

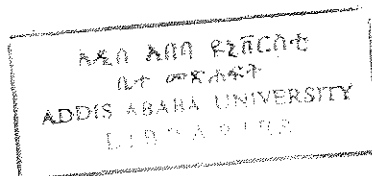
COMPARATIVE STUDIES ON THE ECOLOGY OF BIOMPHALARIA PFEIFFERI

(KRAUSS, 1847) IN NON ENDEMIC AND ENDEMIC AREAS OF
SCHISTOSOMA MANSONI (SAMBON, 1907) IN ETHIOPIA.

A Thesis

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by

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Abstract

The region of Debre Berhan, where schistosomiasis is not endemic, was compared with a well established focus of S.mansoni, Wonji, with regard to climate, snail habitats and snail differences in susceptibility and growth rate. In general rain and changes associated with it have influence on the snail population but this does not account for the lack of endemic schistosomiasis. Snails from both areas were equally susceptible to infection with S.mansoni. The low mean temperature in Debre Berhan increased the incubation period of the parasite. Mortality rate of Debre Berhan snails was higher than these of wonji snails while the growth rate was vice versa.

Despite the relatively unfavourable conditions, the possibility of introduction of schistosomiasis to Debre Berhan as a result of environmental modifications that may bring changes in human activities can not be discounted.

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C O N T E N T S

	<u>Page</u>
ABSTRACT	i
ACKNOWLEDGMENTS	ii
CONTENTS	iii
LIST OF FIGURES & TABLES	vi
INTRODUCTION	1
LITERATURE REVIEW	
1. Factors that influence the distribution of snails.	3
a. General account	
b. Temperature	
c. Light	
d. Water Chemistry	
e. pH	
f. Magnesium	
g. Calcium	
h. Sodium and Potassium	
i. Rainfall	
2. Vector - parasite compatibility	12
a. General account	
b. Temperature	
c. Light	
d. pH	
e. Oxygen	
f. Nutrition	
g. Water velocity	

C O N T E N T S

	<u>Page</u>
MATERIALS & METHODS	21
Description of the study areas	21
Debre Berhan	
Wonji	
Meteorological studies at the two study areas.	27
Chemical analysis of water.....	28
Sampling for snail population	28
Studies on parasites	29
Prevalence surveys	
Natural infection of snails	
Host parasite compatibility	30
Egg-hatching	
Laboratory breeding of snails	
Snail exposure	
Statistical tests	33
RESULTS	34
Physical factors at the study areas	34
Debre Berhan	
Wonji	
Chemical analysis of water	39
Snail population dynamics	42
Correlation of snail population to meteorological factors	53

C O N T E N T S

	<u>Page</u>
Incidence of intestinal parasites at Wonji and Debre Berhan.	53
Natural infection of snails.....	54
Host parasite compatibility	54
DISCUSSION	66
CONCLUSION	74
LITERATURE CITED.....	75

LIST OF FIGURES & TABLES

<u>Figures</u>	<u>Title</u>	<u>Page</u>
1.	Study sites at Debre Berhan	23
2.	Study sites at Wonji	26
3.	Meteorological data and snail population at Wonji	36
4.	Meteorological data and snail population at Debre Berhan	38

Tables

1.	Chemical analysis of water samples from Debre Berhan Site, B, (R.Beres)	40
2.	Chemical analysis of water samples from Wonji Site X	41
3-8.	Monthly collection of <u>B.pfeifferi</u> at sites A-E, Debre Berhan	43
9-11.	Monthly collection of <u>B.pfeifferi</u> at sites X and Y, Wonji	50
12.	Correlation of snail population and Meteorological factors in Debre Berhan.	55
13.	Correlation of snail population and Meteorological factors in Wonji	56
14.	Natural infection rate of <u>B.pfeifferi</u> at Debre Berhan and Wonji	57
15.	Comparative susceptibility of <u>B.pfeifferi</u> from Wonji and Debre Berhan for Adwa.. strain of <u>S.mansoni</u>	58
16.	Comparative susceptibility of <u>B.pfeifferi</u> from Wonji and Debre Berhan for Adwa strain of <u>S.mansoni</u>	59

<u>Figures</u>	<u>Title</u>	<u>Page</u>
17	Comparative susceptibility of <u>B.pfeifferi</u> from Wonji and Debre Berhan for Wonji strain of <u>S.mansoni</u> at Debre Berhan	60
18	Comparative susceptibility of <u>B.pfeifferi</u> from Wonji and Debre Berhan for Wonji strain of <u>S.mansoni</u> at Wonji	61
19	Exposure of <u>B.pfeifferi</u> from Debre Berhan to different number of <u>S.mansoni</u> miracidia under different temperature.1.	62
20	Exposure of <u>B.pfeifferi</u> from Wonji to different number of <u>S.mansoni</u> miracidia under different temperature	63
21	Growth of laboratory bred <u>B.pfeifferi</u> from Wonji and Debre Berhan at room temperature (14 - 21°c)	64
22	Growth of laboratory bred <u>B.pfeifferi</u> in the laboratory (23± 1°c) from Wonji and Debre Berhan	69

Introduction

The characteristics of snail population that allows successful development of endemic schistosomiasis has been discussed by many workers, but our knowledge of the failure of the disease to establish itself in potentially suitable areas is still far from complete. Many factors can cause this failure. Pellüger (1977) considered the following to be the main factors: insufficient snail density, insufficient density of human population, absence of slow flowing or standing water, unsuitable water quality and inability of the parasite to complete its life cycle in the snail under the prevailing climatic conditions. Files and Cram (1949), Files (1951), Wright and Bennet (1967) and Chi et al. (1971) demonstrated that it is due to the inability of snails to serve as intermediate hosts. This incompatibility is well associated with chromosomal-number variation in Bulinus species (Natarajan et al., 1965, Burch and Natarajan, 1966; Burch, 1967; Lo, 1972). In Biomphalaria species such variation in chromosome numbers does not exist. Instead innate internal mechanism which elicit a host reaction whereby the snail encapsulate the miracidia or the mother sporocyst which is unique to the non-susceptible snails was explained (Malek, 1967; Locker et al., 1981).

Biomphalaria pfeifferi, the snail host of Schistosoma mansoni, is found from sea level in Alexandria, Egypt (Malek, 1958b), to 3000 m above sea level in the highlands of Ethiopia and Yemen (Ayad, 1956; Brown, 1964). However, the range of endemic areas are much smaller when compared to the distribution of the snail. In Ethiopia there are several places where the situation seems to be ideal for the transmission of S.mansoni. Snails are available in good numbers and the water bodies are frequently visited by people; however, schistosomiasis is not established. Debre Berhan is one such place (Institute of Pathobiology, unpublished).

The present study was initiated to find out why S. mansoni could not establish itself at Debre Berhan. This was done by observing the physical, chemical and biological factors in relation to the abundance of the intermediate host. This was approached from two angles. The first was to compare the ecology at Debre Berhan (non-endemic area) and Wonji (endemic area) in terms of climatic factors such as rainfall, temperature, relative humidity, water level fluctuation, types of vegetation, water chemistry and prevalence of the disease.

The second approach was to investigate if there was any biological difference between the snails from the two study areas. Accordingly the growth rate in the laboratory, susceptibility and vector competence in the field and laboratory conditions were compared.

LITERATURE REVIEW

1. Factors that influence the distribution of snails.

a. General account.

Deschiens (1957) considered physico - chemical factors such as: temperature of 22 - 26^oc, depth of less than 2 meters, NaCl concentration of 6 ppm as essentials for conditioning the habitat of Biomphalaria glabrata. He also mentioned nutritional and competitive factors involving the flora and fauna as additional favourable factors. Malek (1958a) accounted food, oxygen sunlight and water quality as conditions governing the suitability of snails habitats. Berrie (1970) on the other hand gave, more importance to water temperature and to factors like organic content and light penetration.

b. Temperature

Bulinus and Biomphalaria species in Egypt were found to be tolerant to seasonal variations ranging from monthly average of 11.4 - 29.6^oc (Malek, 1958a). In Kenya Biomphalaria was found to withstand a diurnal variation of 20^oc (Vansomeron, 1946), While in Iraq Bulinus truncatus was found in natural waters ranging in temperature from 8 to 23^oc. Generally temperatures of 20 - 30^oc are said to be favourable to maintain snails (Frank 1963; Standen, 1949), but Biomphalaria and Bulinus species had been found in highland areas of Ethiopia and

Yemen that experience temperatures as low as 6^oc (Ayad, 1956). Hibernating or aestivating snails are more resistant to extremes of temperatures (Hubendick, 1958). Often it is said that continuous moderate variation in temperature is more seriously limiting to reproduction, egg development and growth than temporary extremes of temperatures (Maldonad et al. 1950; Sturrock and Sturrock, 1970).

High temperature in Eastern and Southern Ethiopia is said to have acted as barrier preventing B. pfeifferi from colonizing natural waters in areas below 750 m. altitudes, which are approximately represented by the 20^oc isotherm (Brown and Lemma, 1970). Similar observations were made by Berrie (1970) and Sturrock (1965). The latter considered high temperature to have prevented the establishment of B. pfeifferi along the African coast between 10^oS and 10^oN. These temperature effects seem to extend up to 16^oN in the lowlands of Ethiopia where the rainshadow, produced by the highlands results in a particularly dry and hot climate. Webbe (1962) noted temperature higher than 25^oc forms a barrier for B. pfeifferi in East African coastal areas. Sturrock (1966) considered that temperature of 27 - 31^oc accounted for the absence of snails in the coastal plains of Tanzania, plains of Mozambique and Kenya, around the Red Sea and on the west coast of Africa. Biomphalaria species are relatively more sensitive to higher temperatures than Bulinus spp. The latter survive after exposure to 40^oc while the former die immediately at the same temperature (Gordon et al., 1934).

In general, water temperature is an important environmental factor which influences the number of snails in a habitat by controlling the seasonal pattern of reproduction (Jobin and Michelson, 1970).

Although most snails are seen to live within 10 - 30°C, they have optimal temperatures for breeding, maximum rate of growth, longevity. These optima vary for snails from different areas. Chernin (1967) said that regardless of the initial placement in the thermal gradient, B.glabrata had a preference for 27 - 32°C. Cowper (1946), Frank (1963) and Harrison (1968) have reported 23± 1°C, 24.4°C and 26°C to be optima for B.pfeifferi. Bulinus globosus showed an increase in growth and maturation rate with temperature increase until 27°C while egg - laying had its optimum at 25°C (Shiff, 1964). Under laboratory conditions the optimum temperature for oviposition of B.pfeifferi is between 26 - 28°C and the natural increase in population growth per snail was highest between 20 - 26°C. (Harrison and Shiff, 1966; Shiff and Garnet, 1967; Williams, 1970).

C. Light

Both: Biomphalaria and Bulinus snails prefer an environment in which both sunshine and shade are available (Barlow, 1937; Zakaria, 1955; Watson, 1958). They crawl away from intense light source to the nearest shade and fail to grow well in completely shaded areas (Pesigen, 1958). The long term absence of light suppresses the growth of macrophytic weeds and the microflora which constitute the feed of snails

and release oxygen as they carry on photosynthesis. Welch (1952) observed that snails do not grow well in turbid water caused by dissolved organic matter or silt, but they do well in turbid water due to high content of algae, and suggested that absence of light per se was not crucial to the growth of snails. The few habitats that are reported to have maintained snails in the dark must have had food and oxygen carried from light exposed surfaces. Even then they become smaller and paler and more elongated than usual, which is a kind of molluscan 'rickets' (WHO, 1957).

d. Water Chemistry

In an attempt to gain a better understanding of the relationship between dissolved materials and the vector snails and to develop the ideal conditions for survival of snails in laboratories the importance of water chemistry has been stressed by several investigators (Harrison, 1966; Harrison and Rattary, 1966). The limit of certain cations and anions and the lethal dose for various species and stages of snails have been determined. The relative concentration of cations and anions found in nature, specially in natural fresh waters when compared to that tolerated by snails in laboratories, seems to have little significance (Watson, 1950; Helmy, 1953). In fact DeMeillon et al., (1958) stated that chemical composition in natural waters does not play even a minor part in determining snail habitats but this has been disproved by later workers like William (1970).

Bulinus and Biomphalaria can tolerate concentration of salts up to 1500 ppm and are expected to withstand higher concentrations up to 4000 ppm, (Lanoix, 1958). Single salts like NaHCO_3 and CaSO_4 are tolerated even above 4000 ppm (Deschiens, 1954).

e. pH

pH is rarely a factor limiting the distribution of the snail hosts of Schistosoma. Intermediate hosts of S.mansoni have been found in natural water bodies in which pH ranged from 4.8 to 9.8 and within these limits variation of this factor seemed to have no effect on density of snail population (Vansomeron, 1946; Malek, 1958). B.truncatus and B. adowensis, (B.pfeifferi) have been bred in waters ranging from pH 4 to 10 (Deschiens, 1954; WHO, 1957).

Slight variation in pH preference, as in temperature are observed among snails of the same species. In the Sudan, South Africa and Gambia Biomphalaria pfeifferi were found only in pH ranges of 6 to 9 but in Brazil B.glabrata survived in pH 4 to 10 in both the field and laboratory conditions (Deschiens, 1957). In Iraq B.truncatus showed high mortality below pH 6.3 and pH 7.9 was the upper favourable limit. For Biomphalaria ruppellii, pH 8.3 - 8.7 was said to be the normal range (WHO, 1957). As Lo (1972) has shown with Bulinus guerni, snails although fail to propagate, can survive at pH 3 - 5 but become inactive at pH 12 and die in 5 hrs. High pH, as has been observed with B.glabrata

affects production of eggs. At pH 7.8 it lowered production and the process was completely inhibited at pH 8.2 (Harry and Aldrich, 1958).

These observations infer the fact that pH is not the only critical factor that influence the life of snails unless it is extremely low or high. Nevertheless, from findings of Harry et al. (1957), Harry and Aldrich (1958) and Lanoix (1958) one can safely state that snails can survive over a wide range of pH, with preference to slightly alkaline water (pH 7 - 8).

Calcium

Calcium is one of the essential elements that limit the natural distribution of snails. Besides its role in metabolism and regulation of permeability of tissue, it affects the growth survivorship, fecundity, fertility of eggs and reproduction rate of snails (Nduku and Harrison, 1976). The degree of vulnerability to low level of calcium however, varies according to the species. In laboratory experiments Bulinus (B) tropicus was shown to be restricted to 5 - 40 mg/l only and B. pfeifferi to 5 - 200 mg/l while Bulinus (Physopsis) was able to live in 0 - 200 mg/l calcium (William, 1970).

In the presence of other cations and anions and bicarbonate (5 - 10 mg/l), as a buffer B. pfeifferi survived in water with 2 mg/l calcium, where as for the population to thrive a minimum of 4 mg/l calcium was required (Nduku and Harrison, 1976).

In natural water 5 mg/l calcium has been shown to be critical for the survival of snails. B. pfeifferi in nature is confined to waters with calcium content of at least 5 mg/l (Harrison, 1965 and William, 1970). The former infact reported that he was unable to find aquatic snails of any species. On the other hand Malek (1958a) found an endemic area of S. mansoni with abundant vector snails at concentrations 5.7 - 8.5 ppm.

At higher concentrations calcium seems to have a deliterous effect on the biology of snails. Frank (1963) showed that B. pfeifferi grew much faster, deposited more eggs and survived longer in aquatic aquaria with 13 ppm calcium compared with concentration of 31 ppm. Liang (1973) pointed its retarding effect on egg laying potential of bothe Biomphalaria and Bulinus species. Harrison (1968) demonstrated its decreasing effect on the metabolic rate and fecundity of B. pfeifferi.

The effect of low level of calcium is exagerated when the relative concentration of other cations specially magnesium is higher. According to Nduku and Harrison (1976) the ratio limits beyond which deliterous effects are produced: Ca: Mg= 0.6 ; Ca: Na = 0.1 and Ca: K = 0.33. The reason

for the relatively higher sensitivity of snails to the magnesium ratio is due to its ability to compete with calcium for absorption sites on exposed surfaces and thereby disturb the metabolism.

g. Magnesium

Magnesium is required in small quantities for the snail and the chlorophyll bearing microflora which form their food supply. B. truncatus and B. glabrata could tolerate it at a level as high as 510 ppm. According to Webbe (1962) and Harry et al. (1957) the optimal concentrations for Bulinus and Biomphalaria respectively range between 1 - 90 ppm and 5 - 50 ppm. But when it is disproportionately higher than the concentration of calcium both Biomphalaria and Bulinus species can not survive (Deschiens, 1957; Schutte and Frank, 1964). Harrison and Shiff (1966) failed to get snails of Biomphalaria and Bulinus species in streams with 54 ppm of calcium and 467 ppm magnesium. Similarly Nduku and Harrison (1976) showed that the proportion of magnesium to calcium is more important than mere quantity of magnesium in defining favourable habitat of snails.

h. Sodium and Potassium

Sodium and Potassium occur in the form of chloride, sulphate and carbonate. Their concentration in the water is important for the survival of snails. When concentration of sodium and potassium are higher than calcium the situation becomes unfavourable to snails. According to Schutte and Frank (1964) calcium to sodium, ratio of 0.4 was unfavourable to Biomphalaria spp. but Nduku and Harrison, (1976) reported ca: Na ratio 0.1 and Ca: k ratio of 0.33 to be the limit beyond which deliterious effects could be expected.

i. Rainfall

Major seasonal fluctuations in snail population density occur as a result of rainfall and ecological changes brought by it. In Egypt snails were found plentiful all the year round but with two periods of maximum abundance. These were at cold seasons with daily temperature maxims of 16°C and 24°C respectively. The break in the continuity of snail growth was considered due to the Nile flood that killed large number of them and stopped reproduction, at least temporarily. The presence of B.boissyi in the Nile delta and the Sudan and its absence from the whole of upper Egypt was thought to be due to the sensitivity of the snails to water movement (Helmy, 1953). In Iraq, Watson (1950; 1951) observed the confinement of B.truncatus to stagnant water, due to its sensitivity to water movement. He stated maximum population growth to be in summers and autumns.

B.globosus in Zambia bred during the two peaks of rain-falls. Bulinid snails in Tanzania had population maxima and minima which were directly related to rainfall (Webbe and Mesengai, 1958). Similar observation with B.pfeifferi was made in Siera Leone (Gorden et al., 1934). Cridland, (1957a; 1958b and 1958) in Uganda and Sodeman (1979) in Liberia have also shown distinct seasonal fluctuation in population densities. Cridland (1957a; 1957b) observed an increase in young snails after the long rains, while Sodeman (1979) observed a decrease in the population during the wet seasons and an increase during the dry seasons. This was noted at 62 locations both in Urban and rural areas.

Behavior and activities are also to some extent affected by seasonal climatic cycle. Some snails aestivate when the pools in which they live dry up, other snails hibernate during the cold season.

2. Vector - parasite comatibility

a. General account

Often the disease transmission potential or lack of it is pointed more strongly towards the snail than towards the parasite. But it has been demonstrated that it could be due to interstrain variation in the infectivity towards the snail hosts (McCullough, 1959; Sauod, 1966; Richard and merril, 1972; Dawood and Chu, 1973). Berrie (1970) took Bulinus (Physopsis) species, the intermediate hosts for a Nigerian strain of S.haematobium, and exposed them to an Egyptian strain of the parasite but infection did not take place. On the other hand, when Bulinus species that was refractory

to the Nigerian strain of S. haematobium was exposed to the Egyptian strain, infection was successful. Similarly Roux (1954) using an Egyptian strain of S. haematobium was able to infect Bulinus coulboisi but Cridland (1957a) was unable to infect the Ugandan strain of the same species of snail with the same Egyptian strain of S. haematobium. In the Sudan also Bulinus (Bulinus) species and B. (Physopsis) species occur, but it is only the former species that is susceptible to local S. haematobium infection (Malek, 1958b)

In some areas snails of the same species show different degrees of susceptibility at different ages. For example in Puerto Rico B. glabrata with three different degrees of resistance were found. One group was susceptible at all ages while the other was susceptible only at juvenile and the third one was refractory at all ages (Richard and Merrill, 1972). Complex of genetic factors whose expression could vary in degree are involved. Biomphalaria glabrata from Salvador (Brazil), which was continuously refractory to infection with many strain of S. mansoni, on crossing with susceptible B. glabrata from Puerto Rico, revealed intermediate degree of susceptibility for Puerto Rican S. mansoni (Newton, 1953; Newton, 1954). The phenotypic manifestation of the genotypes governing incompatibility, in the form of a cellular capsule formed around the developing stages of the parasite, was also illustrated. Hybrid snails showed a cellular reaction intermediate between the susceptible parents and the non-susceptible parents (Cridland, 1968; Richard, 1970; Chi et al., 1971).

The refractiveness of snails to S.haematobium should not be considered completely of genetic origin. Sturrock (1968) has reported that specimens of Bulinus nasutus productus collected in the field and those bred in the laboratory portrayed differences in their susceptibility to S.haematobium. It has been suggested that "unidentified humoral elements" that may lyse the parasite may exist. Feng and Canzonier (1970) have also put as evidence of host humoral response to infection, the quantitative fluctuation in hemolymph protein fractions of a mullusk parasitized by a trematode species. Chernin (1970) Bayne et al., (1980) and Locker et al., (1981) have also shown that haemocytes for resistant strains were effective in damaging sporocyst. Locker et al., (1981) showed that the haemocytes contain large number of lysosomal like bodies and that with the help of their pseudopodia phagocytize the sporocyst microvilli and small pieces of the underlying teguments. Resistant snails are dependent upon the specificity of cytophilic factors present both in the plasma and haemocytes. Oliver-Ganzalez (1968) showed that when haemolymph from noninfected snails was injected into infected mice, it killed and disintegrated products became toxic to the worm or stimulate immunological reaction that is not favourable to the worm. In general one can say that the ability on the part of snails to transmit the disease is intimately linked to their genetic and physiological constitution; but its mechanism remains to be elucidated.

In Bulinus spp. the growth rate is temporarily accelerated while the life span is shortened by infection of parasite (Chu et al., 1966; Lo, 1972). Infected snails were more sterile and hatchability of the eggs was less than that of uninfected snails.

b. Temperature

Temperature interferes with the life cycle of the parasite at various stages: egg, miracidium and intramolluscan phase. The effect it produces is dependent upon the duration of exposure. When stools containing S. mansoni eggs were kept for 40 hrs at fluctuating temperatures of 25 - 35°C most of the eggs died; but when they were kept for 18-24 hrs the eggs hatched successfully. At 4°C egg hatching was completely inhibited, but when returned to optimal hatching temperature (22 - 25°C) hatching took place and viability of miracidia did not seem to be affected (Ingall et al. 1949).

The success of miracidial search and infection of intermediate host is affected by extreme temperatures. Temperatures below 10°C inactivated them and infection of snails can not take place. Maximum infection is obtained at 30°C while at 40°C they become hyperactive and die soon. (Stirewalt, 1954; DeWitt, 1955; Lo, 1972).

The miracidial transformation to mother and daughter sporocysts and the cercarial incubation period is also controlled by temperature. In East Africa S. mansoni took 50 - 68 days at 31.75°C to develop to cercariae in B. pfeifferi (Foster, 1964). This confirms Dewitt's (1955) finding; at 23 - 28°C

the cercarial development was completed in 35 - 58 days while between 26 - 28°C it took 22 - 23°C and at 31 - 33°C it took only 18 days. Chu et al. (1966a) in Iran and Schattock et al. (1965) in Southern Africa have noted that the incubation period for Bulinus was short in warmer seasons and that it was longest in colder months. With an increase in temperature the number of cercariae will also increase, to reach the maximum at the highest tolerated temperatures. At higher temperatures the snails become more susceptible to miracidial penetration.

Low temperature is said to retard the metabolism of the parasite as well as that of the host (Stirewalt, 1954; Dewitt, 1955).

The emergence of cercariae from snail hosts could be forced as often as 3 times a day for 8 - 9 days by application of appropriate thermal stimuli. As has been determined with S.mansoni cercariae between 15 - 35°C the mortality is almost exclusively a consequence of the aging process (Lowson and Wilson, 1980). Outside this range, the effects of temperature begin to cause cercarial mortality. At 10°C cercariae suffer a high initial rate of mortality suggesting that some of the cercariae are more tolerant to low temperature or can adapt to it. The cercariae cannot tolerate temperatures above 35°C for long time. The optimal temperature for cercarial penetration, based on the number of adults recovered from mice, was found to be between 30 - 35°C (Dewitt, 1955). It was also noted that penetration does not occur at temperatures over 40°C or below 10°C. Exposure to 32 - 34°C for 8 hrs did

not affect their activities or life span, but when heated to 36°C for an hour their mortality was greater than the controls.

c. Light

Light plays a major role in cercarial shedding. Snails kept in the dark produced less cercariae (20/day/snail) compared to those kept in natural light (70/day/snail). But when they were transferred to the same light conditions, they produced essentially the same number of cercariae as the control groups (Kuntz, 1947). Cercarial shedding under bright light starts in 15 - 30 minutes. In moderate light it needs an hour, but shedding continues in smaller numbers, even in the dark (McClelland, 1958). The periodicity in cercarial shedding which corresponds to the light illumination is also seen in the dark (Barbosa et al., 1954; McClelland, 1965). Temperature has a supplementary effect is in causing greater output than light alone but it is relatively of lesser importance, at least in S. haematobium and S. mansoni (McClelland, 1965). Duke (1952) recorded that temperature served only as a stimulus for shedding and once snails have started shedding they will continue doing so even if temperature fell to 18°C.

Under ultraviolet light the activity of miracidia and cercariae is impaired within 30 minutes and are killed by a 45 minutes exposure.

d. pH

The pH of water affects the survival of miracidia. In a pH range of 7 - 10 miracidia can survive for at least 4 hrs but above and below this range they die quickly. Miracidia have a wide tolerance of pH in terms of successful infection. At pH 3 - 5 or pH 12 no miracidial infection of snails occur, since they die in a very short period of time. But at pH 6.1, 11% of infection, at pH 6.9 - 8.6 an infection rate of 23 - 38% and at pH 9.4 - 9.6 the highest infection rate (33 - 49%) were found (Lo, 1972).

Gumble et al. (1957) stated that a pH of 6 - 8 to have caused high cercarial shedding in O.nosophora while pH above 8 reduced it. On the other hand Bauman (1979) observed no cercarial shedding at pH 6.4 - 7 in rain water and only a few heavily shedding at pH 6.8 - 7 in river water.

e. Oxygen

Lack of oxygen causes B.glabrata to cease cercarial shedding or to shed very small numbers. Snails left for 6 hrs in deoxygenated water, then brought to oxygenated water produced 50 - 189 cercariae compared to 300 - 800 cercariae with controls. When they were kept for an hour in deoxygenated water no difference was observed (Oliver et al. (1953). The cercariae shed from snails previously kept under anaerobic environment showed no reduction in infectivity or activity in general but when subjected to anaerobic conditions after they were shed, they became inactive and lost their tails. It is apparent that anaerobic

conditions do not affect intramolluscan cercariae. Parasites in the snails need oxygen only for synthesis or developmental purposes, otherwise they have an alternative mechanism of respiration that does not involve the cytochrome system but links carbohydrate metabolism to energy production. They can fix CO_2 and reverse the Krebs cycle to produce succinate and acetate (Sa₃, 1970). Biomphalaria pfeifferi also show distress when the oxygen tension falls below 75% of saturation and suffocate when it falls below 10% (Cowper, 1971).

f. Nutrition

Nutrition affects the host-parasite relationship (Kendall, 1964). Malek (1958a) found that undernourished snails harbour fewer parasites and the development of sporocyst is inhibited. The incubation period is elongated and the snails become less susceptible. This indicates that the availability of proper type of food is essential for the development of parasites. In laboratory culture of snails fish-meal as a source of food is recommended to help parasites develop better (McClelland, 1965; Liang, 1973). The fecundity of snails is also directly proportional to the amount of food in the habitat (Jobin and Michelson, 1967; Berrie, 1969).

g. Water velocity

Water velocity of more than 25 cm/ second plays a negative role in the success of parasites penetration. Webbe (1965) accounted the low infection rate and low worm load

in animals exposed to a stream with high cercarial infestation to be due to high velocity of water. Illustrating his point further, he said high velocity of water changes cercarial density per unit volume and influence the contact and penetration of cercariae. At the velocity below optimum fewer cercariae make contact with the exposed animals while at higher velocities some of these are probably swept off. Radke et al. (1965) also suggested that cercariae may become fatigued in fast flowing water particularly over long distances, an effect which was also observed by Webbe (1965). This may be an important factor reducing the infectivity of cercariae in fast flowing turbulent waters. In Webbe's (1965) experiments the highest infection rate and worm loads were obtained at velocities between 0.15-6 cms/second.

The effect of water velocity on miracidial infection of snail hosts is different from that of cercarial infections. Increase in water velocity enhances the effective scanning capacity of miracidia so that snail hosts may be located and infected. With 25 miracidia/snail, high infection rates were obtained between 0.5 - 3.5 ft/sec (Webbe, 1966). Miracidia may show negative rheotropism and be capable of unlimited directional movement against the flow of water.

MATERIALS AND METHODS

Description of the study areas

Debre Berhan (Latitude $9^{\circ} 40' N$, Longitude $39^{\circ} 32' E$)

Debre Berhan is a small town 130 kms North of Addis Ababa (fig. 1) with a population of about 5000. It is located at an altitude of 2800 m. Human activity is largely subsistence farming consisting of barley, pea and bean. The population in the town receives treated and chlorinated tap water. But this does not extend beyond the town population. People living in the vicinity use river water originating from the hills. The people from the town come down to the river sides R. Beresa and R. Dalecha for laundering and watering cattle.

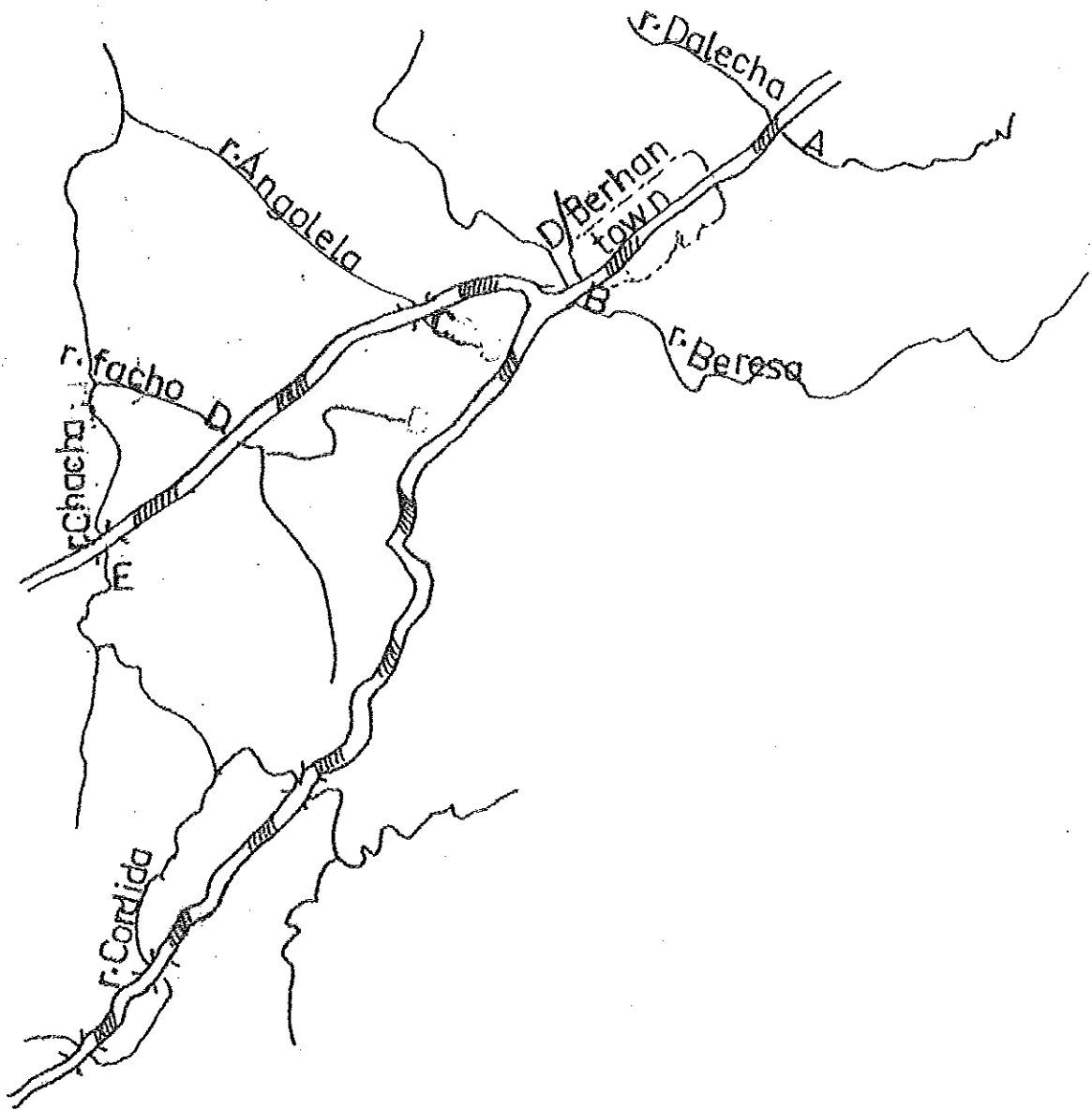
Eventhough . known foci of schistosomiasis are situated about 80 kms away in Shewa-Robi, Gerbi and Senbet (Institute of Pathobiology unpublished), schistosomiasis is not endemic at Debre Berhan.

The vegetation consist largely of grasses and no predominant trees are found except for the eucalyptus that is seen in bunches here and there.

fig. 1. STUDY SITES IN DEBRE BERHAN

fig 1

- 23 -



Wonji (Latitude 8° 25'N, Longitude 39°E)

In the context of this study Wonji refers to Wonji Sugar Estate. It covers an area of more than 120 km². It is found 100 kms East of Addis Ababa at an altitude of 1550 m (fig. 2)

Water for sugar-cane cultivation is obtained from Awash river through an irrigation system. The water is applied by rotation throughout the year except for summer closure, when the factory is closed for maintenance. Moreover the heavy summer rain is expected to satisfy the need for irrigation.

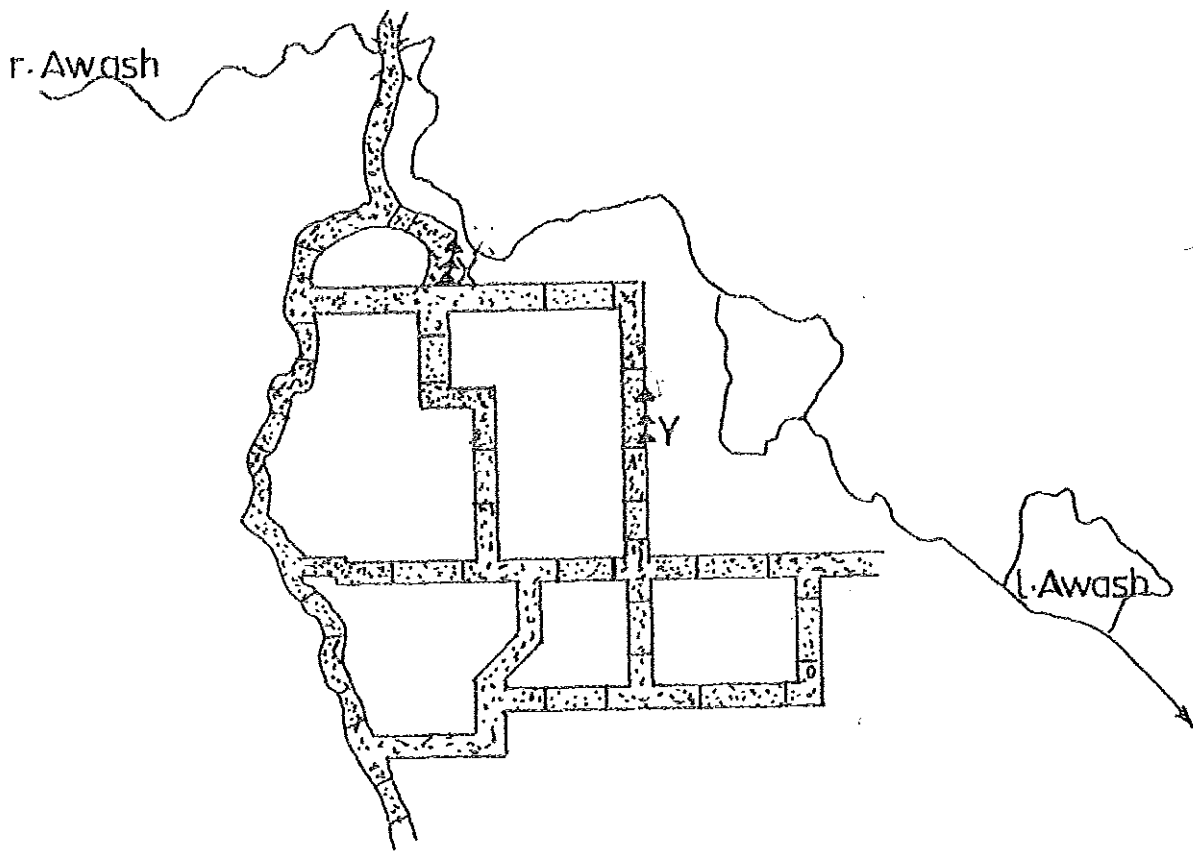
The open canals in the irrigation system have created conditions that are highly favourable to B.pfeifferi, the snail host of S.mansoni. It has given rise to some dense growth of plants consisting of Commelina polygoneum, Cyperies, Gamphoeapys spherathis, Suaveolense gentala and Hypophila species. These have given the snails the opportunity to (propagate) actively and disperse themselves thereby increasing the incidence of the disease (Kloos and Lemma, 1977).

According to WHO Bilharziasis Advisory Board only 10 cases of schistosomiasis were found in the area in 1964 (Duncan and Lemma 1976). They were then considered imported cases. A 1978 report indicated that 32% of plantation workers were infected with schistosomiasis (Teklehaimanot and Goll, 1978). The incidence of infection among canal and irrigation workers was about 60% (Kloos and Lemma, 1979). However survey made since 1980 shows that the infection rate has gone up to

fig. 2. STUDY SITES IN WONJI SUGAR ESTATE

fig 2

- 26 -



major road

study site

river

1km

87% in some of the camps (Wonji Hospital, Personal Comm.)

The snails control unit of Wonji Hospital has 3 staff members who routinely check for the snails and apply molluscicides whenever necessary.

Sanitation and water supplies around the camps are poor. There is a piped water supply but it is inadequate and therefore many people continue to use canal water for a wide variety of purposes. Since the tap water is hard water, canal water is preferred for laundering. At times the tap water stops functioning for weeks and the entire population is left with no choice but to use canal water.

The infection of snails is maintained by the continued defecation of the people in areas where snails are found in abundance. The camps have latrines but they are not well kept and hence people refrain from using them. Instead they go to the nearby plantation and defecate, preferably on the edge of the canals. This provides steady source of infection.

Meteorological studies at the two study areas.

Meteorological data for 1980 and 1981 were obtained from Water Resource and Development Authority and the Wonji Agricultural Research and Weather Service Bureau for Debre Berhan and Wonji respectively. The records for Debre Berhan included rainfall, water temperature and water level fluctuation (only for site 'B'). In addition to the above

data relative humidity and evaporation rate were also recorded for Wonji.

Chemical analysis of water.

During each field trip, pH and oxygen tension of water were measured on the spots, the pH with Beckman Model 2 pH meter and the oxygen by Winkler titometric Method. Surface water samples from R.Beresu and the small canal site 'x' at Wonji were brought to the laboratory in Addis Ababa University and analysis was done in accordance with the procedures given in "Standard Methods for examination of Water and Waste Water" Major cations Na^+ , K^+ , Ca^{++} and Mg^{++} were determined using an Atomic Absorption Spectrophotometer (Model-SP-191, PYE, UNIC) in the Chemistry Department of Addis Abeba University. For the anions the following methods using Hack's Kit were implemented. Cadmium Reduction Method for Nitrate, Diazotization Method for Nitrogen-dioxide, Nessler's Method for Ammonia, Heteropoly Blue Method for Silcondioxide and Periodate oxidation Method for Manganese. Ethylene diamine Tetra Acetic Acid Titrimetric Method for total hardness. Turbidimetric method for Sulphate and Stannous Chloride Method for Phosphate quantitation were also utilized.

Sampling for snail population.

To obtain some knowledge of the seasonal fluctuation of B.pfeifferi a series of observations were made in selected representative habitats. The selection of these habitats

was based on a reconnaissance survey made in November 1980, and the sites chosen were those with highest snail density.

In Debre Berhan five sites A-E were selected (fig. 1). All sites except Site A, which almost dried in May 1981, had water throughout the year. In Wonji, two sites (X & Y) were selected from irrigation canals of the sugar estate (fig. 2).

Snail samples were taken using a scoop net of 20 cm diameter and 1 mm x 1 mm pore size. Snails were collected by scooping for a total of 3 - 6 minutes. Then number of snails /min/man was determined. All the snails collected were measured using a vernier caliper and then were placed back at the sites of collection. To achieve consistency of sampling all sampling was done by the same person.

Studies on parasites

Prevalence surveys

Parasitological surveys on students (grade 1-8) selected randomly were carried out in both study areas. A total of 393 stool samples were taken from Wonji of which 87 were from children around 4 camps. In Debre Berhan 168 samples were taken and all were from school children. Vials were distributed with their names & numbers labelled and faeces in almost all cases were collected immediately. About 3-4 gms of faeces were collected from each and were preserved in 7.5% formalin. Faeces were examined by direct smear at the spot and later

by Ritches concentration technique.

Natural infection of snails

For the determination of natural infection rates, snails were collected from streams, pools irrigation canals and brought to the A.A.U. laboratory 200 snails from each of the study areas were randomly selected and were checked for cercarial shedding under 60 watts electric lamp and in water temperature of 23-26°C. This was done for 3 weeks at regular intervals. Finally they were crushed and the presence or absence of developing sporocysts was observed.

Host parasite compatibility.

Egg hatching

Eggs of S.mansoni were obtained from the faeces of patients from Wonji and Adwa. They were then hatched in filtered water from snail aquaria, as recommended by Chernin (1972), in Erlenmyer flasks covered with carbon papers under 60 watts electric lamp.

Laboratory breeding of snails.

Snails obtained from the various sites were bred in the laboratory. To get parasite-free snails for susceptibility studies and to determine growth rate differences under laboratory conditions, snails obtained from field were bred at the Biology Department, Addis Ababa University. Ten B.pfeifferi snails of the largest size were put in

distilled water or aged water collected from an artificial pool in the Addis Ababa University as described by Liang (1973). After a large number of eggs were laid the larger sizes were selected and put in petridish and were then left under 40 watts fluorescent light for about 8 days. Algae obtained from an artificial pond were provided to the hatched snails and were gradually transferred to a bigger plastic container. In the bigger plastic container they were provided with light from the fluorescent lamps. Water was changed by carefully siphoning. Oven-dried lettuce and ground fish meal were provided with small amount of black-board chalk. When they started attaching themselves to the oven-dried lettuce they were given half cooked lettuce and gradually raw lettuce.

About 3 weeks later 25 snails (1.6 mm diameter) from each set were transferred into different plastic aquaria and were left at room temperature. An equal number were also left in water bath at 23^oc. These sets were left for growth rate study. Measurement of size was taken every other week using vernier caliper for 16 weeks. The remaining ones were allowed to grow before susceptibility studies were carried out using them.

Snail exposure

The snails used for this purpose were mostly those bred in the laboratory and were free from parasites. Snails collected from the study areas were also used after they were checked for cercarial shedding for 6 weeks. Exposure

studies were done three times. The first set-up involved 150 snails from Debre Berhan and another 150 from Wonji. Each of the snails were exposed to 20 miracidia in snail conditioned water as described by Chernin (1972). Of the 150 snails from Debre Berhan 50 were placed in laboratory at room temperatures (14 - 21°C) in Addis Abeba University, 50 in an out door aquarium at Wonji (20 - 28°C) and the remaining 50 at site A in Debre Berhan (7 - 23°C). The snails obtained from Wonji were also divided into three equal groups and placed as mentioned above.

The snails at site 'A' of Debre Berhan were brought to the laboratory, Addis Abeba University, after 28 days while those from Wonji were brought to the laboratory after 32 days and kept at room temperature. All snails died 16 weeks after exposure.

When the experiment was done for the second time an equal number of unexposed snails were added to each set and were kept only for 4 weeks in the laboratory. After that the snails were crushed and the results were recorded.

The last set of experiments were a replica of the second experiments except that it involved greater number of snails. The collection of snails from Debre Berhan (Site A) was done 58 days after exposure while those from Wonji aquaria were collected 28 days later only.

Statistical tests

Coefficient of correlation between the snail population and the meteorological factors and student t-test for growth rate difference between the snails from the two study areas were calculated. In both cases their statistical significance at 95% level was tested (Snedecor & Cochran, 1968).

RESULTS

Physical factors at study areas.

Debre Berhan

The meteorological data obtained from the Water Resource and Development Authority are shown in figure 3. The monthly rainfall for the years 1980 and 1981 was not uniform. In 1980 all the months except December had rain. But in 1981 there was no rain at all in the months of January, February and June. However in both years there were two peaks of wet seasons interrupted by relatively dry months. The wet season that started in March was short with little rain. The rain between July and September was heavy reaching 350 mm precipitation / month. During these three months about 1000 mm of rain accounted for more than 80% of the annual rain falls.

The average monthly water temperature ranged from 7 - 23°C. In cold seasons the morning water temperature fell down to 6°C. The mean average for the months fall was between 14 - 16°C. The water level fluctuation was recorded only for site B. The water level fluctuations showed direct relationships to the rainfall.

Wonji

The meteorological data for temperature, rainfall relative humidity and evaporation rate as recorded by Wonji Agricultural Research and Weather Service Bureau from January 1980 to October 1981 are shown in figure 4.

fig. 3. METEOROLOGICAL DATA AND SNAIL POPULATION

AT DEBRE BERHAN

- A. Rainfall
- B. Number of snails /min/man
- C. Water temperature
- D. Water level fluctuation at site B

fig 3

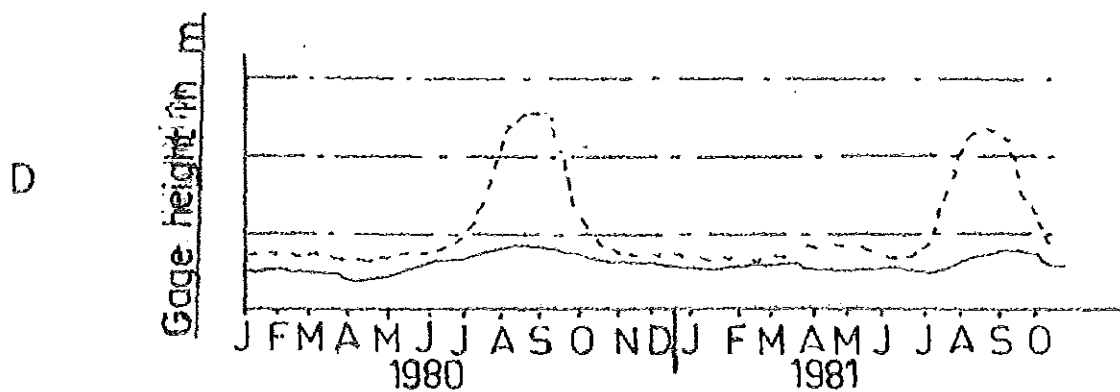
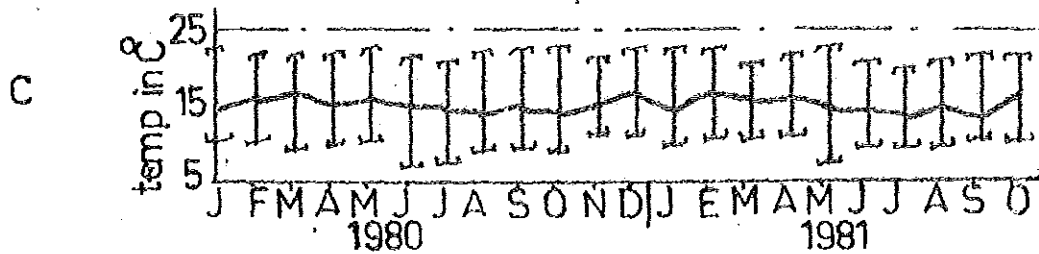
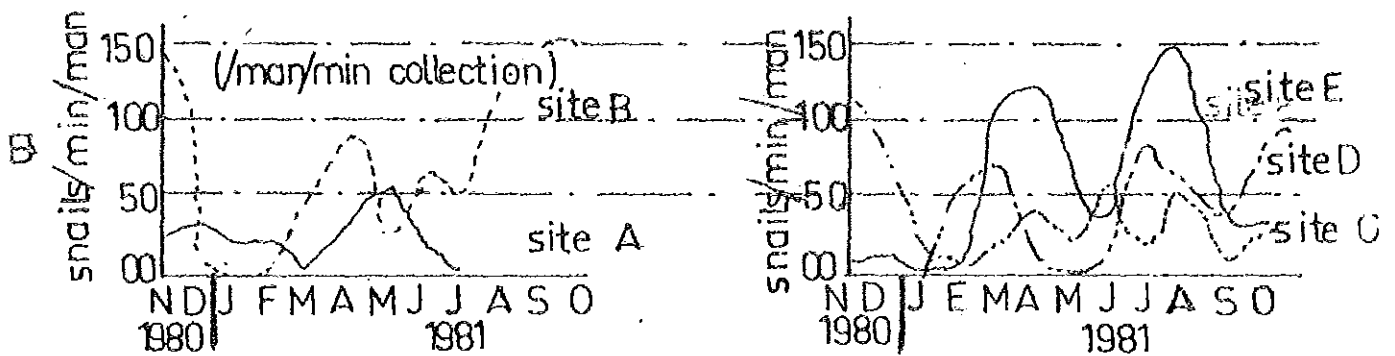
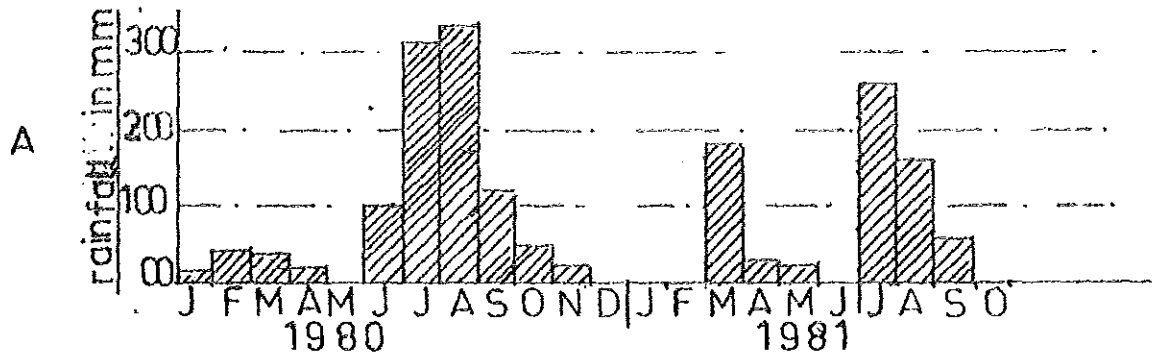
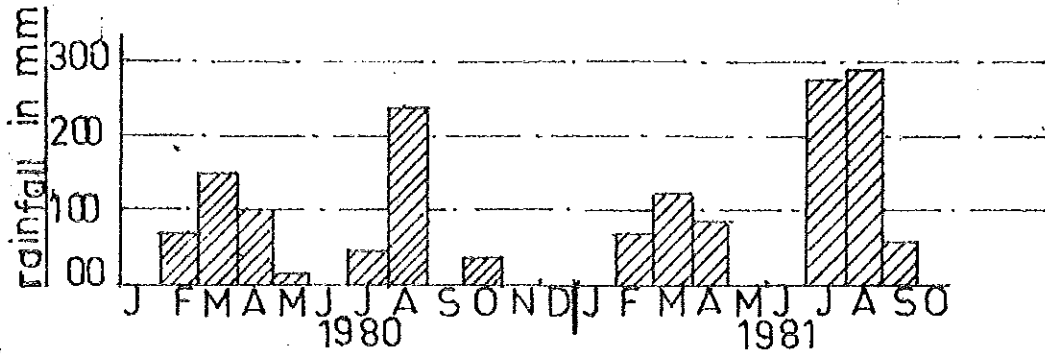


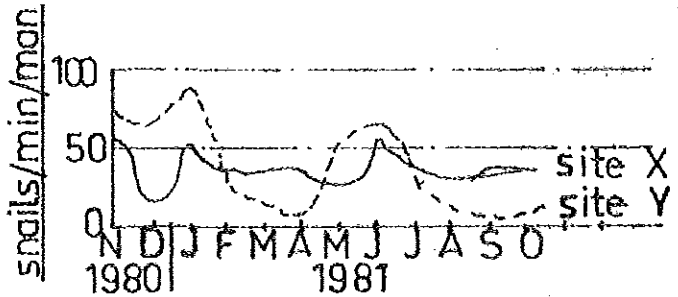
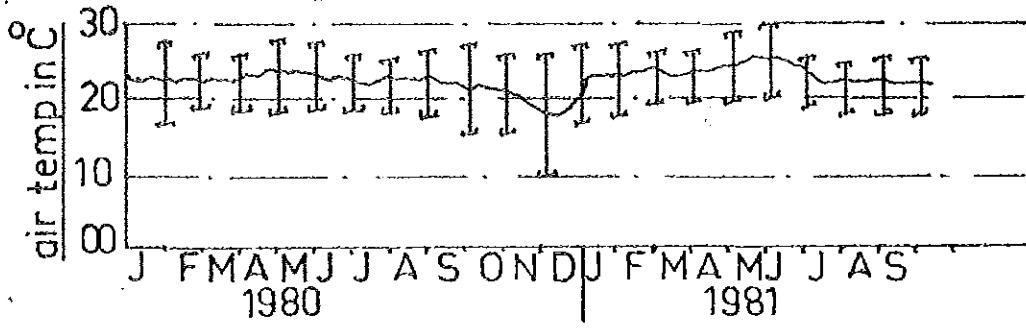
fig. 4. METEOROLOGICAL DATA AND SNAIL
POPULATION AT WONJI, ESTATE .

- A. Rainfall
- B. Air temperature
- C. Snails /min/man.
- D. Rate of evaporation and relative humidity.

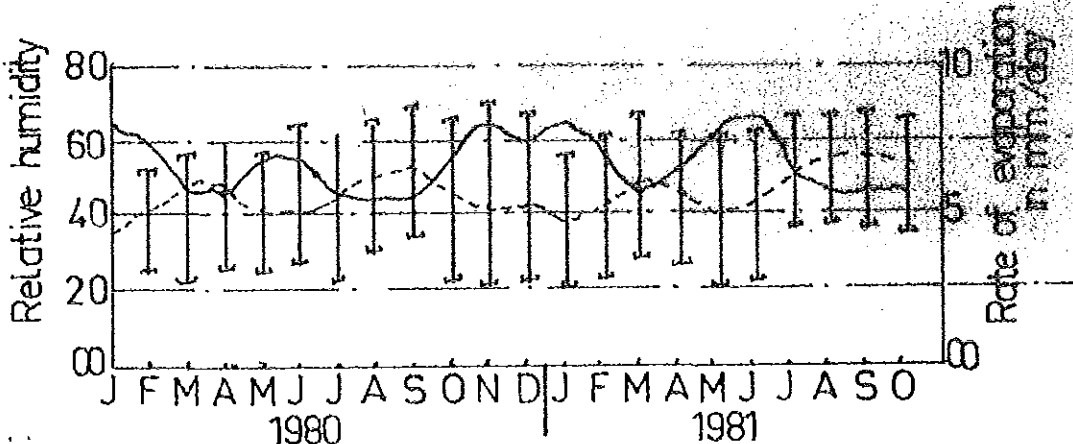
A



B



D



key for D
 broken lines - RH
 block lines = Evap. rate

The rainy season as in Debre Berhan consisted of two peaks. A light rain from February to March and a heavy one from July to September. With the rainfall the relative humidity rises while the rate of evaporation decreases. The average air temperature ranges from 13.2 - 28°C. The surface water temperature for most of the year reach 43°C in the middle of the days.

Chemical analysis of the bodies of water.

The water chemistry for River Beresa as a representative of the study area for Debre Berhan is shown in Table 1. The oxygen concentration was between 10 mg/litre and 70 mg/litre. The highest O₂ concentration (70 mg/l) was recorded in October but relatively speaking all the month after July had more oxygen. The cations concentrations: calcium to magnesium ratio ranged between 1.8 and 5.9; potassium and sodium were on the average 4.12 mg/litre and 11.38 mg/litre respectively while silicon-dioxide was 5.4 mg/litre. The pH ranged from 7.2 - 9.2 and it was above 8 for most of the months in the year.

The cations specially calcium and magnesium were more at Wonji while pH and oxygen tension at Wonji were less than that of Debre Berhan (Table 2). The ratio of calcium to magnesium was highest in the months of June, 9.7 and least was in August 1.6. The average potassium and sodium concentrations were 14 mg/litre and 13.4 mg/litre respectively. The pH was near neutral while the concentration of oxygen was relatively low ranging from 8 - 19 mg/litre.

Table 1. Chemical analysis of water
from Debre Berhan Site B (R.Beres).

Months 1981	Na mg/l	K mg/l	Ca mg/l	Mg mg/l	Mn mg/l	So ₄ g/l	Po ₄ g/l	No ₃ mg/l	No ₂ mg/l	S ₁ O ₂ mg/l	NH ₃ mg/l	pH	Total hardness mg/l	Oxygen mg/l
January	13.2	4.2	20	9.17	-	1.7	-	0.06	1.05	3.71	1.04	9.2	87.6	20
February	10.7	3.7	19	8.72	-	5.9	0.47	0.2	1.013	8.1	1.28	7.2	87.05	14
March	12.1	3.9	15.7	8.47	0.63	4.2	0.89	0.75	0.28	2.03	0.14	7.9	74	10
April	12.4	4.2	18	6.1	1.02	-	-	-	0.66	6.88	0.53	8.7	70	40
May	12.1	4.4	18.7	3.2	-	2.3	-	-	1.03	6.0	1.08	9.6	60	22
June	11.12	3.2	17.3	7	-	1.5	-	-	0.413	1.3	0.01	8.1	80	21
July	10.8	5.0	18	6.34	-	7.6	0.07	-	0.25	6.88	0.76	9.3	80	17
August	8.5	3.7	13.4	7.2	0.82	5.8	0.96	-	0.625	8.3	0.3	8.5	78	50
September	10.6	4.5	14.2	7.93	2.81	3.5	0.44	0.52	1.032	8.13	0.76	8.8	56	60
October	12.3	4.8	16.51	7.5	0.54	7.6	0.013	0.71	0.11	2.7	0.32	8.2	63	70

e 2. Chemical analysis of water samples
from Wonji site 'X' .

Sl	Mn mg/l	SO ₄ g/l	PO ₄ g/l	NO ₃ mg/l	NO ₂ mg/l	S ₁ O ₂ mg/l	NH ₃ mg/l	pH	Total hardness mg/l	Oxygen mg/litre
45	1.35	5.8	0.96	-	-	15.63	-	7.1	128	10
39	1.62	2.1	-	-	0.5	71.25	1.53	7.4	92	13
29	1.89	1.2	0.67	0.33	0.2145	21.25	3.05	7.3	110	12
1	1.62	4.5	-	0.27	0.223	15.63	0.92	7.7	100	19
09	6.75	6.6	0.05	-	0.106	15.0	3.97	7.5	175	8
82	2.7	1.1	-	0.026	-	26.25	3.17	7.2	95	9.2
71	1.62	1.2	0.09	0.08	0.495	11.25	1.93	7.6	11.2	8.8
65	1.08	1.2	0.179	-	0.62	78.0	1.2	7.2	200	6.7
6	-	5.0	0.135	-	0.27	30.0	2.93	7.1	260	6.7
12	-	3.0	0.38	-	0.2	19	1.3	7.3	204	8.4

Snail population dynamics.

The population of B.pfeifferi for the 12 consecutive months (November 1980 to October 1981) after computation for one man per minute collection are tabulated in Table 3 - 7 for Debre Berhan snails. As shown in Table 3, no snail of less than 3 mm size was collected from site A throughout the study period. November to February and April to June were the months that had relatively higher number of snails. More than 50% of the snails obtained during these months consisted of 6 - 10 mm size. The highest count was in May that was 55 per minute/man.

The dry months from December to February were months that had less number of snails at site B (Table 4). In April it showed a leap from 54 to 95 per minute count and in the months that followed the July rain, it grew from 52 to 489. Relatively younger snails appeared in higher number following the rains.

Like the other two sites, site C had a decrease in population from November 1980 to February 1981 (Table 5). In March together with the rainfall it started to grow and showed a small peak of 44 per minute count. There was a fluctuation every month after that.

In November 1980 only 8 snails were collected from site D in one minute (Table 6). In the following two months snails were not found; however, in February and March 50 and 72 snails respectively were collected. Of

Table 3. Monthly collection of B.pfeifferi
at site A, Debre Berhan.

Months	No. of snails in each size group in mm					Total No of snails
	10	8-10	6-7.9	3-5.9	3	
1980						
November	2	12	8	0	0	22
December	0	20	10	1	0	31
1981						
January	3	5	9	0	0	17
February	0	9	0	5	0	14
March	0	0	1	0	0	1
April	9	9	17	10	0	45
May	3	19	28	5	0	55
June	8	2	1	0	0	11
July	0	0	0	0	0	0
August	0	0	2	0	0	2
September	0	0	0	0	0	0
October	0	0	0	0	0	0

Table 4. Monthly collection of B.pfeifferi
at site B, Debre Berhan.

Months	No. of snails in each size group in mm					Total No. of snails
	10	8-10	6-7.9	3-5.9	3	
1980						
November	10	69	45	18	4	146
December	0	0	2	0	5	7
1981						
January	0	0	0	0	2	2
February	0	2	0	0	0	2
March	29	18	2	5	0	54
April	25	47	19	1	3	95
May	26	14	0	0	3	43
June	11	24	18	11	5	69
July	5	21	15	4	7	52
August	0	54	31	26	22	133
September	83	34	30	12	6	165
October	21	181	112	81	3	398

Table 5. Monthly collection of B.pfeifferi
at site C, Debre Berhan.

Months	No. of snails in each size group in mm					Total No. of snails
	10	8-10	6-7.9	3-5.9	3	
1980						
November	27	50	34	8	9	128
December	16	43	19	2	2	82
1981						
January	1	1	3	8	0	13
February	0	0	0	1	1	2
March	0	5	7	3	2	17
April	15	18	1	10	0	44
May	0	13	8	0	0	21
June	11	28	13	3	2	57
July	4	4	0	0	0	8
August	9	6	13	28	0	56
September	3	0	0	4	0	7
October	18	0	8	1	0	27

Table 6. Monthly collection of B.pfeifferi
at site D, Debre Berhan.

Months	No. of snails in each size group in mm					Total No. of snails
	10	8-10	6-7.9	3-5.9	3	
1980						
November	0	3	5	0	0	8
December	0	0	0	0	0	0
1981						
January	0	0	0	0	0	0
February	37	13	0	0	0	50
March	41	22	5	3	1	72
April	1	0	0	3	1	5
May	0	0	0	0	0	0
June	5	0	0	0	0	5
July	21	27	28	10	0	86
August	15	22	7	14	0	58
September	3	20	6	0	0	29
October	6	17	27	15	0	65

Table 7. Monthly collection of B.pfeifferi
at site E, Debre Berhan.

Months	No. of snails in each size group in mm					Total No. of snails
	10	8-10	6-7.9	3-5.9	3	
1980						
November	0	0	4	0	2	6
December	0	5	3	0	1	9
1981						
January	0	0	0	0	2	2
February	0	0	0	1	0	1
March	31	44	22	4	4	105
April	39	51	10	20	3	123
May	7	16	5	0	0	28
June	9	24	2	0	0	35
July	30	39	45	7	8	129
August	14	82	53	19	8	176
September	13	3	13	5	3	37
October	0	11	21	4	1	37

Table 8. Total number of B.pfeifferi collected from the five sites at Debre Berhan in one minute.

Months	No. of snails in each size group in mm					Total No. of snails
	10	8-10	6-7	3-5	3	
1980						
November	39	134	96	26	15	310
December	16	68	34	3	8	129
1981						
January	4	6	12	8	4	34
February	37	24	0	7	4	69
March	101	89	37	15	7	249
April	87	125	56	35	7	312
May	16	62	41	5	3	127
June	44	78	34	14	7	177
July	60	91	88	21	15	275
August	38	14	106	87	30	425
September	102	87	149	21	9	238
October	45	209	168	101	4	527

the collected snails only a few were less than 8 mm in diameter. Again in April, May and June the collection count was found to be between 0 - 5 /min/man. In July and August the count went high up to 86 and 58 respectively. Snails, although few in number, were present from September through October. It was from July onwards that snails of less than 8 mm appeared in good numbers.

Site E had a few snails in the months of November and December (1980) - January and February (1981). In March and April the count per minute was 105 and 123 respectively. All size groups were represented in these counts. In May and June the number decreased and there were no snails of less than 6 mm diameter. The snail population grew to 129 in July and 176 in August. In each month September & October 37 snails were collected. All the 4 months starting from July had all size groups but higher numbers were found in size groups more than 6 mm diameter (Table 7).

The total number of snails from one minute collections at the five study sites representing the area is shown in Table 8. The general tendency of the snail population in almost all size groups was that their number decreased from November (1980) to February (1981). In March and April it showed a peak and declined in May and June. Starting from July there was an ascent that continued until the end of the work in October.

Table 9. Monthly collection of B.pfeifferi
at site X, Wonji.

Months	No. of snails in each size group in mm					Total No. of snails
	10	8-10	6-7.9	3-5.9	3	
1980						
November	8	8	1	27	13	57
December	3	1	9	0	2	16
1981						
January	12	3	9	28	0	52
February	9	12	1	6	4	32
March	9	3	18	8	0	38
April	9	7	4	13	5	38
May	3	0	6	4	8	21
June	9	19	10	6	13	57
July	6	15	2	7	5	35
August	4	3	6	15	1	29
September	13	5	5	7	6	36
October	6	11	4	5	8	34

Table 10. Monthly collection of B.pfeifferi at site Y, Wonji.

Months	No. of snails in each size group in mm					Total No. of snails
	10mm	8-10mm	6-7.9 mm	3-5.9 mm	3 mm	
1980						
November	17	29	10	14	3	73
December	7	29	6	23	0	65
1981						
January	18	32	25	14	0	89
February	17	3	2	4	0	26
March	16	4	0	0	0	220
April	2	0	0	0	0	0
May	2	16	10	18	8	54
June	8	33	18	6	0	65
July	13	6	2	3	1	25
August	6	1	1	0	0	8
September	2	0	0	0	0	2
October	5	4	2	0	0	11

Table 11. Monthly collection of B. pfeifferi from the two sites X, and Y at Wonji.

Months	No. of snails in each size group in mm					Total No. of snails
	10	8-10	6-7.9	3-5.9	3	
1980						
November	25	37	11	41	17	130
December	10	30	15	51	0	117
1981						
January	30	35	34	42	0	141
February	26	15	3	10	4	58
March	25	7	18	8	0	58
April	11	7	4	13	5	40
May	5	16	16	22	16	75
June	17	52	28	12	13	122
July	19	21	4	10	6	50
August	10	4	7	15	1	37
September	15	5	5	7	6	38
October	11	15	6	5	8	45

The snail population at Wonji sites X and Y are tabulated (Table 9 and 10). Table 11 shows the total per minute counts from the two study sites. As is shown in Table 9, the variation in the total number of snails was not so great, however the dominant size group varied quite considerably. In November 1980, for example 70% of the collected samples were less than 6 mm while in December 81% of them were more than 6 mm in diameter. Site 'Y' was a big canal with larger size and denser vegetation than Site 'X'. The snail population showed a marked decrease in the months of April, July and September. This decrease was also noticeable to a certain extent in the months of January, February, July and October. Compared to other months it had the largest population was observed in November.

Correlation of snail population and meteorological factors

The correlation of snail population to meteorological factors for Debre Berhan and Wonji are shown in Table 12 and 13 respectively. In Wonji rainfall is negatively correlated with population size while evaporation rate is positively correlated to the same. In Debre Berhan these factors correlated to the total number of snails but in site D and E they are positively correlated to rainfall.

Incidence of intestinal parasites at Wonji and Debre Berhan

In Wonji out of 306 school children 45 (14.7%) and out of 87 volunteers from the camps 41 (47.1%) were positive

for S.mansoni. On the contrary none of the 168 school children in Debre Berhan were found infected with S.mansoni.

Natural infection of snails

The natural infection of snails in Wonji & Debre Berhan is shown in Table 15. In both cases 200 snails were crushed and those bearing S.mansoni cercariae were recorded.

Host-parasite compatibility

In the first exposure set up (Table 15) only 4 snails were found shedding cercariae and they were all from Wonji. In the second setup (Table 16) shedding snails were found from both Debre Berhan and Wonji. Wonji snails shed in the laboratory of Addis Ababa University and at Wonji aquaria while those of Debre Berhan shed only at Debre Berhan. In all cases they were proved to be S.mansoni on animal exposition. Those that had the parasite at the sporocyst stages are also shown together with the shedding ones. There were 12 Wonji snails and 8 Debre Berhan snails in the Debre Berhan sets and 8 Wonji snails and 3 Debre Berhan snails in the Wonji sets.

In the last set up (Table 17 - 20) snails from both areas were found positive for the parasite, 26% of Wonji and 37% of Debre Berhan snails, from the survivors were found shedding cercariae 58 days later after exposure at Debre Berhan (Table 17. At Wonji 29% and 39% of the Wonji and Debre Berhan snails respectively again from the survivors were shedding cercariae (Table 18). Tables 19 and 20 indicate the number of snails found positive for the parasite at room temperature and $23 \pm 1^{\circ}\text{C}$ after exposure to different dose of miracidia. The last two Tables 21 and 22 show growth rate differences at room and $23 \pm 1^{\circ}\text{C}$ temperatures respectively.

Table 12. Correlation of snail population to meteorological factors in Lebre Berhan.

Months	No. of snails at each site					Total No. of snails	Meteorological factors.		
	A	B	C	D	E		Rainfal (mm)	Mean water temp. (°C)	Relative humidity (%)
1980									
November	22	146	128	8	6	310	22	15.9	52
December	31	7	32	0	9	129	0	16.8	63
1981									
January	17	2	13	0	2	34	0	16.1	72
February	14	2	2	50	1	69	0	16.15	63
March	1	54	17	72	103	249	180	15.5	58
April	45	95	44	5	123	312	20	16.6	57
May	55	23	21	0	28	127	22	15.0	64
June	11	69	57	5	35	177	20	14.7	76
July	0	52	8	86	129	275	260	13.9	79
August	2	133	56	58	176	425	158	15.0	72
September	0	165	7	29	37	236	48	16.0	54
October	0	398	27	65	37	527	0	16.1	46
r- Rainfall	-0.47	-0.09	-0.17	+0.75	+0.76	+0.29			
r- M.wat.temp.	+0.3	+0.11	+0.04	-0.43	-0.44	-0.10			
r- R.E%	-0.07	-0.62	-0.20	-0.03	+0.28	-0.41			

Table 13. Correlation of snail population to meteorological factors in Wonji.

Months	No. of snails at each site			Meteorological factors.			
	X	Y	Total No. of snails	Rainfall in mm	Mean Temp.(°C)	Mean humidity	Evap. rate
1980							
November	57	73	130	0	21.1	45.3	8.1
December	16	65	117	0	20.8	42.8	7.6
1981							
January	52	89	141	0	19.6	45.5	8.2
February	32	26	58	64	22.2	39.5	7.15
March	38	20	58	118	22.5	42.5	5.8
April	38	2	40	82	22.9	49.0	6.6
May	21	54	8	8	22.6	45.5	8.0
June	57	65	122	4	23.7	41.0	8.6
July	35	25	50	276	24.8	43.0	6.3
August	29	8	37	288	22.5	52.5	5.9
September	36	2	38	154	21.6	54.0	5.9
October	34	11	45	0	21.4	53.5	5.8

r- Rainfall -0.22 -0.61 -0.65
r- Temp. -0.06 -0.42 -0.44
r- R.H -0.13 -0.53 -0.47
r- Evap. rate +0.37 +0.90 +0.87

Table 14. Natural infection rate of B.pfeifferi
Debre Berhan and Wonji

1980		1981									
Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug	Sept.	Oct.
0	0	0	0	0	0	0	0	0	0	0	0
4	0	2	0	0	8	17	0	48	60	88	14

Table 15. Comparative susceptibility of B.pfeifferi from Wonji and Debre Berhan for Adwa strain of S.mansoni.

Experimental sets - at	No. of snails exposed	No. of snails alive after		No. of snails shedding during 16 weeks of observation.
		28 days	32 days	
Out - door aquaria (Wonji)	50W	13		4
	50D/B	0		0
Site A Debre Berhan	50W		42	0
	50D/B		24	0
A.A.U Laboratory	50W		39	0
	50D/B		21	0

Key.

1. W= Wonji snails
2. D/B= Debre Berhan snails.

Table 16. Comparative susceptibility of B.pfeifferi from Wonji and Debre Berhan for Adwa strain of S.mansoni.

Experimental sets-at	No. of snails exposed	No. of miracidia per snail	No. of snails found alive 28-32 days	No. of snails shedding cercaria	No. of snails positive by dissection	Total no. of positive snails
Wonji	50W	20	44	2	8	10
	50W/c	0	47	0	0	0
	50D/B	20	13	0	3	0
	50D/Bc	0	34	0	0	0
Debre Berhan	50J	20	37	0	12	12
	50Wc	0	36	0	0	0
	50D/B	20	37	2	8	10
	50D/Bc	0	42	0	0	0
Laboratory	50 W	20	18	3	0	3
	50Wc	0	30	0	0	0
	50D/B	20	2	0	0	0
	50D/Bc	0	22	0	0	0

Key. 1. W = Wonji snails
 2. Wc = Wonji snails control
 3. D/B = Debre Berhan snails
 4. D/Bc = Debre Berhan snails control.

Table 17. Comparative susceptibility of B.pfeifferi from Wonji and Debre Berhan for Wonji strain of S.mansoni, at Debre Berhan.

Snails from		No. of miracidia per snail	No. of snails kept in group aquaria	No. of snails alive 58 days later	Shedding snails from survivors	
					Number	%
Wonji	Exposed	20	100	26	7	26
	Controls	0	100	42	0	0
Debre Berhan	Exposed	20	100	19	3	37
	Controls	0	100	19	0	0

CONTENTS

Table 18. Comparative susceptibility of B.pfeifferi from Wonji and Debre Berhan for Wonji strain of S.mansoni at Wonji.

Snails from		No. of miracidia per snail	No. of snails kept in ground aquaria	No. of snails alive 28 days later	Shedding snails from survivors	
					No.	%
Wonji	Exposed	20	100	37	11	29
	Controls	0	100	48	-	-
Debre Berhan	Exposed	20	150	13	4	39
	Controls	0	150	7	-	-

Table 19. Exposure of B.pfeifferi from Debre Berhan to different number of S.mansoni miracidia under different temperature.

Snails from	Temperature °C	No. of miracidia per snail	Starting No. of snails	No. of snails alive					No. of snails bearing sporocysts
				2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	
Debre Berhan	14-20°C	5	50	45	18	0	0	0	0
		10	50	37	9	0	0	0	0
		15	50	44	40	19	7	3	1
		0	50	40	40	31	7	7	0
Debre Berhan	23-1°C	5	60	18	9	9	6	6	2
		10	60	50	50	43	23	2	1
		15	60	47	19	4	4	4	2
		0	60	51	21	17	2	2	0

Table 20. Exposure of B.pfeifferi from Wonji to different number of S.mansoni miracidia under different temperature.

Snails from	Temperature	No. of miracidia per snail	Starting No. of snails	No. of snails alive					No. of snails bearing sporocysts
				2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	
Wonji	14-21°C	5	50	47	36	8	5	2	2
		10	50	50	31	24	16	14	12
		15	50	50	18	12	9	3	2
		0	50	48	41	35	31	16	0
	23± 1°C	5	60	60	36	36	32	32	13
		10	60	51	27	12	8	6	6
		15	60	22	23	21	21	18	16
		0	60	54	53	42	40	34	0

Table 21. Growth of laboratory bred B.pfeifferi from Wonji and Debre Berhan at room temperature (14-21°c).

Snails from	Time in weeks									Statistical significance at 5% probability
	2	4	6	8	10	12	14	16	t-test	
Debre Berhan	1.6	2.93	4.46	4.8	4.86	5.12	5.44	4.66	$\frac{4.36-4.21}{1.39-1.64}$	not significant
Wonji	1.6	2.93	3.13	4.88	4.92	5.27	5.78	5.84	= 0.6	

Table 22. Growth of laboratory bred B.pfeifferi in the laboraroty (23± 1°C) from Wonji and Debre Berhan.

Snails from	Time in weeks									Statistical significance at 5% probability
	2	4	6	8	10	12	14	16	t-test	
Debre Berhan	1.6	3.05	4.7	5.58	5.9	6.08	6.49	6.8	$\frac{5.00875-5.025}{1.78-1.82131}$	not significant
Wonji	1.6	3.54	4.46	5.48	5.13	6.54	6.46	6.86	= 0.406	

DISCUSSION

The influence of rainfall on the snail population varied depending on the topography, water volume, quantity of vegetation of the particular sites. Except for 1 or 2 months of the year, both juveniles and adults of B.pfeifferi were present in all of the study sites. During high floods the snails accumulated in microhabitats of the stream, such as still water at the edges of the streams or small ponds associated with them. Similar effects of rainfall has also been reported from studies in Uganda, Zambia and Sierra Leone where snails bred rapidly after the rain increasing their population to a significantly higher number. (Cridland, 1957a; Cridland, 1958). However in Iraq and the Sudan the rain created floods and lowered the temperature of the water thus producing a deleterious effect on the snail population (Archibald, 1933; Watson, 1951; Watson 1958).

The five study sites at Debre Berhan received about the same amount of rain, but the effect on the snail population varied significantly. The small rain in March was beneficial to population growth in sites B, C, D, and E. However the March rain created heavy flooding in site A due to its topographic nature. Hence the population almost disappeared in March. In the months that followed April and May, site D did not show a rise while all the rest showed an increase in the number of snails until the next rain in July. The

population increase after the rain was possibly due to the increase in food supply and better protection offered by the growth of aquatic vegetations. In site A for example it was after the rain that young snails of less than 6 mm size appeared in large numbers suggesting those months to be the breeding season.

The effect of heavy rain in July was quite different at site 'A'. The flood completely cleared all emergent and floating vegetation together with snails and the snails could not reestablish themselves until the end of October, 1981. Sites B and C showed slight reduction in snail population density in July but in the former the snail population reached its maximum in October; in the later, however, the snail population density fluctuated until October. This could be because the small pools associated with it acted as an ideal breeding sites and with the rainfall they intermittently released their collections into the stream. In sites D and E where aquatic vegetation a bound similar patterns of snail population density increase were observed. Both were most affected in the dry months of November through January. The heavy rain in July like that of the small rain in March raised the population density. This increase in population density during heavy down pour of rain could be that the sudden rise in the level of water in the streams brought down snails from ponds up stream to the study sites.

From the present study of snail population density the absence of snail is in no way a factor inhibiting disease transmission at Debre Berhan. There is in general a decrease in snail population density in dry months. With slight decrease at the beginning, the tendency is to increase in the rainy month (Table 7). This could hardly be attributed to the hinderance for the establishment of schistosomiasis.

In Wonji, sites X and Y had similar pattern of population density with peaks in November, 1980; January, May and June 1981 (fig.4). There was a decrease in the snail population. In February, March and April 1981. It could be attributed to the increased flow of water due to the rainfall which washed away the snails, but from July to October each site was independently influenced by the rainfall. During these months due to the blockage of water supply from the reservoirs site X was drying. Site Y on the other hand experienced high flood after each rain because it was a secondary drainage. However the rainy seasons were not favourable to population increase.

At the time when the snail population were observed in both sites there was a high rate of evaporation and very small relative humidity. Both are factors associated with rainfall and heat. This might need further confirmation by a wide range of observations but if the correlation holds true these parameters can serve as indices for the population growth pattern in Wonji. Barbosa and Oliver (1958) have shown

that weight loss and survival of the snails were dependent on relative humidity. A decrease in relative humidity resulted in loss of weight and higher death rate, there by decreasing total snail population.

The heavy rain is relatively important for transmission of the disease. In fact it provides a likely explanation of the larger changes in infection rates in August, September and October (Table, 14). The infection rate in these months was as high as 42% which when compared to other months is very high. Rainfall might increase the infection rate of snails in two ways. Firstly due to the closure of the water supply the canals dry. This will concentrate the snails in their residual pools here and there. The increase scarcity of water during this months people will be forced to have closer contact with the water, there by increasing the infection rate in the snails population. Secondly, practically always a small rain now and then through washing of the faeces deposited on the bank provides more opportunity for miracidia to come in contact with the snails before they dry up. This has been shown by many workers for both S.mansoni and S.haematobium (Gisman , 1954 ; McCullough, 1959; Wright and Bennet, 1967; Cridland, 1970; Basch, 1975). Cridland(1970) observed infection rates in snails ranging from 0-100% depending on the strain of S.mansoni. The reason for these variations is unknown. Interspecific differences between vectors

in regard to physiological factors that determine the development of the parasites in the snails have been infected. In Bulinus species group chromosome numbers differences had been accounted for difference in susceptibility. These have been useful in predicting susceptibility to S.haematobium infection, but in Biomphalaria species chromosome counts were not useful because they all have the same number of chromosomes (Natarajan et al., 1965). In this study except that there was a slight increase in the growth rate of Wonji snails, nothing significant in these terms was observed.

Snail exposure studies showed that Debre Berhan snails were not less susceptible than Wonji snails. Thus the absence of endemicity in Debre Berhan could not be due to difference in the snail types.

The infection rate of snails to different numbers of miracidia in the laboratory both at room temperatures (14-21°C) and 23± 1°C did not reflect the effect of the number of miracidia they were exposed to. The probability for the snails to be infected increases with the numbers of miracidia (Standen, 1952), but snails do not absorb more than one or two miracidia at a single exposure (Chu et al., 1966; Lo, 1972).

The average of the daily minima and maxima in Debre Berhan varied from 7-23°C while in Wonji it varied from 13.2-28°C. In Wonji the temperature is optimal for the growth of both the snails and the parasite. Except for

December 1980, the average in Wonji was above 20°C. As Shiff (1964) has noted, at 25°C snails produced eggs at higher rate and could regenerate their population rapidly. Had it not been for the continuous mixing of the water in the canal, the high temperature (40°C) attained by surface water normally at the middle of the day would have been dangerous to both parasite and the snails. That is according to Berrie (1970) highest tolerable temperature for B.pfeifferi is between 28 - 30°C. A mean maximum temperature of 28.6°C in lower and middle Awash is said to be inimical to B.pfeifferi (Meskel et al., 1980). Exposure of snails to high temperature result in high death rates (Gorden et al., 1934; DeWitt, 1955). This does not appear to be the case for snails found in the canals at Wonji. Moreover the snails and miracidia are capable of choosing suitable temperature. This together with the poor sanitary condition of the area will keep it as foci for the maintenance of the disease.

In Debre Berhan the relatively low temperature encountered during certain months (fig.3) does not appear to act as an absolute barrier to the transmission of the disease but it may make the establishment of the parasite more difficult. As Purnell (1966) has shown, low temperature reduces both miracidial and cercarial activity and causes them to sink to the bottom. Snails also withdraw into their shells during cold seasons making it difficult for the miracidia to find the snail host. Exposure of snails to miracidia

at 10°C for one minute did not result in a single infection (DeWitt, 1955). With increased temperature condition become more favourable for contact between miracidia and the snails. Standen (1952) and Stirewalt (1954) have put a minimum temperature of 23 - 26°C to be the requirement for sporocyst development of parasite and an inverse variation in the incubation period for development upto cercarial shedding with temperature. Foster (1964) and Chu et al., (1966d) have also shown that the potential of miracidia of S.haematobium to infect snails was optimal in temperature ranges between 20 - 30°C. Nevertheless infection occurred in temperatures as low as 8°C. In fact the longevity of miracidia increased in cold water. Fain (1963) also conducted an experiment in the Congo, where he exposed snails to S.mansoni in the laboratory at temperature varying between 17 - 24°C and left them at 16 - 17°C and found them shedding cercariae 40 days later. Gumble et al., (1957) working on O.nosophora and Bauman, 1979 on O.quadrasi have also shown that within the range of snail activity (12-30°C) the parasite completes its life cycle although increase of cercarial out put was observed with temperature increase. Eggs could be kept alive at 4°C for long time (Ingall et al., 1949).

From the above, it is not surprising that one finds shedding snails in Debre Berhan that experience a mean water temperature range of 7 - 23°C. It should be noted however that temperature reduces snail infection rate and prolonges

the time for sporocyst development. This is reflected in the present work too (Tables 17 and 18).

Thus the reason for the absence of the disease in Debre Berhan could be due to the presence of only a few people infected in the area which might have kept the infection rate below critical level. MacDonald (1960), discussing the concept of critical level, said that a certain level of parasite density must be reached both in humans and snails before a reasonable chance of perpetuation exists. In Debre Berhan parasitological survey made in the elementary school out of 168 examined there was not a single infection of S.mansoni. Thus, in order to get to the threshold value, the degree of pollution and water contact, daily average egg output, etc must attain a higher level. The location of the town which is high upon the plateau and far from the rivers reduce the chance of faecal contamination. Nevertheless environmental modifications such as installation of irrigation system could change the situation and make it more favourable for the establishment of schistosomiasis.

CONCLUSIONS

1. Snails are found all year round in Debre Berhan area although their numbers fluctuated differently in each study site. The great variation in the seasonal fluctuation of population among the five sites was due to difference in their topography, quantity of vegetation water volume, and flow rate. Population size per se does not seem to be the factor for lack of transmission.
2. Species from Debre Berhan are equally susceptible to infection as those from Wonji.
3. Temperature, water chemistry, pH, oxygen tension and rainfall were studied and none of them appeared to be a deciding factor for the absence of the disease transmission. Lower temperature prolonged the incubation period but was not a barrier.
4. By far the amount of rainfall was the most important factor affecting the snail population size. Floods after the rain washed away the snails, but the population built up in the succeeding months.
5. The supply, of piped water the long distance far from the town to the rivers and the lack of infected people appear to have not favoured the establishment of the disease at present.
6. The B.pfeifferi from Debre Berhan appear to have the same potential of disease transmission as those obtained from Wonji.

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