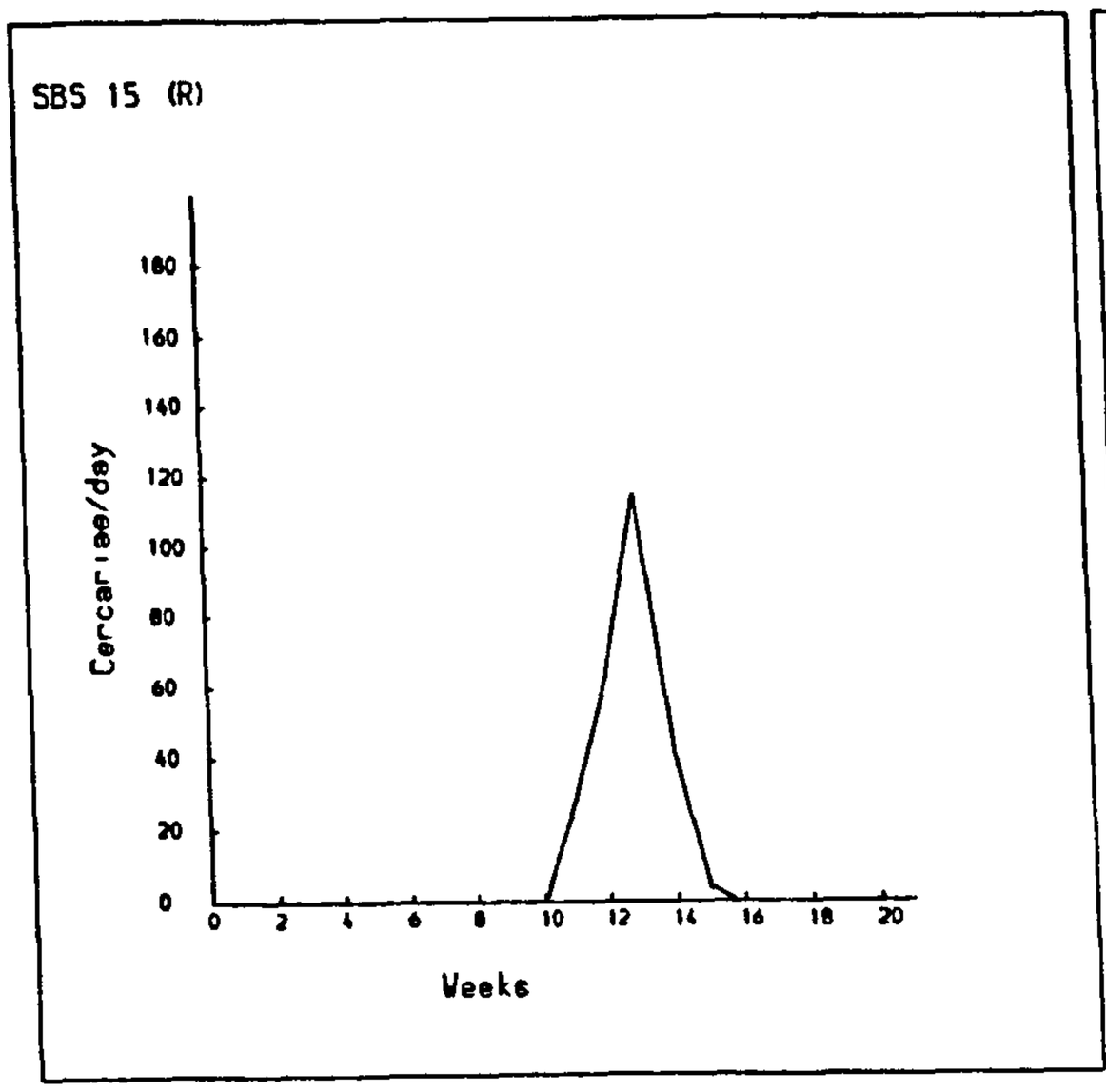

SBS 7 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks

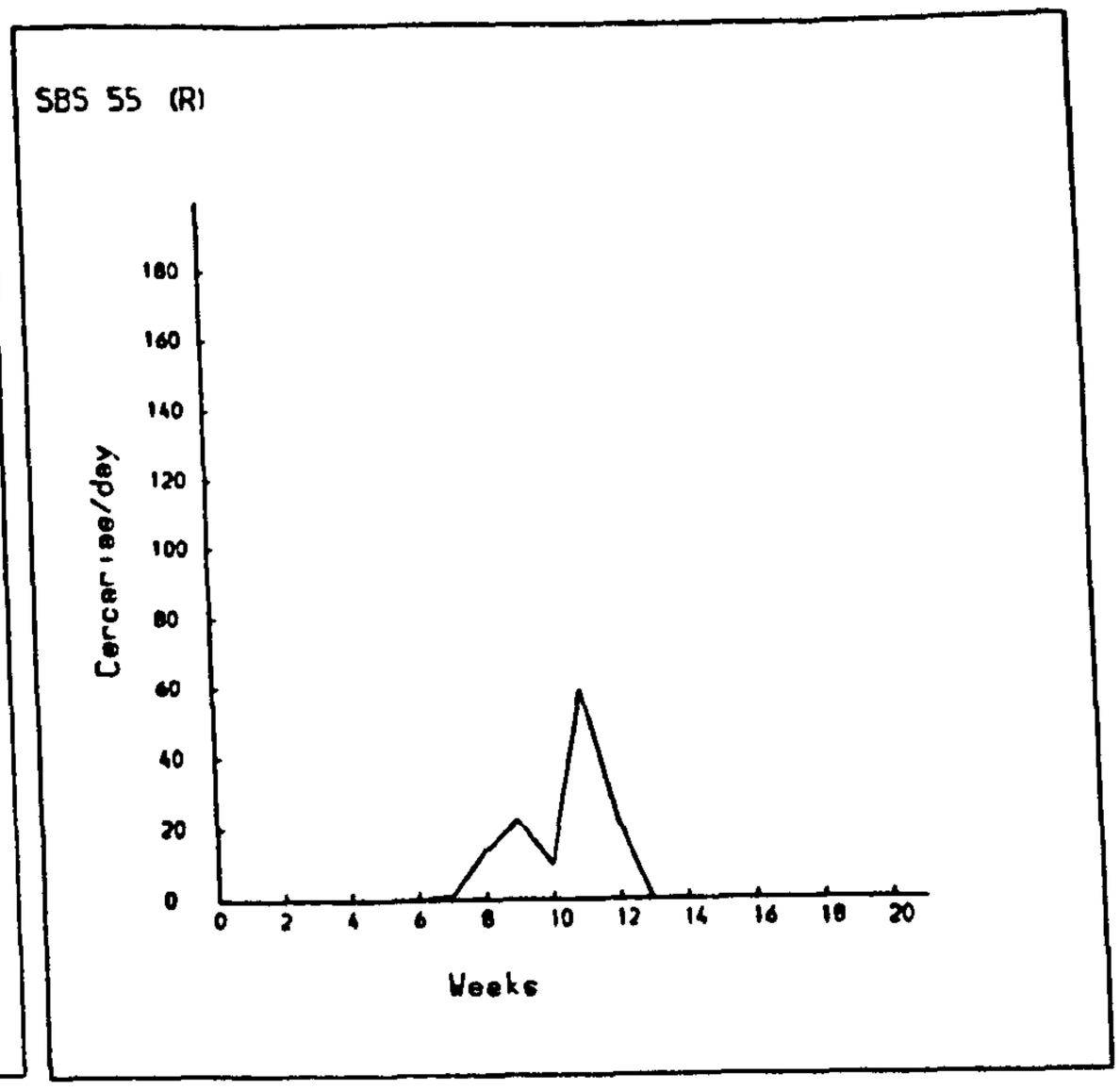

SBS 9 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks

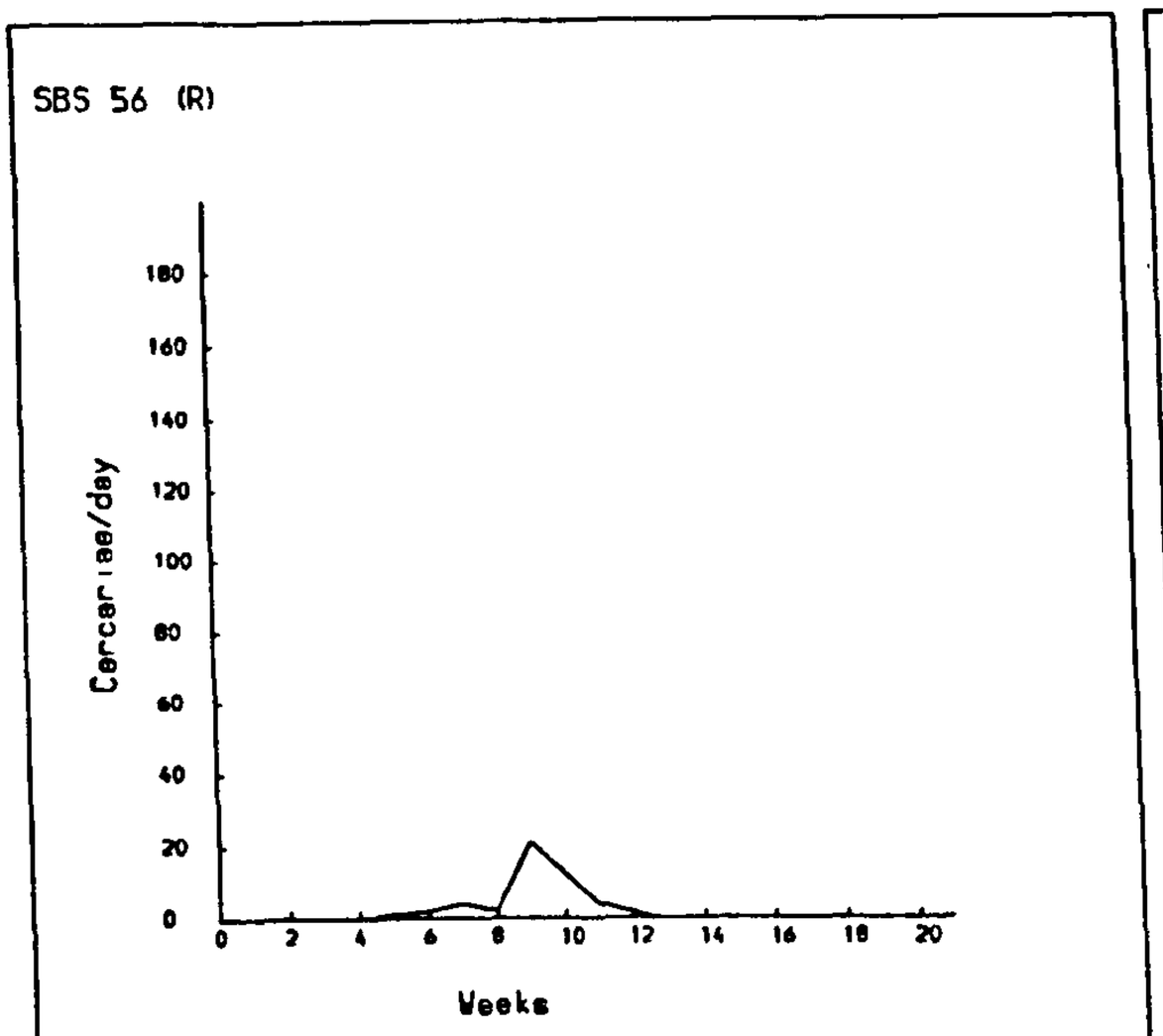

SBS 10 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks

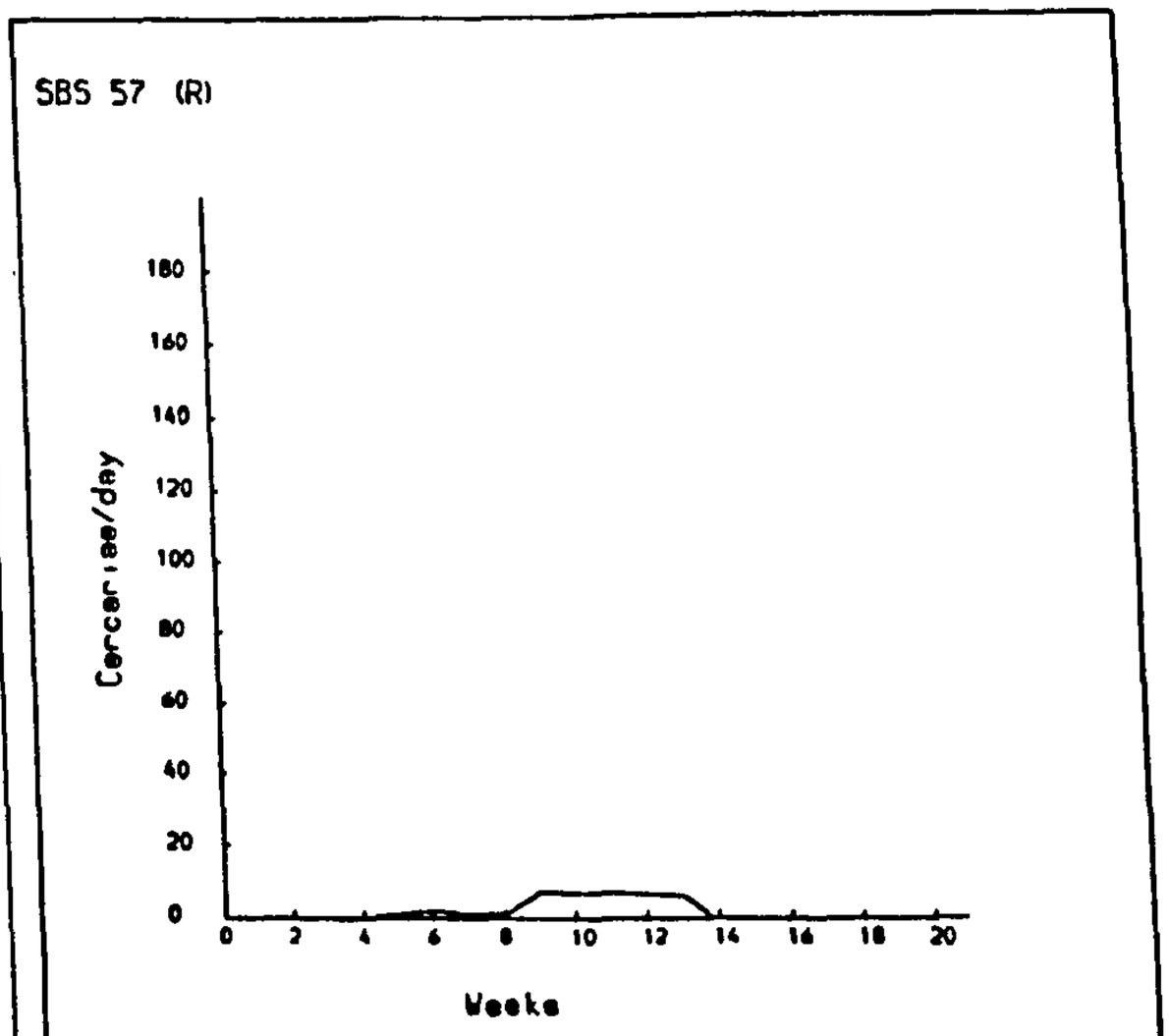

SBS 12 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks

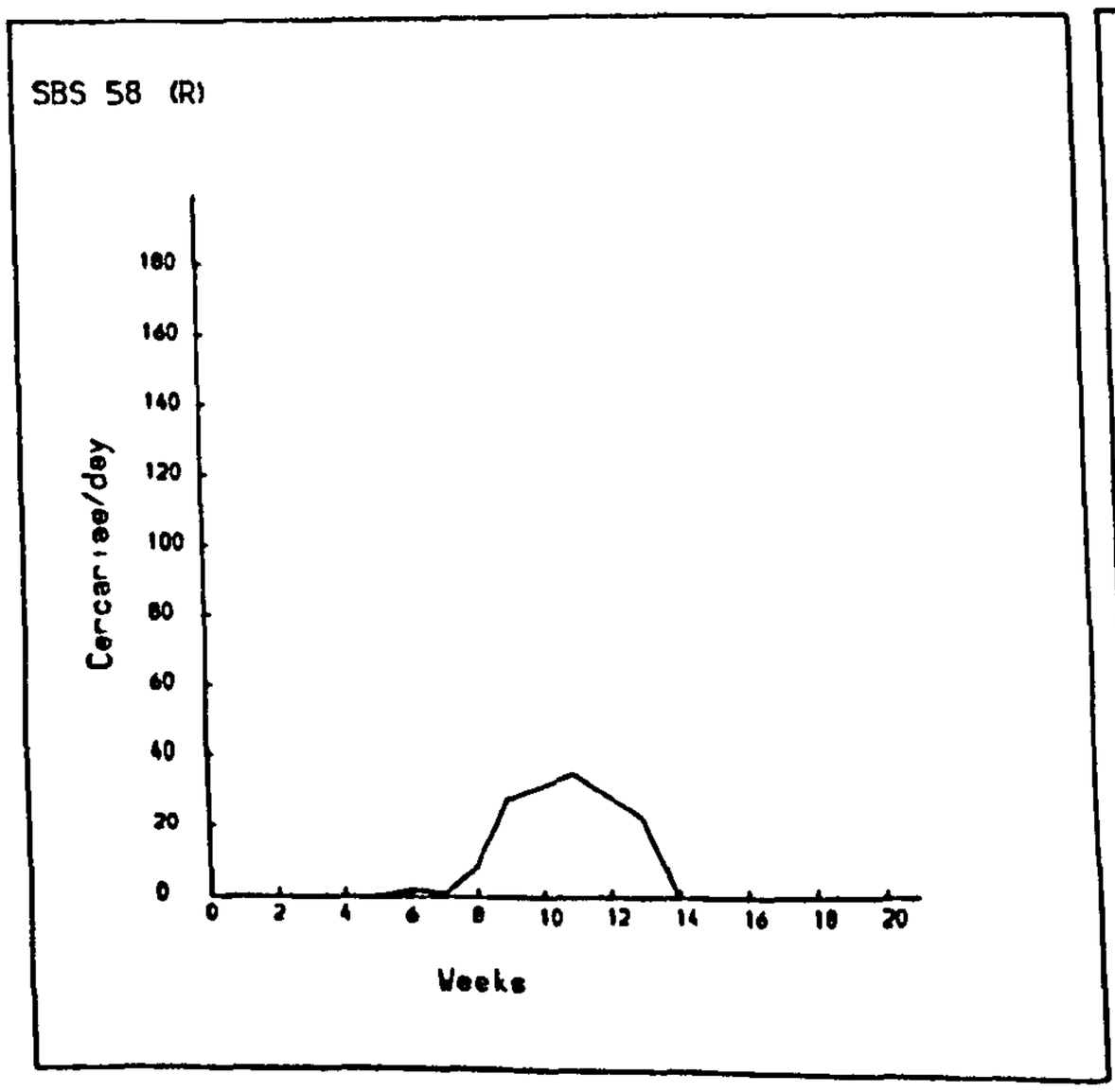

SBS 13 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks

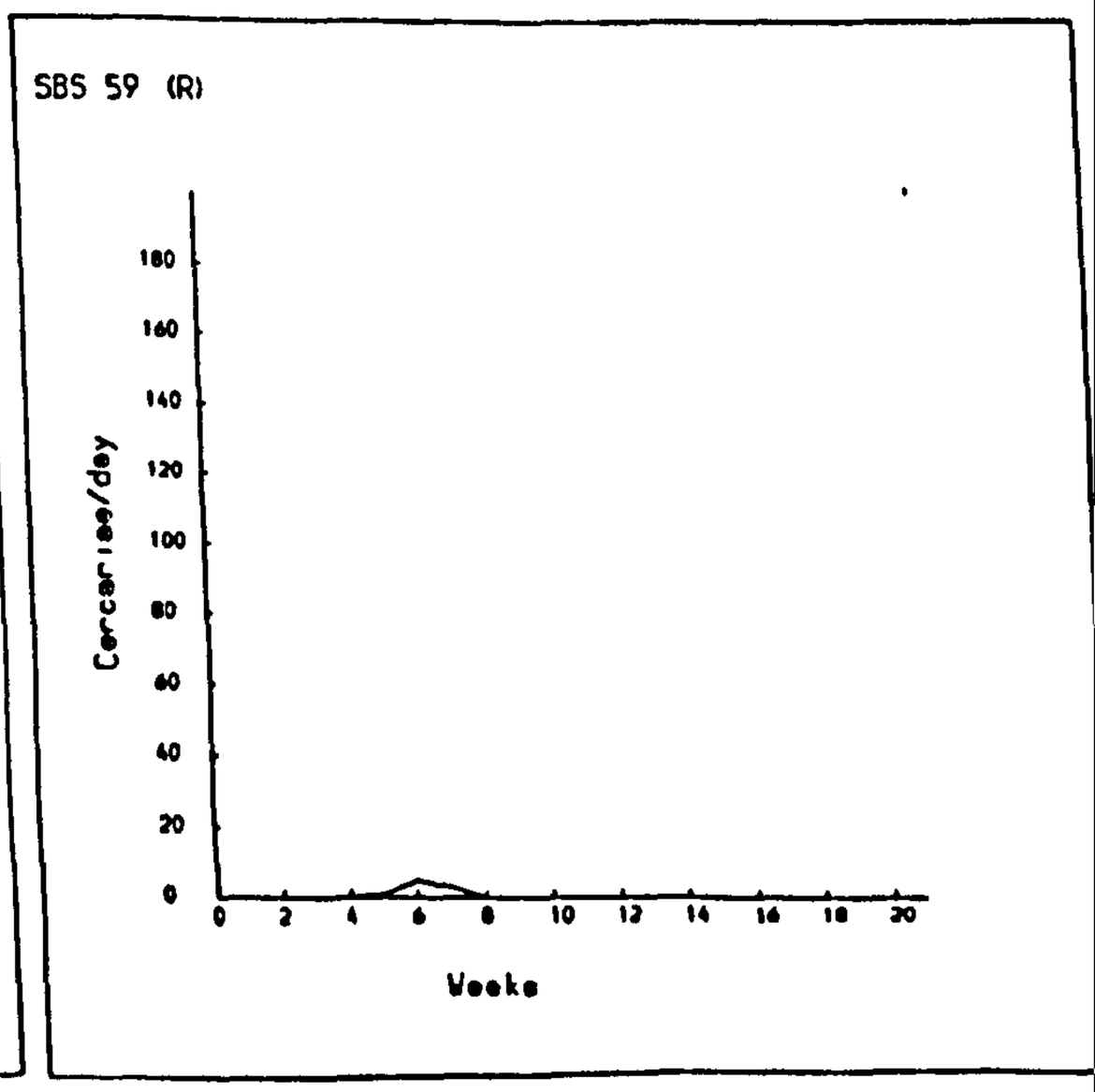

SBS 15 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks


SBS 55 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks

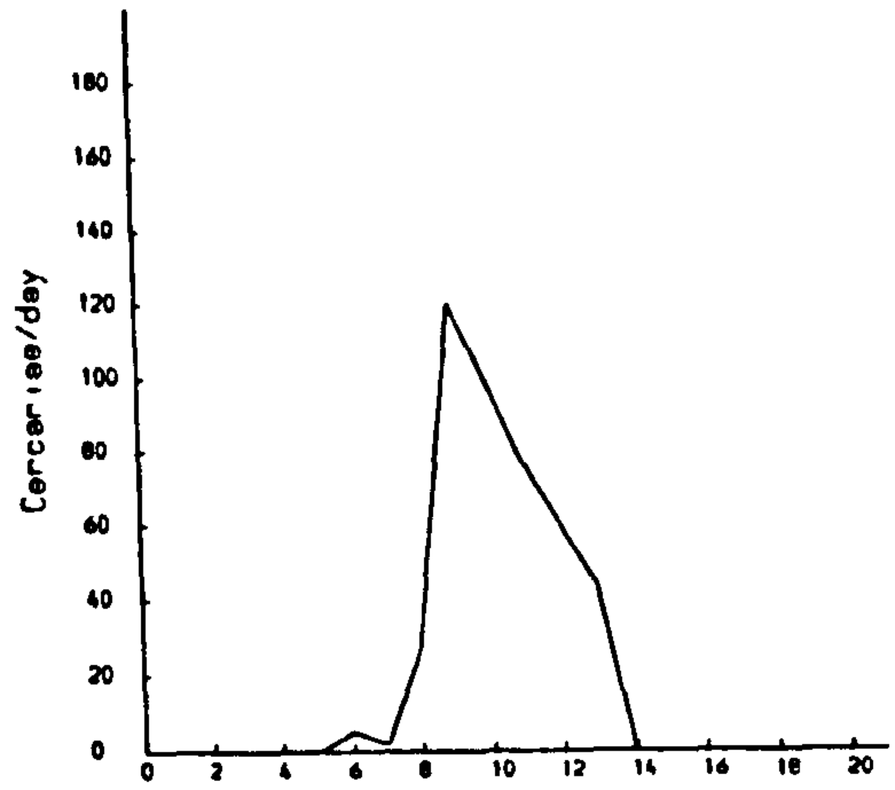

SBS 56 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks


SBS 57 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks

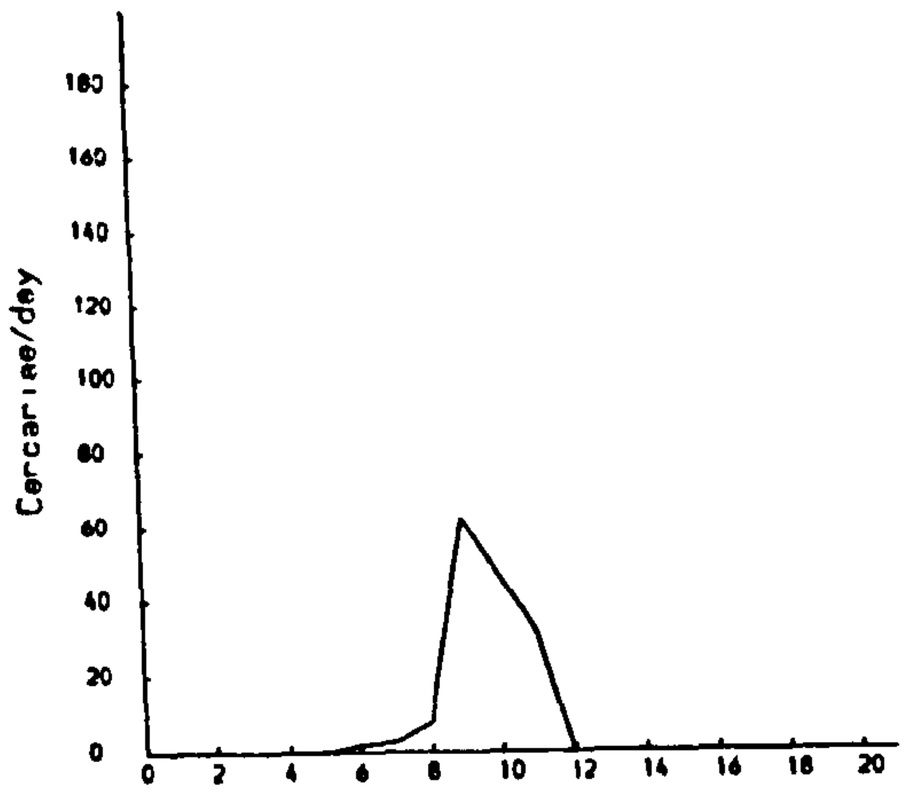

SBS 58 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks


SBS 59 (R)
Cercariae/day
0
20
40
60
80
100
120
140
160
180
0 2 4 6 8 10 12 14 16 18 20
Weeks

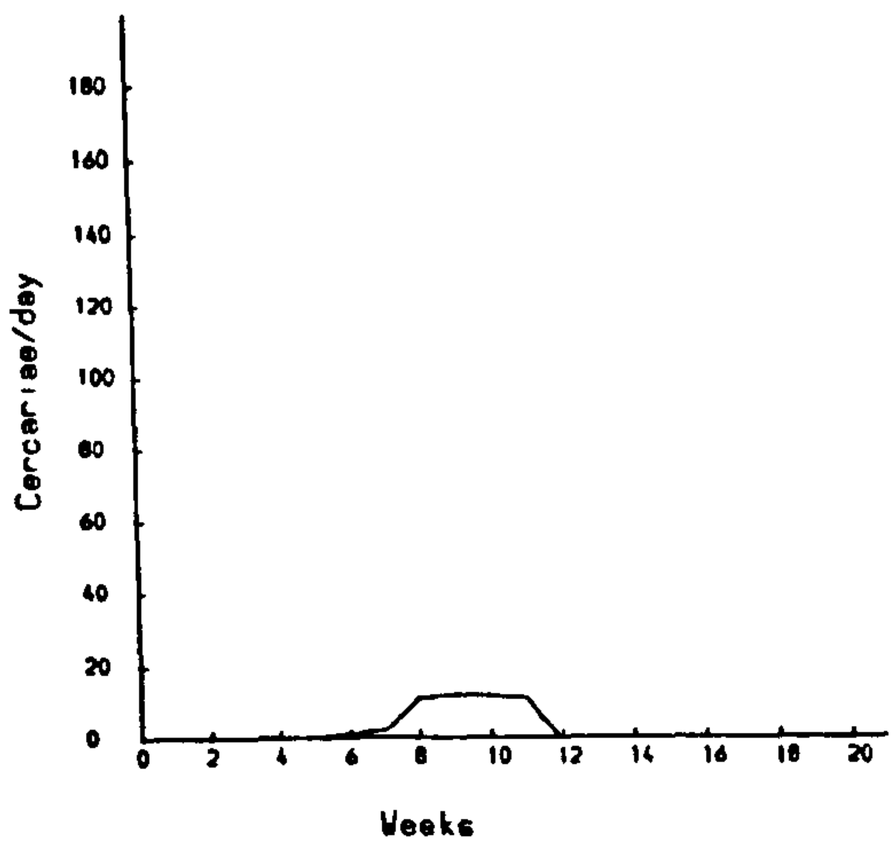
SBS 60 (R)



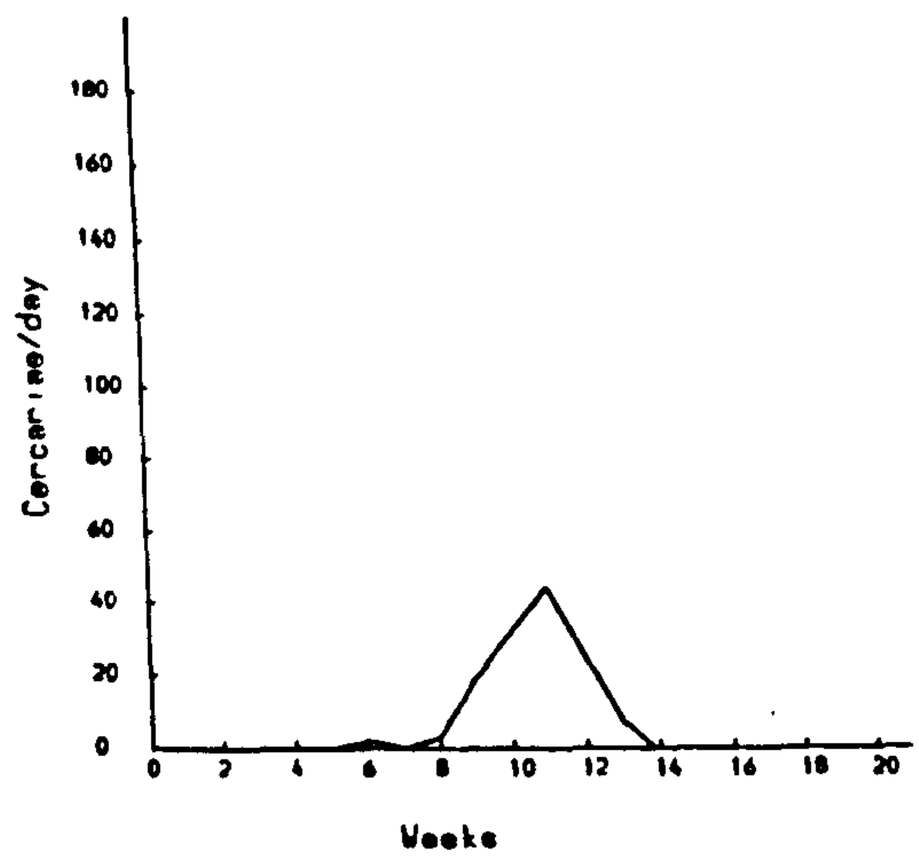

SBS 61 (R)



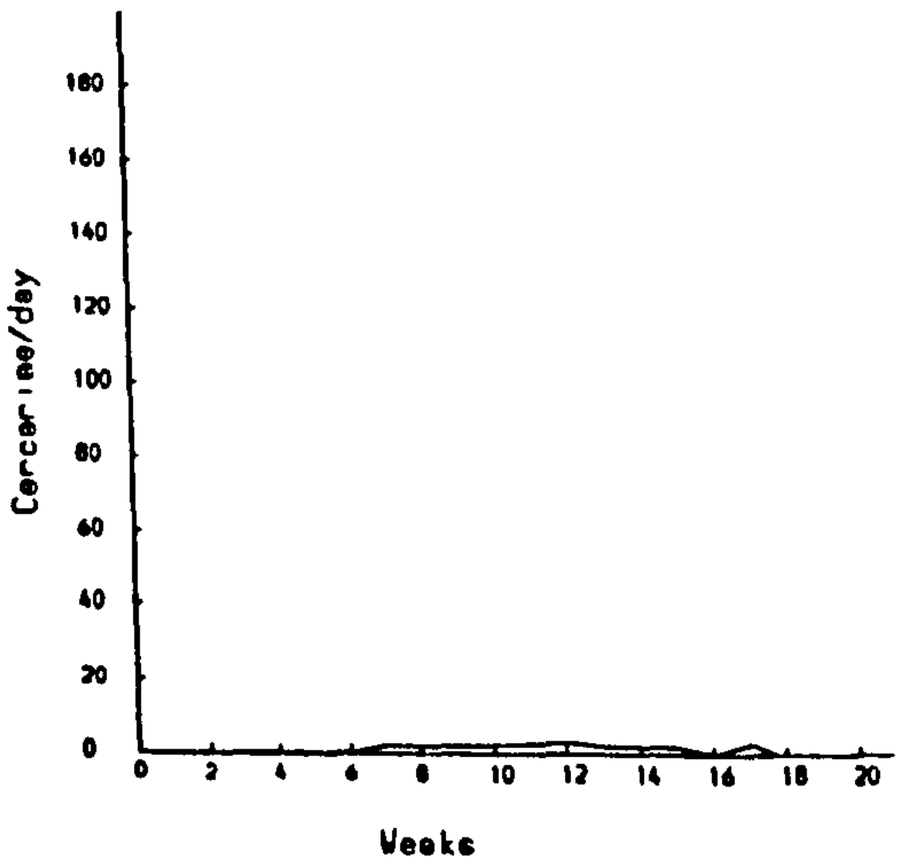

SBS 62 (R)



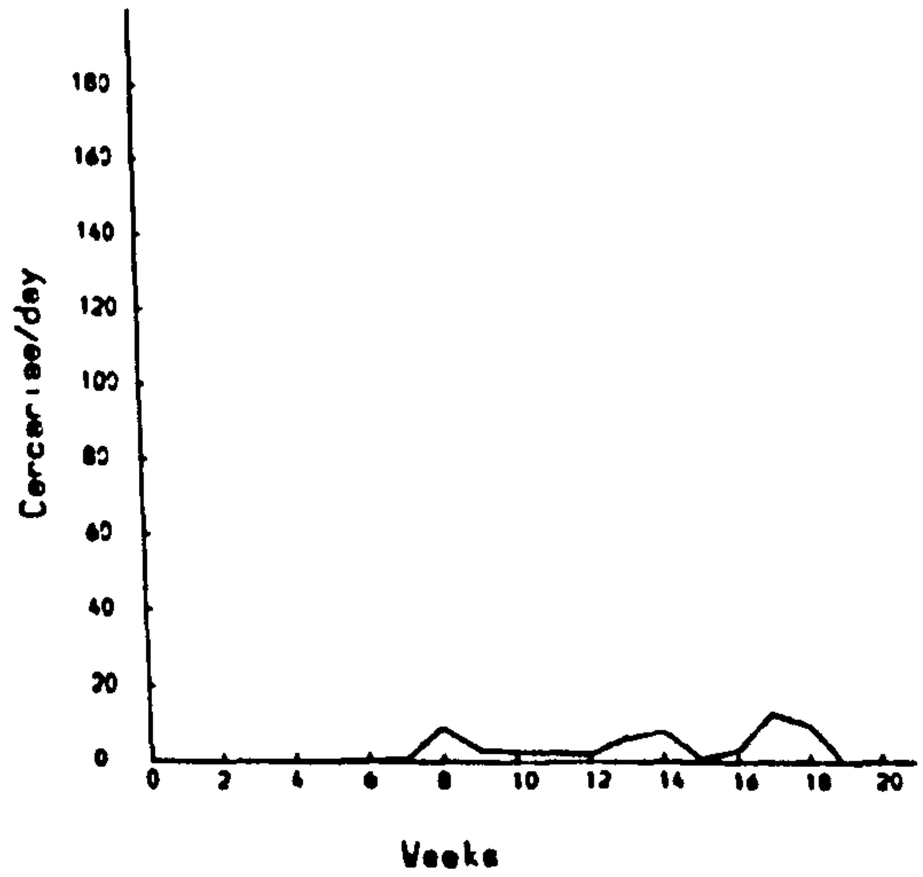

SBS 63 (R)

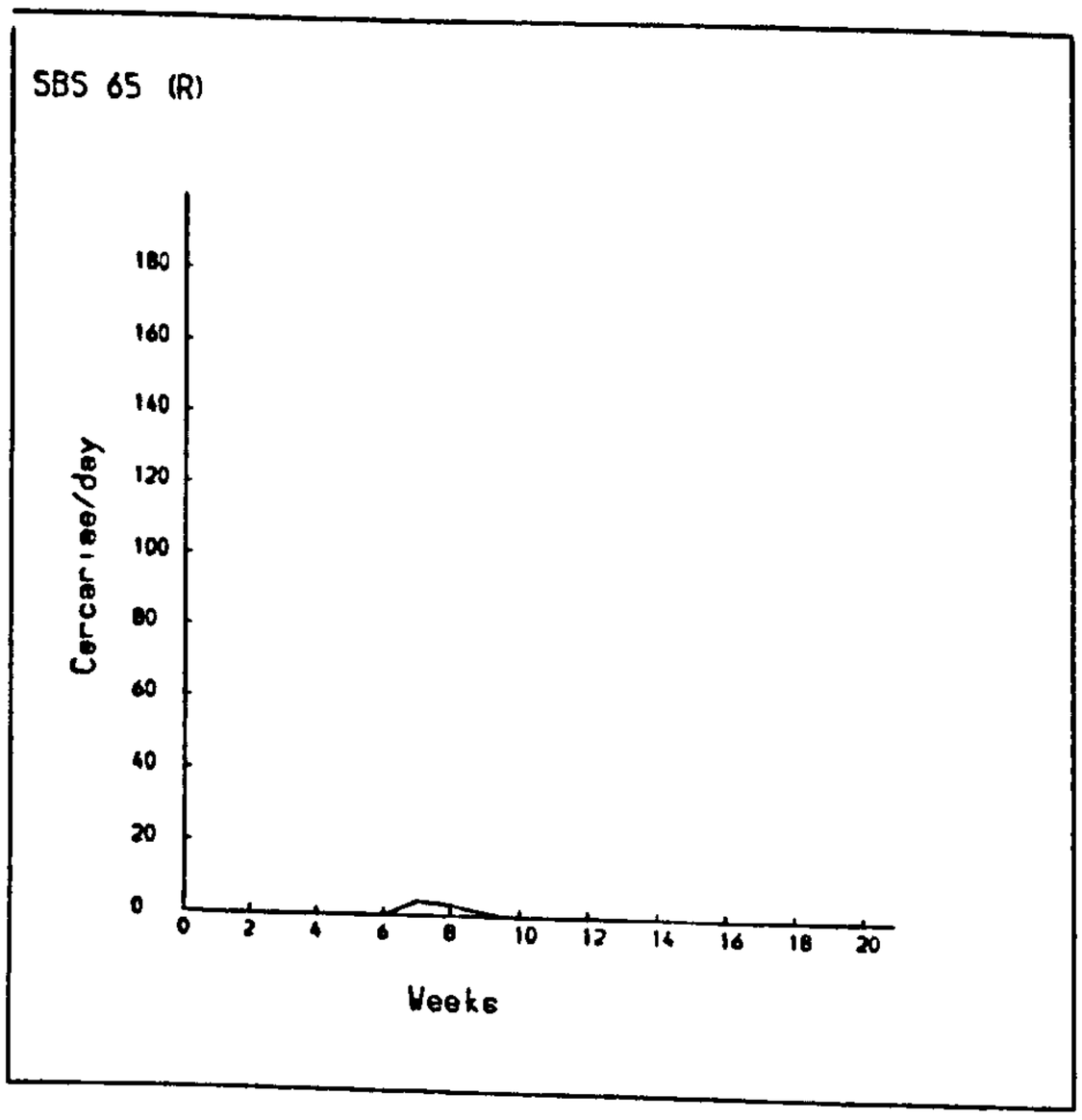


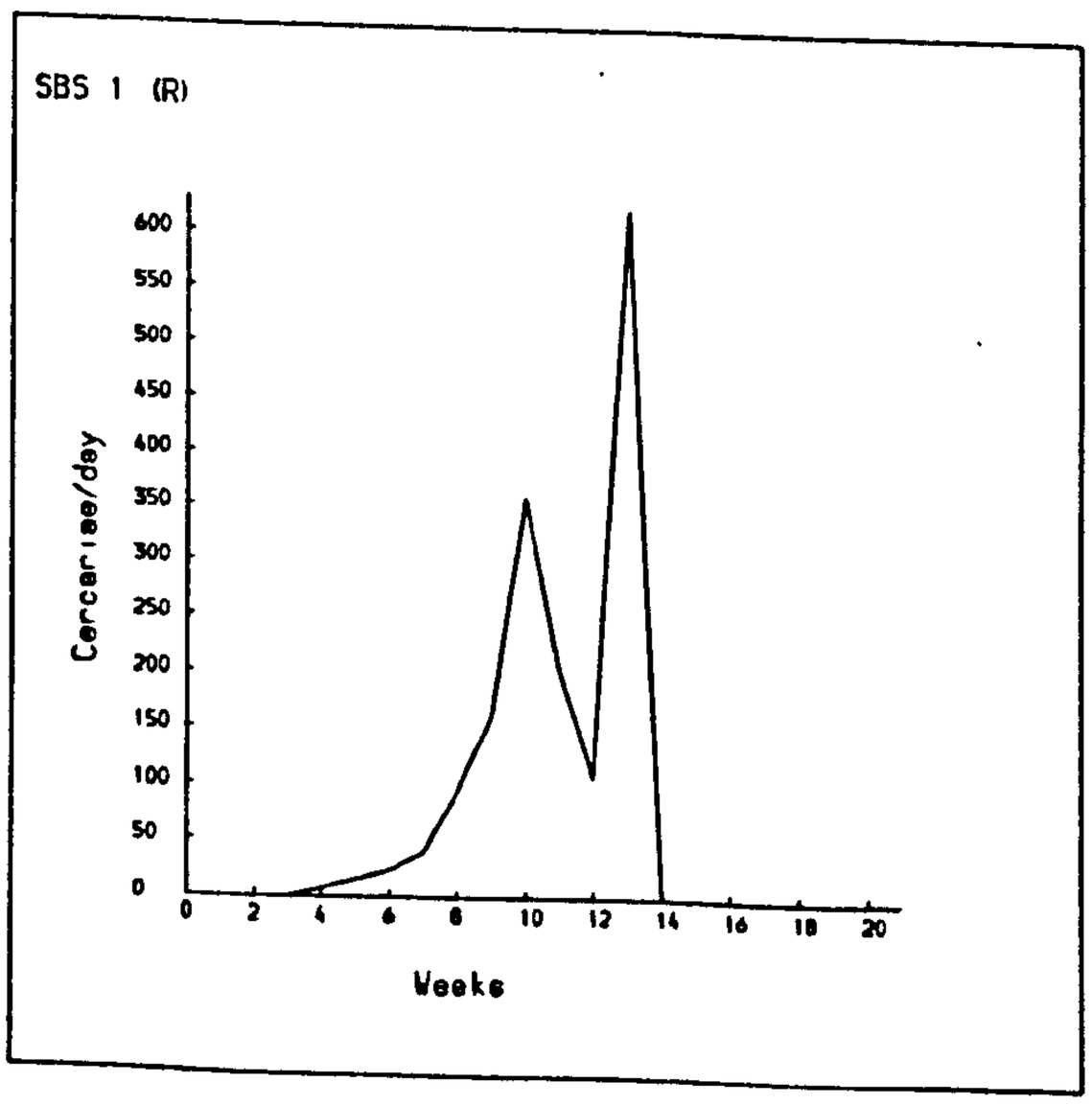

SBS 64 (R)




SBS 66 (R)




SBS 65 (R)
Cercariae/day
180
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
Weeks


SBS 1 (R)
Cercariae/day
600
550
500
450
400
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
Weeks

PAGE/PAGES
EXCLUDED
UNDER
INSTRUCTION
FROM
UNIVERSITY