TECHNICAL GUIDE FOR SCHISTOSOMIASIS CONTROL IN THE PHILIPPINES

SECOND EDITION

Schistosomiasis Control and Research Project
Ministry of Health
Philippines

Published by
The Japan International Cooperation Agency
1980

FOREWORD

This Technical Guide for Schistosomiasis Control in the Philippines is a compilation of the articles on schistosomiasis in the Philippines published in the local and foreign scientific journals. A compilation of the different procedures was also made and in included herein. These procedures have been adopted by the SCRP at Palo, Leyte, Philippines and may be modified or entirely changed as more practical and better ones are introduced. Thus, with the ever increasing knowledge on the various aspects of schistosomiasis, dynamic modifications and changes in the methodology may be expected.

We feel that some points may have been omitted due to oversight. However, in spite of this, we hope that this guide may serve as a useful reference for public health workers interested or actually involved in the control of schistosomiasis in the Philippines and other parts of the Far East where Oriental schistosomiasis is endemic.

In the preparation of the technical guide we appreciate with gratitude the continued encouragement, suggestions and contributions of Prof. Kazuo Yasuraoka and Prof. Hiroshi Tanaka of the R.P. - Japan Medical Cooperation Program; the valuable suggestions of Dr. Tirso C. Banzon of the Bureau of Research and Laboratories; the help extended by Mr. Rogelio Hambre, Mrs. Daisy Trinidad Perez, Miss Lilia Pascua and other malacologists of the SCRP, and for its publication, the JICA.

1976

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Project Director

FOREWORD

It is the pleasure of the compilators of this technical guide to learn that the text has been used widely for the training of new staffs in the schistosomiasis control organizations in the Philippines since it was published in 1976.

They appreciate very much JICA in offering to publish the second edition when they are aware of lack of copies of the first edition and continuing demand for its copies.

The second edition is published after partially revising the first one. The compilators are grateful if the second edition also would contribute much to the education of the persons who are interested in and going to be engaged in the professional position in controlling schistosomiasis in the Philippines.

May 1980
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CONTENTS

SCOPE AND PURPOSE

The presentation herein made is mainly those of *Schistosoma japonicum*, the only species of the parasite present in the Philippines. The other species, *S. mansoni* and *S. hematobium* including their intermediate host and other characteristics are only mentioned in passing in relation to the former.

This manual has been prepared to appraise public health workers and other individuals or groups interested in schistosomiasis control of the different aspects of the disease including its prevention and control.

It is likewise hoped that this manual may interest various individuals or groups in undertaking further researches on schistosomiasis that would provide a more practical way of controlling and eventual eradication of the disease.

HISTORICAL REVIEW

The earliest record account of infection caused by *Schistosoma japonicum* is that of Fujii in Japan, in 1847. The first relationship of the true causative agent to the disease was reported by Katsurada, a Japanese physician, in 1904. He recovered worms from the portal system of a cat and named the species *Schistosoma japonicum.*

The occurrence of *Schistosoma japonicum* in the Philippines was first described by Paul G. Wooley in 1906. Thereafter the infection was detected among the inmates of the Bilibid Prison in Manila, during the course of examination for intestinal helminths (in 1908 by Garrison and in 1914 by Willets), and among patients admitted to the Philippine General Hospital in 1928 (Hison). The infected individuals were found to have come from the islands of Leyte, Samar, Mindanao and Luzon. Between 1906 and Tubangui's discovery of the intermediate snail host in 1932, cases of schistosomiasis were detected by various authors in the Philippines.

As a large proportion of the discovered cases came from Leyte, Tubangui in 1932 visited the area in search of the intermediate host; he found *Oncomelania* sp. in a pond and a small brook in the barrio of Gacao in Palo. He was led by the proximity of the habitat to the residence of the infected individuals and succeeded in establishing that the snail was the intermediate host of the parasite. He was no doubt guided in his search of the snail by the previous discovery of *Oncomelania* sp. as the intermediate host of *S. japonicum* in Japan, China and Formosa. Tubangui's discovery in Palo marked the beginning of the fight against schistosomiasis in the Philippines.

Africa and Garcia in 1935 planned the collection of information on the distribution of the disease in the Philippines through questionnaires sent out to the different hospitals in the country. Mindoro was added to the list of the endemic areas and some of the cases in Luzon were traced in Sorsogon. Africa drew pointed attention to the problem of schistosomiasis in the Philippines in 1938. The first reliable field surveys were organized and reported on by the Bureau of Health of the Philippines in 1940 and 1941 in the islands of Mindanao, Leyte, and Mindoro. Three small field units of the Bureau started detection and treatment of cases and attempted snail control by the use of unslaked lime and fire. The stools of over 3,000 individuals were examined by direct smear in the three islands of Mindanao, Leyte and Mindoro, and prevalence rates of 6.2%, 8.8% and 4.8%, respectively, were reported in the three islands.

Tubangui and Pasco in 1941 found an average prevalence rate of 20% in Mindanao, Leyte, Samar and Mindoro and reported dogs and pigs to be infected as well as humans. They made a rough estimate of between 25,000 and 33,000

human cases in the known endemic areas in the Philippines.

Africa and Garcia (1941) and Tubangui and associates (Tubangui & Aguila, 1941) continued research on the parasite and treatment of schistosomiasis until war broke out in the Philippines in 1941, bringing all progress to a standstill. Paradoxically enough the same was brought the problem of schistosomiasis the forefront when an epidemic of the infection occurred among the United States forces landing in Leyte in October 1944. It involved over 1,700 individuals (Wright, 1950). Starting in November, it reached its peak in January and went down in May 1945 (Carrol & Hunninin, 1948). A similar outbreak at about the same period (December to January) occurred among a Royal Australian Air Force airfield construction squadron which had joined an American task force in Leyte (Dakin & Connollan, 1947). A diagnosis of schistosomiasis was made in 174 of a total of 565 men who spent 16 days on the island.

A rush of papers appeared in the period 1945 - 1947 on the Leyte experience, mostly on the clinical and pathological aspects of the disease relative to the protection of fighting forces, and at the same time some studies were made of the problem as it concerned the local population (Bang *et al.*, 1945, 1946; Magath & Mathieson, 1945, 1946a, 1946b; Faust *et al.*, 1946; Avery, 1946; McMullen, 1947; McMullen and Grahan, 1947).

Bang and associates in 1945 reported 80% of children 10 years and older to be positive for *S. japonicum* in the five barrios surveyed in eastern Leyte, using the sedimentation technique. They considered the area to be hyperendemic and believed that everyone living in the area usually got infected before reaching the age of 15 years. Adults as a group, however, had a lower rate of infection than the children.

In1947, Pesigan (1948a) found other endemic areas in Mindanao around Lake Mainit and obtained a prevalence rate of 21.6%, an estimate close to Tubangui's finding in 1941. The following year, he also established (Pesigan, 1948b) the endemicity of the disease in Irosin, Sorsogon, the first town in the island of Luzon to be so declared, which helped to focus the attention of the Philippine government on the problem of schistosomiasis in the islands.

Tubangui (1948) by now had re-estimated that there were no less than 300,000 cases of schistosomiasis in the country, basing his estimates on a liberal 20% average infection rate in the different endemic provinces. Wright (1950), however, placed the estimate at about 250,000. Wright *et al.* in 1947 had found the Mindanao provinces of Bukidnon, Lanao and Davao to be endemic, which led Pesigan and associates in 1949 to undertake further extensive surveys in that island, leading to the finding of cases in Cotabato, Occidental Misamis and Zamboanga (Pesigan, Pañgilinan & Sarmiento, 1949).

Hunter and associates in 1950 surveyed Mindoro and, on the basis of the two coverslip preparations from direct smears and two from sediment of each faecal specimen, found a prevalence rate of 39.7%, in the region west of Naujan Lake, and reported 8 out of 24 dogs, 9 out of 13 pigs and one out of 6 carabaos (water buffaloes) examined to be positive. This was the first time that the carabao, the most important draught animal in the Philippines, was reported to be a possible reservoir of *S. japonicum*.

During the period July 1949 to June 1950, under the auspices of schistosomiasis research programs established in the Ministry of Health of the Philippines, concerted efforts were made to survey the different endemic areas (Pesigan, 1950). Six field units were organized and they undertook mass stool examinations, snail surveys, treatment of patients, educational propaganda and snail control. This campaign covered 35 towns in 10 provinces in the islands of Mindanao, Leyte, Mindoro and Samar. Of the 35,509 individuals examined by direct single fecal smear method, 4,302 were found positive for schistosomiasis, giving a prevalence rate of 12.1%. The worst affected areas found were: Aurora in Zamboanga: Bonifacio in Occidental Misamis; Naujan in Mindoro; Mainit in Surigao; Butuan City and Jabonga in Agusan; Bobon in Samar; and almost all the towns surveyed in Leyte, with the prevalence rate reaching as high as 50.7% in the newly created town of MacArthur, Leyte, formerly barrio Tarragona.

Another important feature of this survey was that a report was also made on the exceedingly high prevalence rate of intestinal helminths in the areas surveyed, which reached a figure of nearly 99% both in the schistosomiasis-affected and in the free areas. Age and sex distribution of the schistosoma-infected individuals and their clinical gradation into five categories, depending on the stage of the disease, was presented by Pesigan in 1951. Almost 90% of the samples examined by the field units (except Mindoro Unit No. 3) were, however, school children; therefore a true picture in the total population could not be obtained, but a higher prevalence among males than among females was apparent.

Following this survey, the importance of the problem of schistosomiasis in the Philippines came to be recognized, and the Philippine Government created the Division of Schistosomiasis in the Department of Health in 1951. At the request of the Government, the World Health Organization sent a team of consultants to the Philippines in 1952. The terms of reference of this team were: 1) to study the schistosomiasis problem in the Philippine Islands; 2) to examine the control work that was being done; and 3) to make recommendations for a national programme. They spent approximately three months studying the problem and submitted a very valuable report in which the problem of schistosomiasis in the Philippines was described as an extremely complicated one. An estimate of 100,000 to 200,000 cases in the 12 endemic areas of the Philippine Islands was made; this

figure was, however, revised to 200,000 – 300,000 in 1954 (McMullen *et al.*, 1954). The team felt that the disease problem had so far been approached almost entirely from a medical viewpoint, which was but a small segment of the whole, and that, in order that control should be successful, it was necessary to get more information on all aspects of the problem, including biology, engineering, agriculture and education. It was also considered that thinly spread efforts to deal with the disease would be more or less unrewarding, and therefore suggested that they should be terminated and an attack made on the problem from a new angle. The team therefore recommended the concentration of funds and efforts on a pilot project, with the World Health Organization providing three advisers for the programme and the Division of Schistosomiasis supplying collaborating members technicians, assistants and laborers.

The team suggested that parts of Eastern Leyte, which appeared to be the most important endemic area, be used for a pilot project of six years duration, with an absolute minimum of three years.

The Philippine government acted quickly on the recommendation of the WHO schistosomiasis team made in September 1952, in initiating a schistosomiasis control project in Leyte, and WHO assisted the project by supplying three international personnel. Assistance was also received from the Foreign Operations Administration of the USA in the form of supplies and from the Philippine Council for United States Aid. A fully equipped project-centre building, including laboratories, was constructed and staffed, and the Project officially started functioning in June 1953.

The Project has the following objectives:

1) To determine the most effective and economical means of controlling schistosomiasis in the Philippines.

2) To train local professionals and auxiliary personnel in the various phases of the work envisaged in this project.

3) To conduct studies and make observations on the human, other vertebrate and snail hosts of *Schistosoma japonicum* as well as on the parasite itself; to obtain data essential for the rational control of the disease.

4) To make a thorough epidemiological study of the disease in a highly endemic area.

5) To design and test control measures based on the results of data obtained by basic studies and observations.

6) To plan and prepare for an expanded schistosomiasis control programme.

After 1959, various control measures were formulated and found effective in the pilot area of Palo. These measures brought the prevalence of the disease in Palo from 38.9% to 32.8% (Pesigan and Hairston, 1961). These were initially tried in the 3 pilot towns of Mayorga, La Paz and Burauen all in the Province of Leyte. With the encouraging results obtained in these three municipalities, 4 Regional Schistosomasis Advisory Teams were formed and assigned in the provinces of Samar, Lanao, Davao and Mindoro. The team in Oriental Mindoro later on was split into two. One group remained in Oriental Mindoro and the other group was assigned in Sorsogon. These teams up to the present are still operating covering other provinces within their respective regions. More teams are envisioned to be formed, at least one for each endemic province.

The Japanese assistance through the Japan International Cooperation Agency started in August 1972 in supplying necessary laboratory equipment, vehicles for field surveys and a heavy equipment for a small scale land reclamation and sending two Japanese parasitologist to stay for long terms together with several short term consultants annually. Advancement of diagnostic techniques, extraction of new molluscicides of plant origin, epidemiological, ecological and clinical studies has been attained in this program. As a part of this assistance, a laboratory building of about 300 square meters was provided and was open in January 1978 being named as the Dr. Trinidad R. Pesigan Memorial Laboratory. The Philippine-Japan Joint Conference on schistosomiasis research and control was held at rooms Leyte/Samar, Philippine Plaza Hotel, Manila on 20 to 23 November 1979 by inviting about 100 participants from various organizations of the Philippines, Japan and other countries, and international organizations to collect and review the existing technologies for the schistosomiasis control having been developed in the past 10 years to formulate the report of conference. (JICA, 1980).

NATURE OF THE DISEASE AND ITS DISTRIBUTION

Definition

Schistosomiasis japonica is a chronic tropical disease characterized by fever, dysentery, hepato and splenomegaly and a terminal cirrhosis of the liver caused by a species of blood fluke called *Schistosoma japonicum* which is transmitted through the intermediary of a tiny snail known as *Oncomelania quadrasi.* It is also called bilharziasis japonica, snail fever or Katayama disease.

Etiology

There are three principal *Schistosoma* species that mature in man, namely:

1) *Schistosoma hematobium* - causing schistosomiasis hematobia, also called urinary or vesical schistosomiasis. This is found in Africa and the Middle East countries.

2) *Schistosoma mansoni* - causing schistosomiasis mansoni or hepato-intestinal schistosomiasis. This is found in Central America, the West Indies, northern parts of South America and Africa.

3) *Schistosoma japonicum* - causing schistosomiasis japonica or oriental hepato intestinal schistosomiasis. This is found in the Philippines, Japan, China, Sulawesi and Thailand. A purely zoophilic strain is found in Formosa.

Geographical distribution

In Southeast Asia this has recently been discovered in Thailand. An autochthonous case has been reported from Laos. There is a focus of long standing in the Celebes. *S. japonicum* is found in lower animals in Taiwan, but human cases have not been reported. In Japan, the parasite occurs on the main island of Honshu in the prefecture of Chiba, Ibaraki, Yamanashi, and Hiroshima. On the island of Kyushu, it is found in Saga and Fukuoka Prefectures.

In mainland China, *S. japonicum* is endemic in the Provinces of Kiangsu, Chekiang, Hupeh, Hunan, Kiangsi, Anhwei, Kwangtung, Kwangsi, Kukien, Szechwan and Yunnan.

To date, the places endemic for this disease in the Philippines are the following:

I. Luzon

Oriental Mindoro

1. Naujan
2. Pola
3. Socorro
4. Victoria

Sorsogon

1. Bulusan
2. Casiguran
3. Irosin
4. Juban

II. the Visayas

Leyte Del Norte

1. Abuyog
2. Alangalang
3. Babatngon
4. Barugo
5. Burauen
6. Dagami
7. Dulag
8. Jaro
9. Javier (Bugho)
10. Julita
11. La Paz
12. MacArthur
13. Matag-ob
14. Mayorga
15. Palo
16. Pastrana
17. Santa Fe
18. San Miguel
19. Tabontabon
20. Tacloban City
21. Tanauan
22. Tolosa
23. Villaba
24. Tunga

Bohol

1. Talibon
2. Trinidad

Eastern Samar

1. Borongan
2. Canavid
3. Dolores
4. Llorente
5. Oras
6. Salcedo
7. San Julian
8. Sulat

Western Samar

1. Basey
2. Calbayog
3. Calbiga
4. Catbalogan
5. Gandara
6. Jiabong
7. Oquendo
8. Pinabacdao
9. San Sebastian
10. San Vicente
11. Santa Margarita
12. Tinambacan
13. Villareal

Northern Samar

1. Allen
2. Bobon
3. Catarman
4. Catubig

5. Laoang
6. Las Navas
7. Lavezares
8. Mondragon
9. Palapag
10. Pambujan
11. San Jose

III. Mindanao

Agusan

1. Ampayon
2. Bayugan
3. Bunawan
4. Butuan
5. Cabadbaran
6. Esperanza
7. Jabonga
8. Kitcharao
9. La Paz
10. Las Nievas
11. Loreto
12. Prosperidad
13. San Francisco
14. Santa Josefa
15. Santa Fe
16. Talacogon
17. Veruela

Bukidnon

1. Mailag
2. Malaybalay
3. Maramag
4. Valencia

Cotabato (North)

1. Buluan
2. Isulan
3. Matalum
4. M'lang
5. Pikit

Davao Del Norte

1. New Corella
2. Calinan (Davao City)
3. Compostela
4. Davao Penal Colony
5. Mawab
6. Nabunturan
7. Carmen
8. Santo Tomas
9. Tagum

Surigao Del Norte

1. Bacuag
2. Gigaquit
3. Mainit
4. Placer
5. Surigao City

Surigao Del Sur

1. Tago
2. Bislig

Davao Del Sur

1. Digos
2. Hagonoy

Lanao Del Norte

1. Kapatagan
2. Lala

Lanao Del Sur

1. Ramain
2. Tamparas
3. Tanaka

Zamboanga Del Norte

1. Dipolog
2. Nipaan

Zamboanga Del Sur

1. Aurora
2. Dumingag
3. Magsaysay
4. Mahayag
5. Molave
6. Tambulig

Misamis Occidental

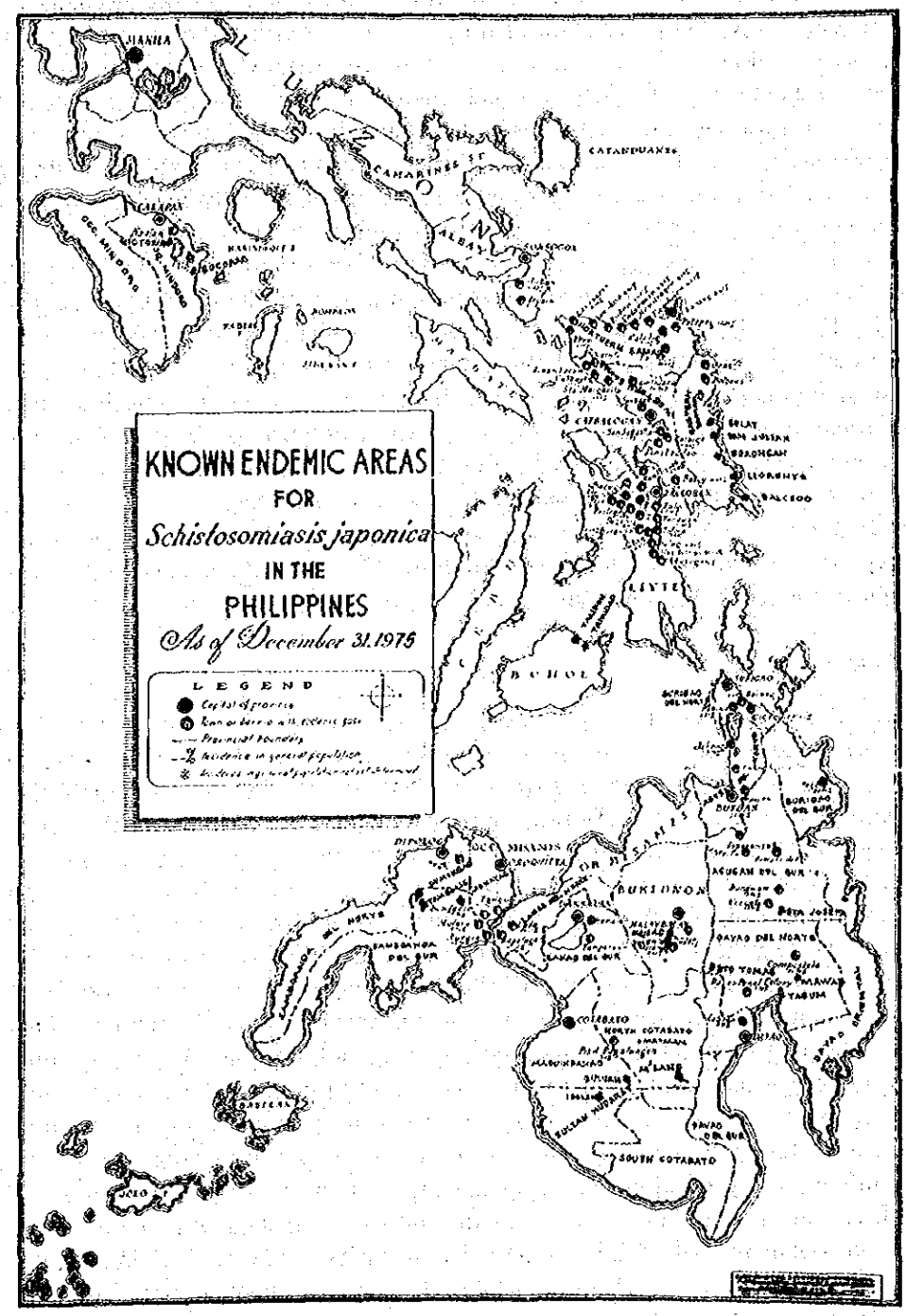
1. Bonifacio
2. Tangub

Life Cycle of the Parasite

The adult males measuring 12 to 20 mm in length by 0.5 to 0.55 mm in width and the female flukes (15 to 26 mm x 0.3 mm) commonly inhabit the mesenteric veins although occasionally they are found in aberrant locations. In the egg-laying operation, the paired worms proceed down to the terminal venules, and the female worm extends without leaving the gynecophoral canal of the male in order to lay her eggs (70 to 100 micra in length by 50 to 65 micra in breadth) adjacent to the walls of the intestine. By mechanical and chemical means, the eggs pass into the tissues, many of them to reach the lumen of the intestine but some to be held up by local inflammatory reaction. Those not so arrested are passed in the feces by which time some of the miracidium contained within the egg has reached maturity, and is ready to hatch if and when the egg reaches freshwater.

On hatching the ovoid ciliated miracidium attachs a suitable molluscan host. If it fails to contact a susceptible snail, the miracidium dies within 48 hours, usually in less than 24 hours. Once penetration has been effected, the larva finds its way to the head, foot, mantle, tentacles or other location within the snail, loses its cilia and transforms into a sacculate sporocyst. The sporocyst in turn produces within its cavity a secondary generation of sporocyst which migrate into the lymph sinuses of the digestive gland of the snail. Here the cercarial (measuring 0.25 – 0.32 mm including body and tail) develop within the daughter sporocysts from which the escape through a birth-pore. When mature, these cercariae emerge from the snail and attack a suitable mammalian host. The cycle

Fig. 1


KNOWN ENDEMIC AREAS
FOR
Schistosomiasis japonica
IN THE
PHILIPPINES
As of December 31, 1975
LEGEND
MANILA
CATANDUANES
CAMARINES SUR
ALBAY
LEYTE
BOHOL
SAMAR
BUKIDNON
DAVAO DEL NORTE
COTABATO
SOUTH COTABATO
JOLO

with the molluscan host requires approximately 8 weeks.

Penetration of the skin of the definitive host is aided by the discharge of the enzymes from the penetration glands. Entrance is effected quite rapidly and may occur within less than 10 minutes. Those cercariae which fail to contact a mammlian host are short-lived and usually die within 48 hours. They are infective only up to 36 hours. During the process of penetration, the cercaria loses its tail. The passage through the subcutaneous tissues is usually effected within 24 hours, and the cercaria now properly termed a schistosomula finally reaches a venule from which it is carried by the venous circulation to the heart. Via the pulmonary artery, it is carried to the lungs, and thence crosses the capillary bed to reach the arterial circulation through which it is transported to the various parts of the body. Except in rare instances, these cercariae which reach aberrant locations never develop further. It is only those that are transported to the arteries of the abdominal viscera, and are able to reach the mesenteric veins that continue their development and reach maturity. From the mesenteric veins, the larva reach the intrahepatic vessels where they continue to develop until sexual maturity is approached. They mate and the worms then migrate to the mesenteric veins where egg production begins. The period between infection and recovery of eggs from the stools of the definitive host may comprise 30 -- 40 days or a little bit longer (Refer to Fig. 2 for illustrated life cycle.)

Pathology

For purposes of description, the pathology is divided into three stages but like the symptomatology, the divisions do not actually exist in practice since in nature all stages may be present at the same time. Briefly therefore, the highlights of each stage will be given. During the first stage or stage of invasion, there may be slight cellular reaction at the point of entry of the cercaria. In the lungs, in cases of heavy infestations, some of the larvae or schistosomulae break through the capillaries and cause hemorrhage and leucocytic infiltration around the young worms. During the second stage or stage of egg deposition, the pathology is mainly in the small intestines and the liver. The young flukes and eggs produce an acute inflammatory perivascular reaction in the small intrahepatic venules which may develop to hemorrhagic congestion in severe infestations. In the intestinal wall, the eggs cause the formation of abscesses infiltrated with leucocytes, which may break through with the formation of scar tissue or may remain intact and be invaded by foreign body giant cells and form a fibrous nodule or pseudotubercle. The inflammatory reaction caused by the eggs will eventually lead to fibrosis of the liver, spleen and lymph glands. In the third stage or stage of tissue proliferation and repair the pathology is mainly due to portal cirrhosis. The liver at this stage is small, hard and cirrhotic while the spleen is enlarged and hard. The muscles are atrophied, skin pale and edematous and the ascities is marked.

During the second and third stages, granuloma of the brain simulating brain tumor may be produced.

Symptomatology

The earliest stages of schistosomiasis in man, before the worms have reached maturity, produce relatively unimportant signs, which are usually disregarded. A transient skin lesion may follow invasion of the cercariae, followed by cough and other transitory pulmonary reactions as the parasites pass through the lungs. A little later, when the young worms are growing to sexual maturity in the liver, such minor toxaemic symptoms as loss of appetite, malaise, headache, fever, and temporary enlargement and tenderness of the liver may occur. The major symptoms and pathogenic effects, however, which are not observed until the worms have reached sexual maturity and oviposition has begun, are mainly due to the presence of the eggs in the tissues and to the irritation and reaction causes thereby. Such lesions may be of two kinds - closed or open. In the former, which occur principally in the liver and lungs, the eggs are trapped in the host tissues and are unable to leave the body, while in the latter, which occur principally in the intestine, the eggs are passing through the tissues, from which they eventually escape to the exterior. Closed lesions are generally more serious than open lesions, and may produce indirect effects upon the heart and some major blood vessels.

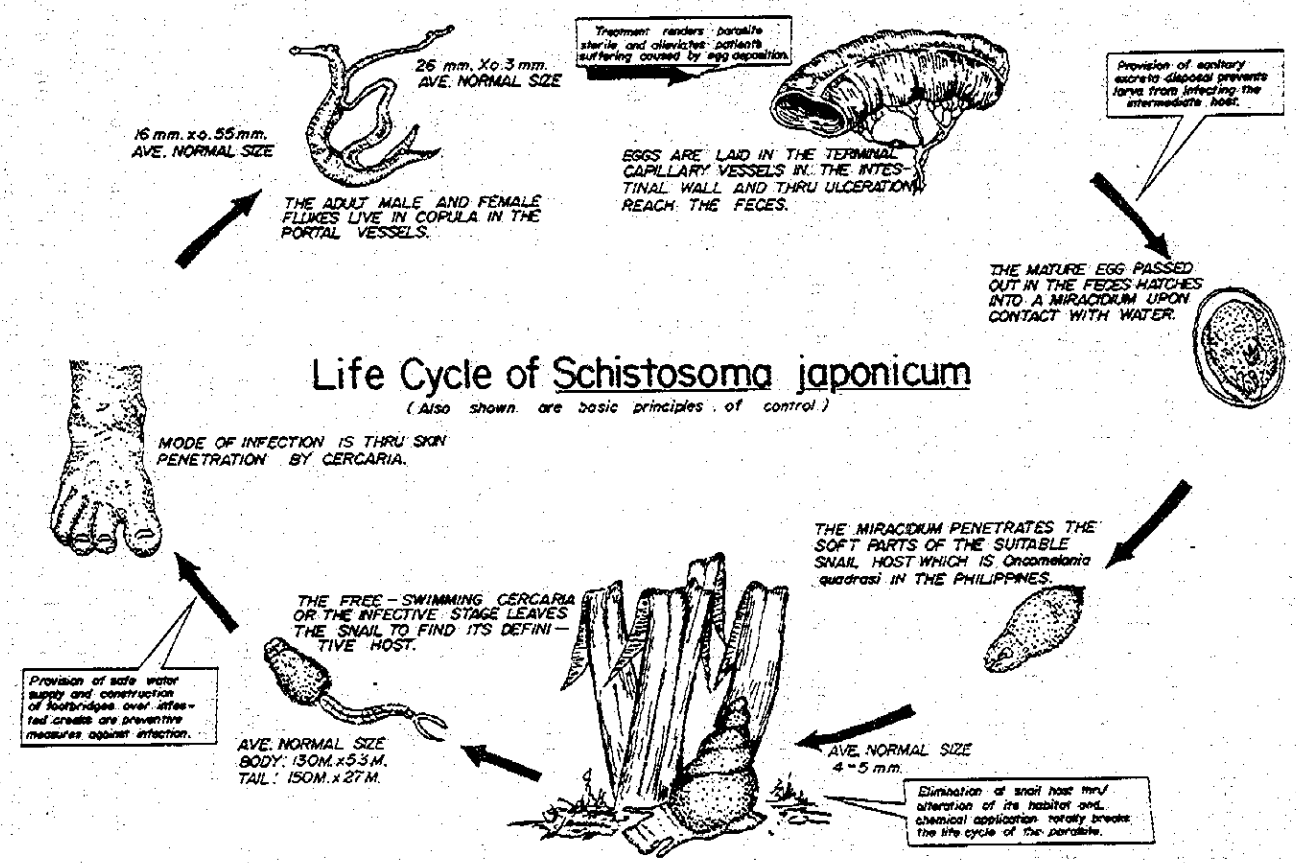
Since serious lesions are due to the effect of the eggs in the tissues, a high rate of infection is very pathogenic and may sometimes be fatal. On the other hand, light infections may be essentially symptomless and lacking in obvious pathogenic effects. All grades of severity occur between these two extremes. The higher the degree of infection and the more frequent the occurrence of re-infection, the more serious will be the damage to the host.

Immunity and toxic allergic reactions play an important and little understood role in pathogenesis. Immunity frequently operates to reduce the appearance of symptoms in infections of long standing. On the other hand, severe toxic-allergic reactions may complicate the issue.

Similar to *S. mansoni*, the lesions in *S. japonicum* occur initially in the wall of the intestine, particularly the caecum, colon and rectum. Dysentery with abdominal pain is often severe. Later closed lesions in the liver and lungs are frequent, with resulting enlargement and disfunction of liver and spleen, and arterial obliteration in the lungs leading to secondary effects upon the heart.

Anaemia is often observed in all forms of schistosomiasis; but whether is due to the disease or to accompanying malnutrition is not certain.

Fig. 2


Life Cycle of Schistosoma japonicum
(Also shown are basic principles of control)
26 mm. X o.3 mm.
AVE. NORMAL SIZE
16 mm. x o. 55 mm.
AVE. NORMAL SIZE
THE ADULT MALE AND FEMALE FLUKES LIVE IN COPULA IN THE PORTAL VESSELS.
Treatment renders parasite sterile and alleviates patient's suffering caused by egg deposition.
EGGS ARE LAID IN THE TERMINAL CAPILLARY VESSELS IN THE INTESTINAL WALL AND THRU ULCERATION REACH THE FECES.
Provision of sanitary excreta disposal prevents larva from infecting the intermediate host.
THE MATURE EGG PASSED OUT IN THE FECES HATCHES INTO A MIRACIDIUM UPON CONTACT WITH WATER.
THE MIRACIDIUM PENETRATES THE SOFT PARTS OF THE SUITABLE SNAIL HOST WHICH IS Oncomelania quadrasi IN THE PHILIPPINES.
AVE. NORMAL SIZE 4 - 5 mm.
Elimination of snail host and/or alteration of its habitat and chemical application totally breaks the life cycle of the parasite.
THE FREE - SWIMMING CERCARIA OR THE INFECTIVE STAGE LEAVES THE SNAIL TO FIND ITS DEFINITIVE HOST.
AVE. NORMAL SIZE
BODY: 130M. x 53 M.
TAIL: 150M. x 27 M.
Provision of safe water supply and construction of footbridges over infested creeks are preventive measures against infection.
MODE OF INFECTION IS THRU SKIN PENETRATION BY CERCARIA.

Infection is almost always acquired early in life, usually in the first decade. Constant reinfection and slow development of immunity produce a tendency to maximal infection in age-groups ranging from 12 to 20 years.

Physical weakness and reduction of the ability to work, increasing with the severity of the infection, is characteristic to some degree of all schistosomiasis patients; and, especially in children, may be coupled with retardation of mental development and intellectual dullness. Incapacity is progressive. Thus infected persons may be grouped in four categories, as follows:

1) Those with light infections, who display slight or occasional symptoms and are able to work normally.

2) Those with moderate infections, who display more marked and frequent symptoms, and on account of weakness are incapable of hard work.

3) Those with severe infections, who display recurrent or continuous symptoms of a disabling character and are consequently often absent from work.

4) Those with severe infections who, in addition to continuous, severe disabling symptoms, display ascites and/or emaciation, and who, therefore, are permanently unable to work.

This depressing sequence of events remains unchanged unless treatment is given. The drugs at present available are not perfectly satisfactory since a 100% cure rate is rarely obtained, rest and cessation of work is necessary during treatment, and relapses are frequent. Nevertheless, mass treatment of the infections appears to prevent the development of more severe manifestations.

In 1949, Pesigan *et al.* adopted the following conventional classification of schistosomiasis cases based on the pathogenesis of the disease.

Grade	Stage and Important Features
A	Pre-Egg Deposition Stage - (not usually seen): With urticarial rashes, itchiness, localized dermatitis, cough, anioneurotic edema, fever and other allergic manifestations. May have diarrhea.
	Laboratory Aid: - Leucocytosis with marked eosinophilia - may try skin and other serological test; stool still negative.
B	Early Egg-Deposition with Early Hepatic Irrigation: With bloody-mucoid stools or diarrheic attacks of recent onset. Abdominal and right hypochondriac pains. Liver may be felt on deep inspiration, with slight tenderness toward the right of the epigastrium. Spleen not yet palpable.

Duration of this stage - fifth week from date of exposure to one year or even earlier depending on the heaviness or severity of the infection.

Laboratory Aid: - Stool examination easily positive; slight leucocytosis with further increased eosinophilia.

C Late Egg-Deposition with Definite Liver Enlargement (Pre-cirrhotic Stage): May have recurrence of diarrhea or dysentery-like symptoms. Abdominal and right hypochondriac pains increased. Liver enlargement marked especially below xiphoid process. Spleen only slightly enlarged. Duration of this stage - 1 to 1.5 years from date of exposure.

Laboratory Aid: - Stool examination - usually positive; eosinophilia still notable, but leucocyte count may be normal or slightly higher than normal.

D Frank Cirrhosis: Size of liver receding but still palpable. Spleen markedly enlarged. Superficial abdominal veins visible. Ascites beginning. Emaciation is visible especially in under-nourished individual. Patient may die of severe hemorrhage due to rupture of esophagial varices. Duration of this stage - Second year onwards.

Laboratory Aid: -- Stool examination almost always requires concentration technics. Protosigmoidoscopy, rectal biopsy or rectal crypt aspiration, skin and other serological tests often of value.

E Advanced Cirrhosis with Marked Emaciation: Very marked splenomegaly, liver small and contracted or non-palpable. Distinct ascites and very prominent superficial veins. Emaciation and anemia marked.

Laboratory Aid: - Usually negative on stool examination (unless with another recent exposure). May try rectal biopsy, rectal crypt aspiration, or serological and skin test. Leucopenia and moderate anemia present.

Complications

The complications which have been observed consist in extension of the lesions from the abdominal viscera to other organs and tissues of the body, and secondary infections or disease processes. Among these are:

a) Neurological manifestations. This is frequently of the Jacksonian type of epilepsy demonstrated to be caused by the lodgement of *S. japonicum* eggs in the brain.

b) Hematemesis resulting from rupture of esophageal and gastric varices.

c) Cor pulmonale characterized by enlargement of the right ventricle due to increased pressure in the pulmonary circulation caused by pathological changes in the pulmonary arteries or massive embolism where the schistosome is involved.

d) Hepatic cirrhosis with or without splenomegaly may supervene.

e) Supervening diseases. These may consist of chronic processes from which the patient may suffer like tuberculosis, malaria, deficiency diseases and the acute infectious disease such as pneumonia and infectious hepatitis.

Diagnosis

1. Clinical Diagnosis

A history of exposure particularly when such exposure took place in a gruop of individuals who develop similar clinical course. The clinical findings of outstanding significance are fever, diarrhea, abdominal pain, blood in stool coming on and off and later palpable liver and spleen. The diagnosis should be confirmed by laboratory diagnosis.

2. Laboratory Diagnosis

A. Fecal Examination

Eggs of schistosomes, together with those of worms which live in the intestine, liver or lungs, are eliminated in the feces the presence of these worms in the body can therefore be detected by finding their eggs in the feces.

The most important consideration in the collection of fecal samples is that they be accurately identified with individual persons, fresh when examined or preserved, and uncontaminated by water, soil or other substances which may contain organisms or their eggs.

The size of the sample, i.e., the amount requested from the individuals to be examined, need not be more than 5 – 10 g (a mass about as big as a thumb). If suitable containers (paper cartoons of 200 ml capacity or larger) are available it is often more satisfactory to request the whole stool. The chief advantage in this arrangement is that it permits defecation directly into the container and a proper

sample for examination may then be selected.

For the diagnosis of schistosomiasis, more so than for some other types of worm infections, preservation of fecal samples for later examination is less satisfactory than immediate examination of the fresh specimen. When, for special reasons, preservation of fecal samples for later examination is less satisfactory than immediate examination of the fresh specimen. When, for special reasons, preservation must be resorted to, the preferred preservative under usual circumstances is 10% formalin (commercial formaldehyde diluted with 9 parts of water). Approximately 1 gram (1 ml) of feces is transferred to a vial or wide-mouth bottle of about 10 ml capacity or larger, and enough preservative is added, with stirring, to bring the feces to a fluid-pasty consistency. Additional preservative is then added to half-fill the vial which, after being stoppering and then added to half-fill the vial which, after being stoppering and vigorously shaken is then nearly filled with preservative, stoppered and labelled. The speciman can be examined later by sedimentation in water or by the formalin-ether technique.

The technique of fecal examination used by the SCRP are: 1) the direct fecal smear and 2) gravity sedimentation in aqueous solutions of 0.5% glycerol or 0.85% sodium chloride and 3) merthiolate-iodine-formaldehyde concentration technique. The details of these method are given in the annexes on diagnostic procedures.

B. Liver and Rectal Biopsy

The eggs in the tissues are demonstrated by pressing the tissue between slides and examining it under the microscope or by the usual pathological technic of preparing slides from a block of suspected tissue.

C. Immunologic Methods

a) Skin test
b) Circumoval precipitation test (COPT)
c) Complement fixation test
d) Ouchterlony test

The COP test with lyophilized *Schistosoma japonicum* eggs is the method of choice for the laboratory diagnosis of schistosomiasis japonica.

The employment of lyophilized eggs and dried blood in filter paper makes the test practicable for routine use and for epidemiological surveys.

Prognosis

The prognosis of early cases is good particularly if the patient received

adequate treatment and is removed from the endemic area. However, late cases, even with treatment have a bad prognosis since the parenchymatous tissue destroyed either by the adults or the eggs can no longer be regenerated and only connective tissues can take their place. As a rule, schistosomiasis japonica has the worst prognosis of the three types of schistosomiasis.

SALIENT EPIDEMIOLOGICAL FEATURES

The Parasite

Prevalence of Schistosome Infection in Man in Relation to Age, Sex, Occupation and Envrionment:

In the pilot area of Palo and three environmental divisions, out of the total number of 2,909 individuals examined on 1953 – 1954, 48.0% were found infected. It was observed that the infection during childhood and adolescence built up rapidly until it reached the maximum and downward trend followed.

As far as age and sex distribution of infected individuals are concerned, there are significant sex differences between age groups in childhood with the prevalence rates being higher for males than for females, the overall rate being 49.6% for males and 46.4% for females. Children of both sexes before school age run an equal risk but the difference becomes clear when males become more active in the field and run greater chances of acquiring the infection.

Prevalence in respect of occupation showed that farmers as a class have the highest infection rate (74.1%). When not planting or harvesting rice, they engage themselves as fishermen, unskilled laborers or tuba coconut milk gatherers, which would explain the next highest prevalence among this group of professions which is over 60%. The occupations that would bring people most in contact with infected waters are farming and inland fishing. Seagoing fishermen who live mostly in the coastal division, would naturally be the people most exposed to infection in rivers, swamps and streams. The class of workers with the lowest rates (exclusive of the pre-school children) is for obvious reasons the office workers and the professional group with an overall infection rate ranging between 16% to 26%. The rest, students, housekeepers and jobless persons, occupies in intermediate position between the first two groups mentioned, with a range approximately 51% to 58%.

Significant differences in the general prevalence of infection exists in the 3 environments with the highest prevalence rate (61.1%) on the inland division. The differences as one would expect are due to the opportunities for infection and the general sociological attitude of the population.

The prevalence, therefore, in respect of age, sex, occupation and environment follows a pattern explainable on the basis of oppotunities for contact with infection. A factor in respect of age, namely, the downward trend with advancing age after the peak prevalence may be explained on the basis of a host reaction

arising from humoral response to infection with a possible immunity mechanism coming from host-cell reaction around infiltrated eggs.

Egg Laying Habits of *S. japonicum*

Studies made on mice showed that the eggs of *S. japonicum* are found in densely packed clusters in the gut wall of the experimental animals. The eggs in a cluster are in the same stage of development, indicating that they are all laid at one time by one female, which probably empties her uterus in the act. Examination of mice sacrificed 22 – 46 days after infection has shown that both the frequency of egg-laying and the number of eggs laid at one time increase for a period of at least two weeks after the previous egg-laying. Counts of egg clusters in the gut wall with comparative study on those in the feces, gut contents, liver and other organs show that 17 days after maturity a female fluke lays more than 100 eggs each time, 12 times a day.

Possible Strain Differences of *S. japonicum*

Early workers in schistosomiasis japonica assumed that the parasite was the same in all vertebrate hosts, although minor local differences in egg-morphology were noted (Faust & Meleney, 1924; N.G. Hairston). More recently, some significant biological differences have been discovered, the most important of which is the failure of the Formosa parasite to attack man. Hsu and colleagues (1955) and Dewitt (1954) found that parasites of different geographical origin varied in their ability to develop in different snail hosts, including the North American *P. lapidaria*.

A large series of experimental crosses of flukes were therefore made, the origins of which were five different mammal hosts—man, dog, pig, cow and rat—all naturally infected. Since sex is determined prior to the miracidium stage, a laboratory-hatched snail, infected with a single miracidium, yields cercariae of only one sex. The sex can be determined by experimental passage through laboratory animals. As snail infections of the appropriate strains and sexes have become available, crosses of flukes in a variety of experimental animals were carried out. The cross has been judged successful upon the appearance of eggs, either in the stool or in the gut wall at autopsy.

All crosses of flukes of different origins have proved successful. Because miracidia are difficult to obtain from the stools of wild rats, and are frequently too weak to penetrate snails when they are obtained, the crosses between flukes of rat and human origin were not completed. The nine different successful crosses obtained, however, indicate that non-human mammals do not carry strains distinct from the human in Leyte.

In order to be doubly assured of the validity of this conclusion, the fertility of the hybrid flukes in a series of eleven crosses was tested. Again, all crosses attempted were successful, and there can be no further doubt that in the light of these findings, all Leyte schistosomes belong to a single strains. The control of schistosomiasis in the Philippines would seem to involve more than sanitary disposal of human feces, and an assessment of the relative role in transmission played by each species of definitive host is therefore of considerable importance.

Relative Role of Human and Other Animal Reservoir Hosts in Transmission

An index that would express the relative importance of the different mammalian hosts would be a composite of several factors. These factors are the numerical strength of the host concerned, the proportion of the host population infected (prevalence of infection), and the miracidia-producing capacity of the host. The latter is dependent upon the average output and hatchability of eggs from the host. All these factors for the seven mammals known to be involved in the transmission of *S. japonicum* in this area; humans, pigs, dogs, goats, carabaos (water buffaloes), cows and rats were therefore determined.

Table I shows that humans and cows have relatively high rates of prevalence; rats, dogs and pigs would fall into an intermediate category in the order named.

Carabaos and goats have low rates of less than 3%. The carabao is the most important draft animal, and the two animals found positive both calves (not more than 2 years old). The texture of the dermis in older carabaos perhaps makes penetration by cercariae difficult.

The highest mean daily egg output is from dogs and cows, with over 12,000 eggs, followed by the carabao with close to 10,000 eggs (Table I). Humans and goats fall into an intermediate group; pigs and rats come last in the series.

Table 1. Relative Transmission Index of Different Hosts of *Schistosoma japonicum* in Palo

Host	Population	Prevalence (%)	Mean daily-egg output	Hatch-ability (%)	Mean daily miracidia Produced	Trans-mission Index (AxB) x (CxD)	Rel. trans. index (%) of total $\frac{(AxB)x(CxD)}{E} \times 100$
	A	B	C	D	C x D		
Human	14,819	48.0	1,123	42.4	476	3,385,845	75.7
Dog	1,517	18.2	13,106	17.8	2,333	644,127	14.4
Cow	76	38.2	12,212	71.9	8,780	254,901	5.7
Pig	3,193	13.3	481	31.9	153	64,974	1.5
Rat	148,190*	22.7	21	10.6	2	67,278	1.5
Carabao	1,318	1.5	9,166	29.4	2,695	53,280	1.2
Goat	204	1.4	952	71.4	680	1,942	0.04
					Total (E)	4,472,347	

* Estimated number of 10 rats per person, which equals 60 rats per hectare.

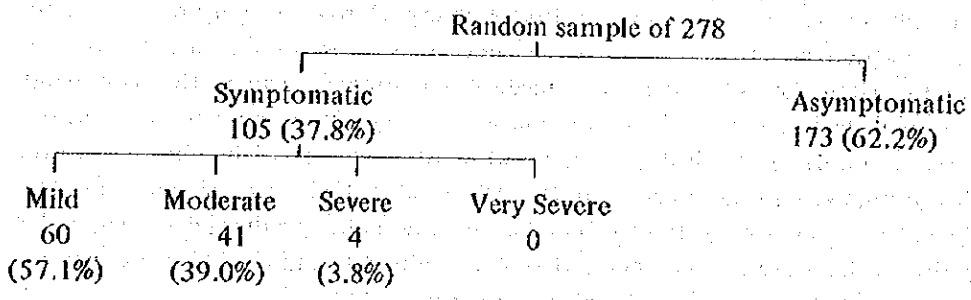
Hatchability from the different hosts bears no relation to either prevalence or the mean daily egg output. Cows and goats have the highest rates (over 70%) and dogs and rats are the lowest (less than 20%).

Humans therefore constitute the major source of infection. In the light of these findings, the sanitary disposal of human faeces assumes greater importance and it becomes imperative to keep the cow and the dog population in check in the endemic areas. Other animal hosts seem to play a comparatively minor role in the transmission of *Schistosoma japonicum*.

Clinical Gradient of the Disease

The term "clinical gradient", here is used to express the morbid process from its simplest manifestation to its severest form.

In a study of a random sample of 278 schistosome-infected individuals examined in Palo from 1954 only 37.8% were symptomatic as shown below:



In the study of the clinical gradient of the disease, it was also noted that although the prevalence of infection reaches its peak on the age-group 20 – 24 years, manifest disease and it severe forms dominate the picture during childhood. This is especially true of the age-group 10 – 14, in which period 73% of the infected children manifest symptoms referrable to schistosomiasis and among them the majority of cases with moderate to severe symptoms are found. The clinical picture changes thereafter and manifest disease and its severity decline considerably there being no significant differences in either respect among the different age-groups above 14 years. A similar epidemiological feature was observed by Fujinami (1916) among the rice farmers in the endemic areas of Japan. Vogel and Minning (1953) report that they made a similar observation in Chekiang Province, China, in 1934 and that Tang and co-workers did likewise in 1950 – 1951 in the Chinese Province of Fukien.

Results of the studies on the clinical gradient of the disease, therefore, will indicate that schistosomiasis provided another instance of a successful parasitism which allows a large proportion of individuals to live in a state of natural balance with the parasite without their normal life being too profoundly disturbed. A growing tolerance to the infection appears to suppress clinical manifestations, and acute attacks became infrequent once the individuals get past the most vulnerable age (10 – 14 years). Infection among adults therefore is not synonymous with disease. This can only be explained on the basis of immunity developing as a result of constant exposure to schistosome infection from the early years of life up to the point where a further quantum of infection is prevented. The nature of this phenomenon however, needs precise definition in terms of immunology.

Observations on the Natural History and Public Health Significance of Schistosomiasis

Schistosomiasis or bilharziasis is one of the most important public health problems of the tropics and subtropics. Conservative estimates place the number of infected individuals at 150 million of which half a million are in the Philippines. (1969 – 1970). It is a chronic insidious disease. Unless the initial exposure is unusually severe, symptoms of the disease emerge slowly. The intensity of infection is extremely variable. In most endemic areas, cases are diagnosed only incidentally when the individual seeks medical attention for some other complaint or through mass surveys. As a consequence, many cases go unrecognized and the disease is rarely established as a cause of death. Because of its chronic nature, schistosomiasis saps the energy of the individual, reduces his resistance, renders him prone to attack by other infections and lowers his productivity. These deleterious effects are exceedingly difficult to evaluate but in the aggregate they represent a social and economic burden of great magnitude.

Reliable data on mortality being difficult to obtain and meaningless in areas where deaths occur without medical attendance, the possibility of using this as a measure of the public health significance of schistosomiasis has to be ruled out. Severity of the disease was therefore, used as the main criterion irrespective of the stage or speed of the disease process. As loss of working capacity is an important element in the severity of the disease, an estimate of the man-days lost per year and the economic loss sustained by the community can be made. To sort out the effect of helminthic and other intestinal infections which could cause some of the symptoms included in the classification of the disease, a paralleled study in a representative sample among schistosomiasis free individuals in a check area has been undertaken, this area having a more or less equal prevalence of helminthic infections. By this the effect of conditions other than schistosomiasis could be eliminated by measuring the morbidity in these two areas in respect to the symptomatology. The findings from this study are as follows:

1) On the average, an infected individual in an endemic area suffers an incapacity equivalent to 5.4 days of total loss of gainful employment attributable to the disease.

2) The disability rate for the 6 month period in the check area was equivalent to 0.74 days of complete loss of capacity to work per person per year.

3) Based on the difference of 4.7 days loss of working capacity per year and assuming that one-half of the 500,000 infected individuals are breadwinners, the amount lost annually in wages can then be calculated. To this must be added the cost of treatment to obtain total loss due to disease and disability resulting from schistosomiasis infection.

4) To be able to calculate total loss from schistosomiasis, estimates of human mortality from schistosomiasis, as well as that of livestock should also be made. A case fatality rate of 1.78% was obtained from a randomly selected 278 infected individuals followed up for 12 years.

5) As of 1975, it has been calculated that the total loss due to death, disease and disability amounted to one hundred million pesos annually.

THE MOLLUSCAN HOST

Description

The anatomy of *Oncomelania quadrasi* (Möllendorff) are based on the studies of the general appearance and micro-dissection of entire soft parts of the animal.

1) The External Anatomy

1) *Shell* - The shell of the adult snail is from 3 to 5 mm in length although many individuals reach more than 5 mm particularly the females. The whorls number from 6 to 7, however, mature snails in the field often show only 5 because the nuclear whorl have been eroded. Except for the fine axial lines of growth, the shell is rather thin and smooth (see Figure 3, the anatomy of *O. quadrasi*) and has a translucent chestnut brown to purely black color.

2) *Operculum* - The operculum is very thin and translucent especially at the peripheral side. The general shape is ovately conic with a much sharper apex.

3) *Head Region* - The body of the animal is divided into two parts namely: the head region and the visceral mass. All the organs behind the head which includes the gills, stomach, heart, kidney, digestive gland ("liver") and the reproductive system constitute the visceral mass. The head is bilobed anteriorly and is almost cylindrical in shape. The snout or the rostrum is the blunt and extended anterior part. The buccal mass can easily be perceive through the transparent dorsal part of the head region due to its reddish tint in live specimens. A pair of non-contracting protruded organs, the tentacles, can be seen extending from the dorso-lateral sides of the head. It is not uncommon to find individuals with branching tentacles. Immediately behind the slightly swollen base of each tentacle are the eyes which are charcoal black in appearance. Arched above each eye is a streak of yellowish "eyebrow" which is made up of a compact mass of bright yellow granules. This appears to be the most prominent color feature of the snail.

4) *Foot* - The mascular foot is simple. It appears to have no fold or division but in relaxed and preserved specimens portions of the foot seems to be very much folded.

5) *Mantle* - The thin mantle covers almost the entire soft parts of the snail with

the exception of the head. It has a thickened anterior portion, the mantle collar, which is located immediately behind the head region. On the lateral side not very far from the collar (left side of the head), the mantle becomes folded forming the gill filaments. The mantle is attached to the shell by way of the columellar muscle.

2. The Internal Anatomy

1) *The Digestive System* - The alimentary tract of the animal is composed of the following parts: (a) The *mouth* is a median vertical slit on the blunt and thickened anterior end of the proboscis or snout. (b) The *buccopharyngeal cavity* (often referred to as the pharynx) is a tubular and somewhat laterally compressed structure directly posterior to the mouth. It is about 300μ in relaxed state and is lined by a layer of columnar epithelium cells.

The two bean-shaped structures located on each ventro-lateral sides of the junction between the pharynx and the esophagus are the (c) *lingual organs* or the buccal mass. Each organ measures about 295μ in length and 105μ in width.

The (d) *radula* is located between the posterior region of the bucco-pharynx and the esophagus. It is an elongated ribbon-like organ about 850μ long and is made up of seven rows of teeth attached to a tough transparent membrane whose anterior third lies at the floor of the bucco-pharyngeal cavity. The remaining part of the ribbon is contained within a tube that coils downward to the buccal mass. The lumen of this tube is continuous with that of the bucco-pharyngeal cavity and the esophagus.

The teeth of the snail are classified into rachidian, lateral, inner marginal, and outer marginal. The teeth are arranged in rows along the entire length of the lingual ribbon. The rachidian teeth comprise the middle row and on both sides are the laterals and marginals arranged in transverse but symmetrical order.

Along the dorsal surface of the esophagus are a pair of elongated club-shaped organs, the (e) *salivary glands.* The ducts of these glands empty into the dorso-posterior part of the bucco-pharyngeal cavity just above the point of attachment of the lingual ribbon.

A folded tubular structure, the (f) *esophagus,* runs mesio-dorsally from behind the buccal mass and follow the coils of the columellar muscle. Then it inserts itself to the posterior part of the stomach near the junction between the second and third whorl of the snail.

The (g) *stomach* is located in the posterior portion of the second coil of the

shell. It is an irregularly shaped sac which immediately follow the esophagus. On the anterior part of the structure where the intestine begins to leave, a slightly projected structure is found, the style sac. This sac is lined by a layer of ciliated cells which causes the rotation of the style. The head of the style rotates against the gastric shield, the cuticular and much folded layer of the stomach, to aid in the rotation and digestion of food particles. All the undigested material find their way out into the instestine, where they are transformed into egg-shaped fecal pellets.

The (h) *intestine* is a much coiled tubular organ extending from the left anteroventral surface of the stomach to the anal opening located at the right side of the mantle cavity just behind the thickened mantle collar. In live animals, the organ is often filled with egg-shaped fecal pellets, which are arranged in rows.

The last three whorls of the snail is generally occupied by a brownish-orange organ, the (i) *digestive gland* or "liver". This is loated directly behind the stomach, to which it is joined by a very short duct.

2) *The Reproductive System* - The components of the *female reporductive system* are the following: The (a) ovary is a lobulated bright yellow organ which lie alongside the mid-ventral surface of the "liver". Its anterior portion follow the initial turn of the digestive gland while the hind part is located near the base of the second coil. The whole organ is covered with a thin transparent layer of squamous epithelial tissue.

Starting from the base of the ovary, the (b) *oviduct* is almost straight and then it runs diagonally across the ventral surface of the stomach and parallel to the posterior portion of the esophagus. Further it follows the coil of the olumellar muscle down the body whorl, and after a much convoluted region, it joins a tiny sac-like body, the bursa copulatrix.

The (c) *Bursa copulatrix* is a very small sac-like and silky structure embedded in the thick connective tissue in the anterior portion of the seminal receptacle. As the name implies, this sac-like structure is the place where fertilization takes place.

The *seminal receptacle* (d) is a bean-shaped brownish organ located on the dorso-posterior region of the last coil of the columellar muscle. In gross dissection, this body can be observed filled with spermatozoa without any definite orientation. A duct which originates from the seminal receptacle empties into the oviduct just anterior to the attachment of the bursa copulatrix. This same tube before it reaches the ovidust, it joined by another duct, the spermathecal duct.

The (e) *spermathecal duct* is a very small tubular canal that starts from the genital opening of the female snail and then joins the duct of the seminal receptacle. It runs parallel to and side by side throughout the entire length of the accessory gland, and is attached directly by a connective tissue to the right side of the mantle below the accessory gland.

A very large structure which may be observed running parallel to and intimately in contact with the right side of the intestine, is the *accessory gland* (f). It is opaque and whitish in appearance. The accessory gland appears to have two main functions: the production of albumen and the shell for the eggs. At the anterior end of the accessory gland is a cup-like and bilobed structure, the genital opening. It has a dual function, that is, passage of the egg from the oviduct to the outside and then as a point of insertion for the penis during copulation. The spermathecal duct joins the duct of the accessory gland anteroventrally at a point not very far from the genital opening.

The male reporductive system consist of the following parts: (a) *testis* is an organ which is very much similar to the ovary with respect to color, shape and relative position to the digestive gland. However, it is smaller and has numerous ovalshaped lobes.

The lobes of the testis possess tubules which empty into a common duct, the (b) *vas deferens*. This whitish organ which is about 17μ in diameter has a very much coiled posterior portion. It originates from the base of the testis and then crosses the ventral surface of the stomach. After this the tube becomes almost straight, as it pass along the dorsal side of the distal part of the esophagus, and finally it turns dorsad and perpendicular to the columellar muscle. The vas deferens connects midventrally to the prostate gland.

The (c) *prostate gland* is a whitish mass located on the postero-dorsal surface of the second coil of the columellar muscle to which it is attached by connective tissue ventrally. Its position is the same as the location of the accessory gland of the female animal, but it does not resemble the above gland either morphologically or histologically. The size and shape of the prostate gland is variable. During period of intense sexual activity, sexually mature males have exceptionally large prostates, which indicates that this is connected with the mating habits of the snail.

After the vas deferens connect with the prostate gland, it emerges from the same side (a little anteriorly) of the above organ and proceed towards the posterior portion of the head cavity to the (d) *penis* (or verge). The penis is an elongated broad structure measuring about 2 mm long and 175μ wide.

This organ originates from the columellar muscle and the width of its base is attached to the dexto-lateral portion of the anterior coil of the muscle. It tapers anteriorward over head. When retracted, its anterior half is coiled sinistrally into the mantle cavity. A papilla is present at its anterior tip.

3) *The Nervous System* - The nervous system of the snail is composed of paired and prominent ganglia together with their nerves and commissures. The largest and most prominent pair of nerve centers are the *cerebral ganglia.* These are located on each side of the esophagus, somewhat dorsal to the anterior end. These ganglia are more or less flat, forming a rooflike "canopy" over the anterior esophageal region. They are joined together by a small band of commissures mesially. Several nerves arise from these pairs of ganglia and several ganglia are connected to it by commissures.

The Sense Organs - Below each pleuro-pedal commissure are the otocysts whose function is probably hearing and balancing. These are spherical bodies which are connected to the pedal ganglia by thin connective tissues and minute nerves. They come into close contact with the muscles that run beneath the esophagus and those that retract the buccal mass. Each otocyst is made up of thin circular wall of squamous epithelial cells enclosed within which is round yellowish structure, the otolith.

The eyes are located at the slightly swollen base of the tentacles. A nerve that originates from the lateral surface of each cerebral ganglion supplies the eye.

The tentacles are slender extensions from each of the dorso-lateral sides of the head. Their walls are made up of stratified, layer of small columnar epithelium continuous with that of the head.

At the left side of the gills inside the mantle cavity are folds which run parallel to the lateral sides of the posterior portion of the head region. These are the components of the osphradium. This organ, like gills, is inward out-growth of the mantle. Its function is primarily for testing the water that passes through the gills.

4) *The Muscular System* - The muscular system of the snail is composed of the following major muscles: The columellar muscle is the largest muscle in the body of the animal and it give rise to others in the head. This muscle serves to retract the operculum, foot and head.

The median longitudinal muscle arises from the mid-dorsal surface of the columellar muscle inside the head cavity. It is broad and flat, and extends forward giving rise to three pairs of branches:

The first pair, the buccal muscles retract the buccal mass or lingual organs.

The radular muscle is small and ribbon-like and functions to retract the radula.

The third pair that arises from the medical longitudinal muscles are the proboscis muscles. They serve to retract the proboscis.

The columellar muscle sends off a branch to the foot after giving rise to a branch going to the operculum. It also send out branches to the sides of the head. These muscles are almost inconspicuous and form part of the wall of the head and give rise in turn to smaller muscles that retract the tentacles.

The foot is made up of a network of transverse, oblique, circular and longitudinal muscle fibers interlaced in all directions forming the floor of the foot musculature and are responsible for its movement.

5) *The Circulatory System* - The circulatory system is composed of a tubular heart and is immediately posterior to the gills and anterior to the kidney. It consists of two chambers, an auricle and a ventricle. The auricle gives rise to a blood vessel which divides into two anteriors. The main branch goes to the gills and the other empties into the postmantellar cavity and the lacunae which surround the entire length of the intestine. The blood vessel that goes to the gills break up into smaller tributaries which supply each gill filament.

6) *The Respiratory System* - This system is made up of a set of gills or branchiae arising as folds from the mantle. They occupy the left side of the anterior part of the mantle parallel to the distal half of the rectum, extending from near the edge of the mantle posteriorly to near the heart. There a set of gills with from 33 to 38 filaments is located. A lacuna exists in each filament forming the space between the two layers of cells and through which blood passes. A typical gill fold is almost triangular in shape and possesses a short afferent blood sinus and a long slender efferent one. The lower half of each filament is ciliated.

7) *The Excretory System* - The excretory organ is composed of a mass of finger-like structures called the kidney, located on the antero-dorsal surface of the proximal portion of the intestine in close contact with the stomach. This organ is separated from the heart by the posterior wall of the latter. The kidney has no duct and thus it may be surmised that the only means of eliminating waste products of metabolism is through the sinuses or body cavities.

Fig. 3-A The External Anatomy of *Oncomelania quadrasi* (Möllendorff)

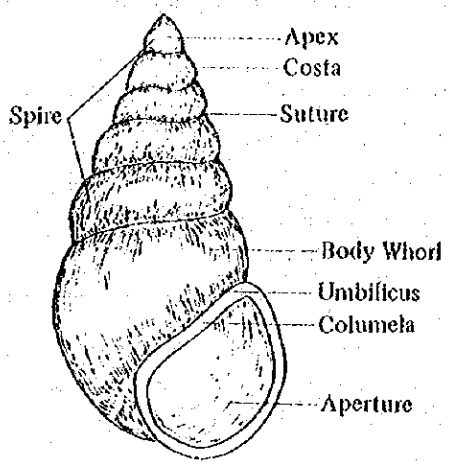


Fig. 3-B The Internal Anatomy of *Oncomelania quadrasi* (Möllendorff)

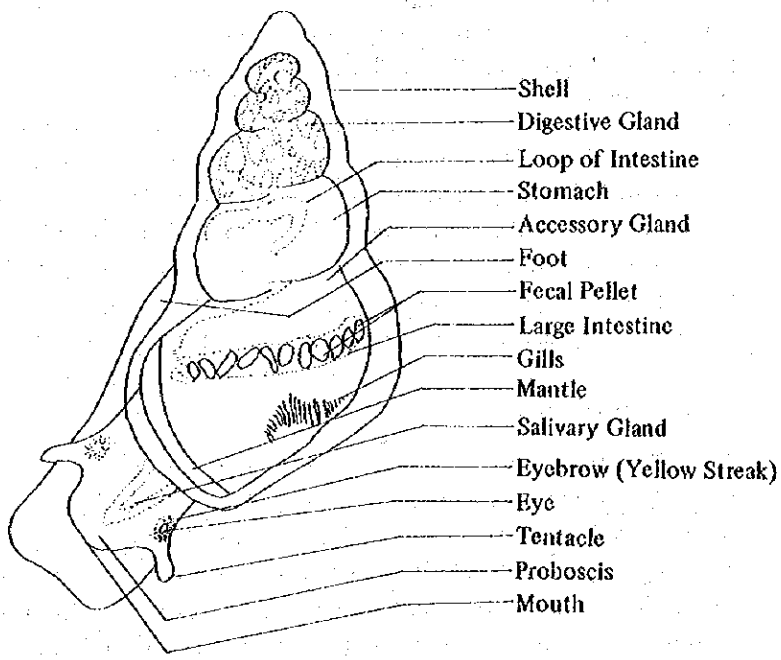
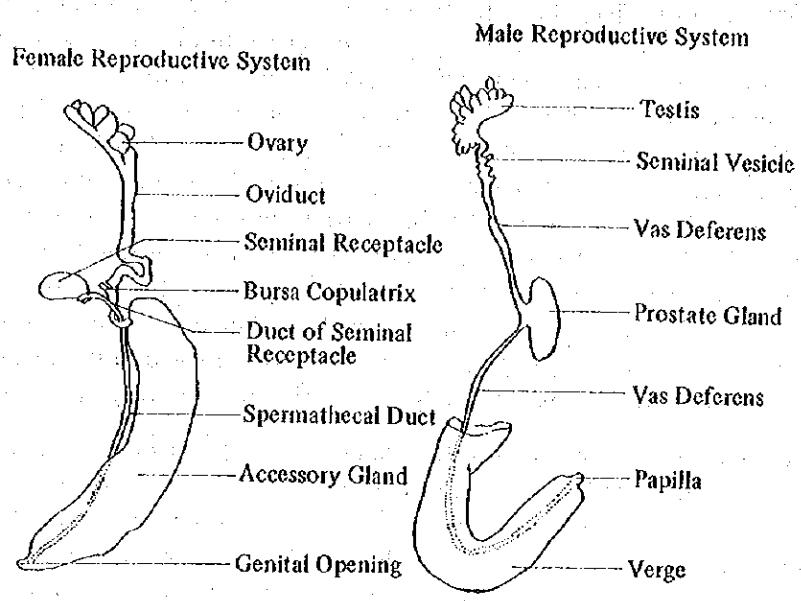


Fig. 3-C Reproductive System of *Oncomelania quadrasi* (Möllendorff)



General Geographical Distribution

Molluscan intermediate host of *S. japonicum* are as follows:

Oncomelania quadrasi	- Philippines
O. nosophora	- Japan
O. hupensis	- Mainland China
O. formosana	- China (Taiwan)
O. lindoensis	- Indonesia (Sulawesi)

Oncomelania quadrasi is apparently confined to the Philippine Islands. It was first described by Mellendorf (see Bartsch, 1963) on specimens from Surigao Province in Mindanao, and first recognized as the intermediate host of *Schistosoma japonicum* by Tubangui on the basis of observations made at Palo, Leyte in 1932. The subsequent history of information concerning its range is completely bound up with that of the parasite. With certain minor exceptions, its known distribution is that described by Pesigan (1953). The snail known to occur over of Mindanao and Samar Islands, eastern Leyte, and in relatively small areas in extreme southeastern Mindoro. It has also been found on the minute island of Bani off the northwest coast of Samar, and on Surigao Island, northeast of

Mindanao. The only probable range extensions will be along the coast of Mindanao, including the adjacent islands of Dinagat and Bucas Grande. None of these areas has been surveyed. The reasons why other areas are not suspected are two. The first is the correlation between the distribution of the snail and the annual rainfall pattern; Tubangui & Pasco (1941) and Pesigan (1948, 1953) have pointed out that the snail is confined to areas that get not annual dry season. Second, the areas with this rainfall pattern, aside from those listed, are so located that foci endemic for schistosomiasis would probably have come to the attention of health authorities by now; or else they have been surveyed thoroughly for the presence of the disease. The most interesting confirmation of the theory that the pattern of rainfall limits the distribution of *O. quadrasi* is the case of the north coast of Mindanao, where a large area, including almost the entire province of Misamis Oriental, has a marked dry season, and is conspicuously lacking in *O. quadrasi*, although the snail is found adjacent to this area in every direction.

Within the endemic islands, the general distribution of the snail is related to local topography. Although most often found near sea level, the snail is known to occur at elevations up to nearly 900 m.in Bukidnon Province in Mindanao. At whatever elevation, the outstanding characteristic of snail-inhabited regions is their flatness. This features makes for retention of water, a point of obvious importance to an animal with an aquatic stage in its life-history.

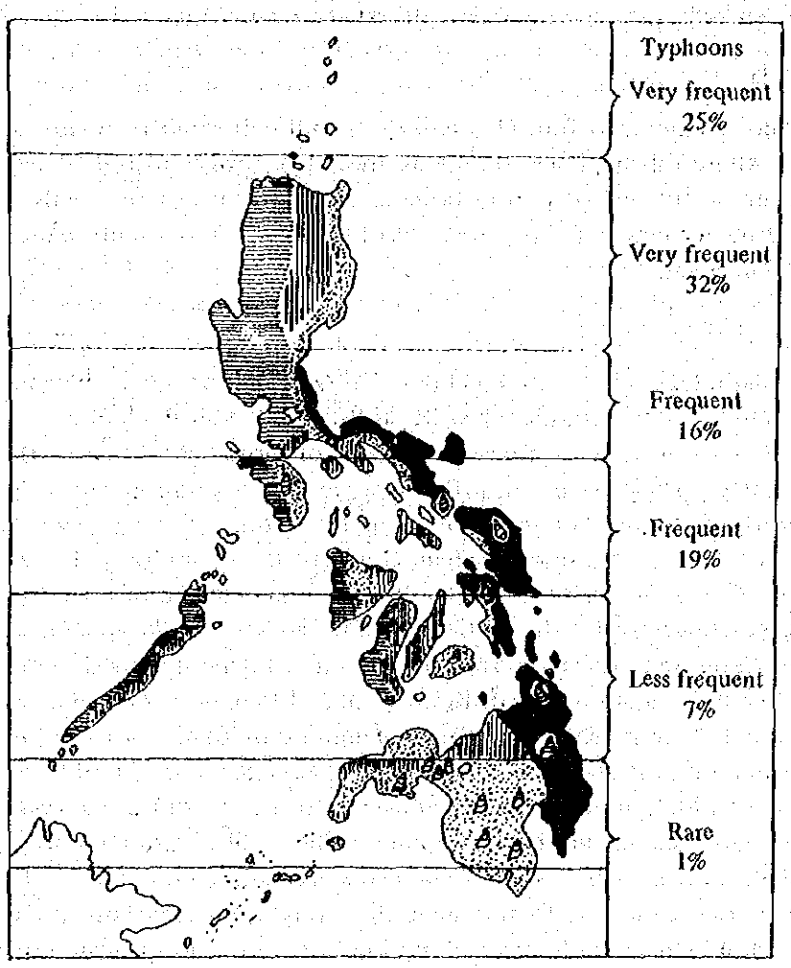
Habitats of *Oncomelania quadrasi*

It is the wet places, of course, that actually harbour snails. Those places are of many different types, but they can be grouped into a small number a general categories, as follows:

a) Flood-plain forests and swamps

These apparently represent the most extensive original habitat of the snail. Because the land is desirable for growing lowland rice, these forests are gradually destroyed, and it is only under the pioneering conditions in certain parts of Mindanao that we can find the habitat in an undisturbed condition. The largest snail habitat yet discovered covers the entire Manat River swamp in Davao Province. It is approximately 20 km long by at least 2 – 3 km wide. The Manat River at this point is contained within banks, and the forest can be entered readily. Snails were found on the forest floor in places that seemed scarcely lower than the general ground level, and that contained now water at the time that the place was observed. Elsewhere, as at a trail-crossing near the center of the swamp the river fingers out and has no defined channel, covering a large part of the forest with water. Snails were found all along this trail from the point of its entrance into the swamp onwards for least 2 km.

Fig. 4 Map of the Philippines Showing Geographical Distribution of Schistosomiasis in Relation to Climate



Types of climate:

First type		Two pronounced seasons, dry in winter (November to February) and Spring (March to May), wet in summer and autumn (June to October).
Second type		No dry season; with a very pronounced maximum rainfall in winter.
Third type		No very pronounced maximum rain period; with a short dry season lasting only from one to three months.
Fourth type		No very pronounced maximum rain period and no dry season.
		Declared Endemic Areas

The sample description could be repeated with minor variations for many other places in Mindanao. As mentioned above, the land is desirable for rice farming, and all such habitats seen under agricultural encroachment.

b) Ricefields

It is not common to find *O. quadrasi* in well cultivated ricefields, and in the localities where this happens, the fields are either newly cut out of swamps, or else they are abandoned or poorly farmed. The presence of the snails, therefore can only be accounted for on the basis of the primitive agriculture practised in some endemic areas.

c) Streams and creeks

Throughout all the areas inhabited by *O. quadrasi*, occur meandering, sluggish streams, densely populated with snails. The typical picture seen is a stream bed 3 – 15 m wide, at a level one meter or more below that of the surrounding country. The stream bed is flat, clogged with vegetation, and very soft. In spite of the vegetation, there is usually a moderate flow of water. The amount of low and the absence of a defined channel constitute a paradox. The explanation seems to lie at least partly in the habits of the people and of their domestic animals. Several species of fish, particularly the mudfish (*Ophicephalus* sp.) inhabit these streams and are widely sought for food. The method used in catching them is to dike off or dam a part of the stream, and then to wade in and capture the fish by hand. The repeated temporary damming up of the streams prevents the water from making anything like a permanent channel. In addition, the streams are very widely used as a wallowing sites for the water buffalo (carabao), the principal work animal in the Philippines. The wallows are temporary, and many different sites may be used by the same animal. This wallowing has the same effect as the fishing; it forces the water away from any defined channel, softens the whole stream bed, and making the flow more sluggish promotes the growth of semi-aquatic grasses and other plants, which in turn impede the water still further.

d) Small Swamps

These areas are much more like the streams in appearance than they are like the large flood-plain swamps. The principal differences from the streams are their small size and the lesser flow of water through them. Like the streams, they are fished and used as carabao wallows. These small swamps, which we have called "pockets" are always located at the foot of rather high and steep banks, and the source of their water are seepages and springs emerging below the banks.

In many places, but particularly in the Sorsogon area, spring outlets may support snails without forming a well-defined swampy area. Often the banks

around such a spring, though rather steep, and even sandy, are continuously wet from seeping water. Such places may be very small. Omagom spring in Irosin, Sorsogon, forms an *Oncomelania* habitat whose maximum dimensions are 10 x 2 m. It is particularly these small swamps that have given such vivid impression of discontinuity in snails distribution that the term "colonies" have been given to individual snail-inhabited area.

e) Road ditches and borrow-pits

In the construction of roads in lowland areas, it is often necessary to do extensive filling in order to raise the road bed above ordinary high-water levels. For economic reasons the sources of the fill-dirt cannot be far removed, and the most common practice seems to take it from the immediately adjacent land. The result of this is that a rather large ditch is left on either side of the road. These ditches remain wet because of their low level, and in many places provide good habitats for *O. quadrasi.*

Discussion: Not every snail colony can be fitted exactly into the categories described above, as many of them are really combinations of types. The classification intended mainly as an aid in description.

One interesting feature of the snail colonies is their permanence. Not only have all those that have been followed up constantly since early 1953 continued to maintain themselves, but there is good evidence of much longer continuity. The swamp behind MacArthur (formerly Tarragona, Leyte) is discussed by Sullivan & Ferguson (1946) as a snail habitat, and there is every reason to suppose that the snails found by Tubangui in 1932 came from either Binog or Gacao streams - both flourishing colonies at present. The indications are that the factors making for suitability do not ordinarily change over a period up to some tens of years. The example of the road ditches, however, is enough to warn us that it is possible to alter places so that snails can breed in them.

A consideration of all habitats of *O. quadrasi* reveals some striking differences. The extremes of conditions would appear to be the gravel-bottomed springs and seeps in Sorsogon, the moist and not water-logged forest floor in Davao, and the Cogon-Anahaway ricefield in Leyte. These areas are so different that any features common to all of them would appear more or less to define the requirements of the snail.

One characteristic appears to be that they never dry out for any length of time. Drying is prevented by constant seepage of underground water in Sorsogon, by the dense forest cover in Davao, and by the flat topography and clay subsoil in the ricefield. Therefore, the right amount of water proper to the slope and soil would appear to be the most important requirement for *O. quadrasi.*

As seen above, any further disturbance of the ricefiled through more intensive cultivation would probably eliminate snails there, just as removal of the forest cover would permit the ground to dry enough to make that place unsuitable. It can be concluded, that a relatively undisturbed situation is necessary for snails to thrive. These two features would thus appear to make the major differences between the places suitable and unsuitable for snails, and the obvious implication is that control measures should be designed with them in mind.

Physical and Chemical Components

The analysis of snail habitats presented above, while quite satisfactory in general, becomes inadequate when detailed surveys are made within a single area. The reason for this is that there are many places that look suitable for *Oncomelania*, but do not harbour them. Such places apparently have the necessary moisture, topography and vegetation. Some of these negative areas can apparently be explained by the fact that the water is stagnant and foul. This is particularly evident in the case of the large swamp behind the town of MacArthur, Leyte. In some parts, the water has a moderate flow, and such places support snails, whereas other parts of the swamp, where there is a foul smell to the habitat, have none.

Even after discarding the stagnant areas, however, there still remains a residue of apprarently favourable places that have no snails, and in a few cases, these are actually connected with snail colonies. It was this observation of apparently suitable but negative habitats that led to the hope that a detailed chemical analysis of such places would provide a clue to some easily controllable requirement of the snail.

However, as shown in Table 2, it was conclusively demonstrated that soil chemistry has nothing to do with the distribution of *Oncomelania* in the Palo area.

Table 2. Soil and Water Analysis in *Oncomelania quadrasi* Habitats and in Similar Places without *O. quadrasi**

Chemical	34 Snail Colonies		8 Areas without Snails	
	Range of values (p.p.m.)	Average (p.p.m.)	Range of values (p.p.m.)	Average (p.p.m.)
NO_3	0.8–20	5.9	0.8–4.0	1.65
P	12.5–100	90.8	75–100	93.3
K	45–185	90.4	50–1,110	49.37
Ca	250–5,000	1,286.8	50–1,000	781.25
NH_3	0.5–2.5	0.85	0.5–2.5	0.75
Mg	0.5–5.0	3.5	2.5–25	6.25
M_n	0.5–5.0	3.5	0.5–12.5	3.8
Al	0.5–50	23	12.5–25	23.4
NO_2	1–5	1.5	1–1	1
Fe	0.5–25	15	0.5–25	94.4
So_4	50–250	85.2	50–250	81.25
Cl	25–100	51.0	25–100	43.75
pH (soil)	4.6–7.2	6.6	5.4–7.2	6.35
pH**(water)	6.2–7.8	7.2	6.2–7.8	7.4

* Soil analysis performed by LaMotte colorimeter method; water pH values determined with Beckman electronic pH meter.

** 50 habitats, 35 negative areas.

From the ecological and evolutionary standpoint, these results are not surprising. A species, in order to maintain itself, must be adapted to its whole environment, and it is the components of the environment, interacting in innumerable different ways, that determine the survival of the species. Ecological results have indicated that the biological part of the environment is ordinarily more important than the physico-chemical part. To select an example from the field of public health, it was once thought that *Anopheles atroparvus*, a malaria vector in Europe, could only breed in brackish water, an obvious restriction due to chemicals. It was later found that this was not true, and that the apparent restriction was due to the fact that other species of *Anopheles*, better adapted to fresh water than *A. atroparvus*, prevented it from entering fresh-water habitats through competition (Hackett, 1937). Although there are no other species of *Oncomelania* in the Philippines, it might be of value to investigate the local gastropod fauna and the association of the various species with *O. quadrasi*.

Distribution of Snails within the Habitat

Like practically all other species of animals, *O. quadrasi* is distributed

neither evenly nor randomly over its habitats. This fact increases the complexity of sampling and statistical analysis.

Behaviour and Activity of *O. quadrasi*

Field Studies:

Experiments carried out showed clearly that snails are active at all times of the day and night. However, both the amount and the kind of activity do change during a 24-hour period. All forms of activity, but especially copulation, are greater at night. Rate of horizontal movement changes least among the activities measured. Mid-afternoon is the time of least activity.

The Influence of Physical Factors on Movement

It is clear that snails respond to weather changes, but it is not possible from field observation to be sure of the relative importance of the four factors of humidity, temperature, light and atmospheric pressure. The first three change regularly over the 24 hours, and any or all of them could therefore cause the observed changes in snail activity. A decrease in humidity resulted in reduced movement. Increase in temperature and light, increase movement, and it is interesting to note that the effect was more pronounced for females than for males.

The reaction of the snails to light is clearly one of avoidance. It was shown that snails invariably crawl from the strong light source placed at the side of the container. During the day, the progress of the sun across the sky exposes many snails to its direct rays, and under these conditions they can be seen moving rapidly into the nearest shade. The effect would probably be sufficient to produce considerable movement during the day as shade positions change. There would be some reinforcement of this effect by temperature increases, although these are probably not as great on the surface of the mud as those observed in air. During the day, climbing would be inhibited because of the lower humidity above the mud and also because of light from the sky. At night, increasing humidity would encourage movement, especially climbing and copulation, because of the increased exposure of soft parts to air. Thus, the effect of temperature and light during the day seems almost to balance the effect of humidity a night so far as horizontal movement is concerned.

The Daily Cycle of Egg-laying

Studies show that nearly three times as many eggs were laid at night as during the day. Inasmuch as most eggs are laid above the water, the advantages

of egg-laying at night are obvious. The lower temperature, higher humidity and absence of sunlight would all contribute to the protection of the female snail during the process of egg-laying.

The Daily Cycle of Feeding

Returning to a general consideration of 24-hour activities, those that relate to reproduction (copulation and egg-laying) are more pronounced at night, whereas feeding is more pronounced during the day. *Oncomelania* appear to show preference for food rich in cellulose. They feed on the algae and decaying organic matter which occur on moist soil above the water level, and on the micro-organisms found on living and dead vegetation in shallow water. Decaying organic matters, mainly of vegetable in origin, assist in the formation of a suitable substratum for the snails and provides additional food material.

Life History of *O. quadrasi*

Growth:

McMullen (1947) was the first investigator to make observation on the rate of growth of *O. quadrasi.* He reported on the basis of field observations that snails grew approximately 0.25 mm per week. He made bi-weekly collections and by measuring all snails collected was able to follow apparent broods over several weeks by comparing the modes of the successive size-distribution curves.

In general, the SCRP findings confirm McMullen's over the part of the growth curve that he observed. After the snails reach maturity, they grow at slower and slower rates, requiring an estimated two weeks to grow 0.1 mm after reaching an age of 6 months, at which time males are 4.5 mm long and females 5.0 mm. Above these sizes, no reliable data was obtained because only a small proportion (less than 10%) of the collectable snails reach these sizes. Very small snails are also too rare in field collections for their rate of growth to be estimated by this technique.

From the available data, growth curves was drawn and a table was made that represent the best estimate of the average state of affairs. It was found out that there is little if any difference between growth rates of the two sexes up to about 35 days at which time the snails are slightly larger than 2.0 mm. After that age, males grow less rapidly than females.

Maturation and Reproduction:

Egg-laying probably begins in nature at a size somewhat smaller than the observed 3.7 mm but probably not much before the snails are 3.5 mm long.

From growth figures, this would be at an age of 90 – 94 days. The observations that almost none of the copulating males was less than 3.0 mm long would tend to that roughly the estimated age of maturity of male snails is 78 days.

In a number of observations, both in the laboratory and the field, snails were seen to copulate with an average of once a day. The duration of copulation varied from 25 minutes to more than two hours, with an average between 60 and 70 minutes.

Egg-laying:

Oncomelania quadrasi prefers to lay its eggs at or above water level on a solid substrate like decaying coconut husk. The eggs of *O. quadrasi* were first discovered by Abbott (1946), but apparently no serious attempts were made to estimate the rate of egg-laying quantitatively until B.W. Halstead and E.D. Wagner in 1954 made observations on them under laboratory conditions in California. They reported a maximum rates of 0.34 eggs per female per day. Repeated experiments in the laboratory in Leyte have essentially confirmed their observations.

In round numbers, the figure of two eggs every five days is a good estimate of a female snail's output.

Survival of newly hatched *O. quadrasi:*

As has long been recognized, the newly hatched snails are aquatic and remain so for sometime. Two weeks in the duration of the aquatic stage.

Both laboratory experiments showed a survival of about 21% at 70 days after hatching.

Population Dynamics

Observations on snails density:

The density of snails were found to change independently in different colonies. It is clear then that the mean density of *O. quadrasi* is a function of place, and is much less dependent upon changes in weather, which would affect all colonies alike.

Population structure:

Population structure, like density, is characteristic of a place, and that month to month changes in population structure may be quite large.

Reproduction rates and survival in the field:

Males reach maturity at a smaller size and grow slowly than females. With

the average daily mortality rate of 0.76% the average female *O. quadrasi* lives for only 47.6 days. This is the obvious explanation for the unbalanced sex ratio consistently found in field collections. This has averaged 1.4 females per male for the 10 colonies in Palo over the 18 months of observation.

It comes as something of a surprise to find such short average lives in the field, since it is rather easy to keep snails much longer in the laboratory (Ward, Travis & Rue, 1947). Laboratory snails, however, do escape a number of hazards to which field snails are subject, such being carried away by rising water, predation, and local changes of the habitat by large animals such as wallowing carabaos.

Potential repopulation of snail colonies:

Studies have shown that continuous presence of males is not necessary, as females, once they are laying fertile eggs continue to do so for three or more months, or longer than the average adult life. Therefore, continuous or repeated contact between the sexes is not necessary.

It has been estimated that the population would recover its original density from a 75% killing in 90 days, from 85% killing in 105 days, and from 95% in 175 days.

The studies carried out indicate that prevention of brooding is more important than success in killing the snails that are present. The potential for repopulation can only be attacked through radical alteration of the habitat, making further breeding impossible or reducing it to the point, where it does not keep pace with normal mortality rates. Under these circumstances the population of snails would disappear, even though the initial effect of the control measure might appear slight. Moreover, once the original changes in the habitat have been made, maintenance costs should be slight compared to the cost of repeated application of molluscicide. If the land thus changed could then be put into agricultural production, the repeated disturbance of farming might well hasten the disappearance of snails and further reduce the cost of maintenance.

Laboratory Procedure for Infecting Snails

It was shown that cercariae can be obtained from about 44% of snails penetrated by one miracidium and 75% of snails penetrated by five miracidia.

It may be worthwhile to mention here that field data show more infected female snails than males. The general infection rate obtained for males was 4.04%, while that for females was 5.16%. Statistical analysis of these figures showed that this difference in infection rates was not due to chance.

This difference must be due to either physiological or ecological factors

and the following possible explanations can be considered:

a) Males might be more resistant to penetration by the miracidia than females;

b) Miracidia do not develop to cercariae as well in males as in females;

c) Miracidia are more attracted to females than to males;

d) Males may have different habits from females, i.e., the males may be out of the water more of the time and hence not exposed so frequently as females.

Since it has been shown that the miracidium has no clear preference for either sex, and since studies so far show no great dissimilarity of habits between male and female snails, it is held that the other two possibilities are true. Further confirmation to these assumptions may only be made by a careful study of prepared slides of infected snails.

Snail-Parasite Relationship

Effect of parasite on snail reproduction:

So far, the pathology of the infected snail has not been thoroughly explained. Some have claimed that the sporocysts develop in the region of the liver and through pressure and irritation cause liver damage which eventually leads to the death of the snail. Others contend, however, that it is in the gonads that the sporocysts develop and consequently affect the reproduction of the snail.

When the data from all 10 colonies in Malirong were summarizes for the 18 months they were studied, the proportion of young was found to be inversely related to the infection rates, i.e., the highly infected colonies tended to show lesser proportions of young snails than did the colonies with lower infection rates.

However, when the infection rates were compared to the proportion of young individual colonies by dates, the relation was not striking, especially where the infection rates were less than 10%, as is frequently observed in nature. In colonies where great variations in infection rates occurred, however, the proportion of young was found to be affected.

Effect on Growth:

Like reproduction, the growth rate is reduced among infected snails. They show retarded growth, and the effect was more severe among the younger snails. The curves followed by the infected snails were similar in both sexes.

Effect on longevity:

Of a group that was followed for 20 weeks, mortality rates among the in-

fected snails exposed to one miracidium each was noted to be high. A survival rate of 16.6% was observed as compared with 59.5% in the unexposed controls during that span of time.

Cercarial Shedding:

Cercariae are shed in considerable numbers during the first 10 – 20 days of shedding and that the peak of production is reached 20 – 40 days after they begin to be liberated. After this time, shedding becomes more or less intermittent and fewer cercariae are liberated each time. It seems then that after this initial shedding, some time is required before an appreciable number can mature and accumulate in the snail. In view of this, it would seem that prevention of the release of cercariae for several days would be an appropriate method of obtaining large number.

It was shown that the efficiency of shedding increase with the number of days of previous drying. Of the physical factors that change over a 24-hour period, light is most likely to stimulate shedding, inspite of various statements in the literature that it was not effective (Bauman, Bennet & Ingall, 1948). The periodicity of shedding, which was not altered by the time of placing the snails in water, suggested that it was perhaps the duration of exposure to light that brought about the release of cercariae.

There can be no doubt that drying and exposure to light both stimulate shedding. However, four days' drying causes a high mortality rate among the snails. In cases where the survival of the snails is important, the use of light alone may produce sufficient number of cercariae for laboratory experiments.

Daily cercarial output:

The singly infected snails shed twice as many (15.07) cercariae per shedding days as those with multiple infections (8.44). The pattern of shedding is influenced by the number of miracidia used, but the total number of cercariae is not, because opposing factors cancel each other.

Presence of Cercariae Downstream from Snail

During the military campaign on Leyte Island in 1945, about 1,700 members of the United States ground forces contracted schistosomiasis and nearly half of these belonged to the engineering battalion. Sullivan and Ferguson (1946) have presented good evidence that many of them were exposed during bridge-construction work at places considerably removed from snail colonies. Since their survey of the area disclosed that the snails were located in the tributaries that join the river upstream, it would imply that they got the disease through either (a) the cercariae that travelled or were carried downstream, or (b) the cercariae that were

released from the infected snails washed down from the snail colonies.

From these observations, no fresh water downstream from any snail colony can ever be considered safe.

PRÉVENTION AND CONTROL

Engineering, Ecological and Other Snail Control Measures

The WHO consultant team on schistosomiasis in the Philippines expressed the opinion in 1952 that[a])

"theoretically the most effective method of attack on a trematode disease is to control the snail intermediate hosts. Some effective molluscicides are known but they are not economically practical, unless combined with drainage, agricultural methods, etc. In Japan almost complete land utilization usually confines the snails to small, neat irrigation ditches between ricefields. In the Philippines the snails are still in wet, swampy places covered by dense vegetation, in former ricefields left to fallow during the drier season, etc. They often cover large areas. The situation in Japan approaches the terrain believed to be ideal for the use of molluscicides. The opposite is true in the Philippines and molluscicides will be of little or no value until environmental control has been developed".

This point of view is widely accepted here, especially as complete land utilization in Leyte and other endemic areas is still far off, and chemical control is regarded as something of a terminal measure which should be applied in eradicating or further reducing the snail populations that remain after there have been major reductions in areas suitable for snail breeding.

It was the hope at the start of the project at Palo, Leyte that some weak point could be found in the life-history of the snail that would permit a relatively inexpensive attack to be made upon it. However, no such well-defined target has appeared, but there are certain facts about the biology of *Oncomelania quadrasi* that are important in considering possible methods to be tried with a view to its eradication.

The first of these in the permanence of known snail colonies with no spontaneous disappearance. The major significance of this observation is that not all freshwater areas need to be considered. For whatever reasons, areas not now inhabited by snail are not likely to become dangerous, unless subjected to human interference, as in the case of road construction or irrigation canals that hamper natural drainage, thus creating what may be regarded as "man-made schistosomiasis".

The second observation of possible importance is the absence of major fluctuations in density within *Oncomelania* colonies. It follows that the snail population might not be able to withstand the continual pressure of permanent-

a) See Bull Wld. Hlth. Org., 1958, 18, 481-544

ly changed conditions, or even the pressure of repeated disturbance. The desirable changes have been indicated by the distribution of snails within the habitat, their life history, and their behaviour under field and experimental conditions. One important example is the fact that newly hatched snails are aquatic. Water, then, must be present more or less permanently for snails to thrive, but the marked decrease in density with increasing depth shows that too much water may be as damaging as none at all. It is also clear that both completely stagnant water and very swift water are unfavorable for snails. The reduced activity during the day and the avoidance of bright light indicates the necessary of cover, which in nature is usually provided by vegetation.

Even given these promising leads, there remains the overriding question of cost. The control of water and of vegetation is expensive whichever attempted, and the expense is greatly multiplied in the tropics (rainfall is hardly ever less than 250 cm per year in the endemic areas, and may be more than twice that amount). For this reason, measures that would result in other benefits than those of mere snail control were taken into account. Land reclamation and improved land use have therefore, been important considerations in controlling or eradicating the snail host.

a) Vegetation Removal

The effectiveness of clearing is quite substantial in terms of snail population reduction (34%). The principal difficulty with this control method is the frequency necessary to be repeated.

b) Drainage

1. Stream Channelization

Marshy margins, seepages and small tributaries of streams in many regions provide important breeding-sites for host snail. Streams with these characteristics are always costly and difficult to treat with molluscicides. The vegetation-checked margins and the seepages along the banks tend to keep the chemical introduced upstream from panetrating many snail-infested areas. Unless they are treated separately, each tributary is a potential pocket from which surviving snails may emerge to repopulate the treated portions. Stream channelization does not only improve the flow characteristics, but also facilitate effective application of molluscicides. After channelization, any remaining snail colonies can usually be eliminated by mollusciciding.

Although stream regulation is a specialized field of civil engineering, the problems encountered in the channelization of streams as a snail control measure are not so particularly serious. It is, of course, desirable that engineers entrusted

with stream channelization should have adeqaute training, experience, facilities for field investigations for topographic, hydrographic and geological surveys.

For snail control work, stream regulation generally involves improvement of grade and stabilization, channel contraction and bank protection works. The stream bed should form a continuous gradient without depressions. At no point along its length should it be possible for water to accumulate so that snails can develop in residual pools if the stream dries out. Limited dredging may be carried out by manual labor or by use of heavy equipment including bulldozers and dragline excavators.

The light trenching machines for use in relatively soft ground would facilitate the drainage work. One model only 3 feet wide, 3 feet high and 7 feet long, with a builldozing blade 3 feet by 18 inches which weighs less than one ton, has tracks that spread the weight to 518 lb/sq. foot and can be easily transported on a trailer, can dig a trench 12 inches wide by 30 inches deep excavating over 100 feet per hour.

It should be pointed out that drainage and reclamation of extensive mashy areas are highly specialized and relatively expensive operations, requiring the use of a wide range of excavating and earth-moving equipment. For these reasons, large-scale drainage schemes ordinarily are impractical as a disease-control measure alone, and must be a part of a land reclamation and utilization programme to be economically feasible. Fortunately, the long-term value of such reclaimed land is often far greater than the cost of the drainage works.

2. Seepage Control

Seepage along the margins of streams or foothills may give rise to boggy areas, pools or even to swamps and marshes in which the intermediate hosts find favourable conditions for breeding. It is therefore important to eliminate seepages wherever possible.

Where a stream is flanked by low-lying, level ground, a considerable area of this may be affected by seepage. In such cases it is necessary to lower the water table on either side of the stream by the construction of drainage ditches or by installation of a sub-surface drainage system such as French drains or perforated pipes laid in trenches filled with broken bricks or stones.

3. Diversion and Intercepting Channels

Location, design and construction of diversion works and intercepting channels play an important part in the efficient control of snails. This is especially important in snail areas that could hardly be drained due to the water coming from an upland area.

4. Canal Linings

The relative absence of aquatic vegetation, and the relatively high velocities which are possible in lined canals make them less attractive as snail habitats than earth canals. Moreover, lining reduces seepage to low-lying areas which may otherwise become potential breeding-sites. Finally, the application of molluscicides, where advisable, is more effective and less costly in lined canals than in earth canals. However, it is first necessary to establish the cost against the practical benefits to be derived from such a step.

In the design and construction of hard-surface linings such as concrete and bitumen, the preparation of the sub-grade is most essential in order to provide sufficient support for the lining and to avoid cracks caused by settlement. The principal types of canal linings are Portland cement concrete, bituminous concrete, bituminous membrane, plastic membrane, asbestos cement lining and clay lining. While the various types of linings have their particular fields of application for snail control the most suitable is concrete.

5. Drainage and Irrigation Schemes

Lack of drainage in irrigation schemes results in the formation of stagnant pools, wet areas, and seepages which are often good snail habitats. It is therefore important that in irrigation schemes in endemic areas adequate drainage be provided if snail control is to be successful. In addition to facilitating snail control work, adequate drainage has important beneficial effects on the productivity of an irrigation scheme. There are instances of irrigation schemes that have failed to achieve the expected productivity because of lack of adequate drainage. In some places waterlogging have become so serious within only a few years of the introduction of irrigation that valuable lands have been rendered useless. The reclamation of such lands may be feasible, but at considerable cost. It is therefore essential that in planning irrigation schemes provision for adequate drainage at the outset, even though under certain conditions the implementation of this part of the development may be carried out in stages as the need arises, should be made. For example, drainage for the removal of surface run-off from rains and from excess irrigation should be provided at the outset, whereas drainage required for conditions brought about as a result of several years of irrigation practice may be provided later.

The design of drainage schemes is far more difficult than the design of irrigation schemes. There are such factors as rainfall intensities and frequencies, the quantity of excess irrigation water, the length of time crops can be subjected to flooding without affecting their yield, the soil properties that control the rate of movement of water, and the nature of the ground surface as it affects the movement of water overland. It must be pointed out, however, that even the approximate determination of the above factors requires a great deal of investiga-

tion and long periods of observation, and often the engineer has to fall back on his experience and judgement. Nevertheless, steady progress is being made in the various aspects of drainage design, and it is now possible to attempt such design with some confidence.

c) Combined Vegetation Removal and Drainage

After the vegetation had been cleared once, it has been shown that combined clearing and drainage further reduced snail density by 96% of the original numbers.

The question arises here whether the cost of clearing was justified in view of the fact that the subsequent drainage might have accomplished the purpose alone. From the stand-point of snail control, the clearing may have been superfluous but for reclamation and for the land to become productive, clearing would still be necessary.

d) Earth Filling

Certain snail colonies were so located that it appeared feasible to eradicate them by covering them with earth. There can be no doubt that the method is completely effective, and will require no maintenance. Unfortunately, the method is of somewhat limited application because it requires the presence of a nearby source of earth, and such situations are not often encountered in the endemic areas.

e) Flooding and Ponding

Snail densities declined in the flooded areas showing that excess water may be as harmful to the snails as lack of water. The effect of excess water suggested that digging a series of ponds might make an area uninhabitable for snails because of the excessive depth of water. The vertical banks used in the drainage experiment, and those observed in the construction of fishponds, appeared to be an added hazard to the snails.

Data obtained showed that the act of ponding reduced the number of snails but some remained after the work was finished. However, it was gratifying to observe that a continual decline in snail densities occurred to the point where none could be found eight months after the construction of the pond. This indicated that the 100% water-habitat (with a depth of 2 m) is totally unsuitable, since the snails that remained were unable to repopulate the place.

The difficulty with this method lies in the requirement of vertical banks, which can be made permanent only if clay or some similar cohesive material is present. If the pond banks are of sandy nature, maintenance costs will be much higher.

Ponding, although expensive, has a definite value to the people because the ponds can be utilized for fish culture.

f) Improved Rice Culture

The system of rice culture in most of the endemic areas involves rudimentary preparation of the land by either a single shallow ploughing or by trampling the weeds into the mud by teams of carabaos, close and irregular planting of seedlings and no weeding at all. The system seemed obviously open to improvement, and when improved showed their effectiveness in reducing or eliminating snails.

g) Chemical Control

Experience with molluscicides, especially since 1945, has shown that they can effectively control the snail intermediate hosts in many types of habitat. Successful snail control by means of molluscicides alone has been shown to be possible in certain areas in Japan. In the Philippines this is primarily used after instituting ecological methods such as clearing of vegetations and drainage of waterlogged areas. With limited funds, it will be vest to apply molluscicides as a terminal measures when the snails are already confined to the drainage and irrigation canals and resistant pockets. So far, the two best chemicals for snail control are Sodium pentachlorophenate (NaPCP) and Bayluscide (Bayer 73).

h) Biological Control

This method for snail control has long had a wide appeal. It has been suggested that snails might be controlled by predators such as ducks, fish, insect larvae and leeches; by competitors such as the snail Marisa cornuarietis, which in Puerto Rico has rendered some habitats unsuitable for the schistosome vector *Biomphalaria*; or by parasites and diseases of snails, which might keep down their numbers.

With the possible exception of Marisa introduction in Puerto Rico, trials of such methods in general, however, have not been encouraging and at present there is no practical technique for the application of biological control against the snail hosts of schistosomes in endemic areas situated on large land masses.

Discussion:

Experience has shown that snail habitats are so varied that no single line of attack can be devised that will appear to all places harbouring snails. Removal of vegetation and the digging of fishponds are obviously unsuitable for a ricefield or a swamp covering several square kilometers, and drainage is impossible in streams; earth-filling demands sufficient earth near by; and scientific agriculture requires that the area be suitable for rice-growing. Clearly each snail colony

demands individual study before a decision can be made as to the best approach for snail control.

From the relative effectiveness of the various methods for snail control, it is clear that the degree of success in related to the amount of charge brought about in the habitat. The more radical the change, the greater the reduction in snail density. It also appears that after the more redical changes, less maintenance is required. The lesson to be learned here is that half-way measures are likely to be ineffective, and that compromising to reduce the initial cost will prove to be false economy. None of the methods are really new to public health engineering - most of them have proved their worth in malaria control. The cost of such measures is high, so that efforts should be exerted at simultaneous improvement in land use, so as to provide a broader basis for costing and also to make the method more attractive to the individual landowner.

Environmental Sanitation

The importance of sanitation in rural areas has been stressed frequently as a potential method of control not only of schistosomiasis but also of intestinal pathogens of all kinds. After perfect sanitation has been achieved as far as human faces is concerned reduction on transmission by about 75% can be achieved. The other 25% will be contributed by lower mammals. Further effect of a good water supply and the building of footbridges would be obvious on the prevalence of the disease.

It has been argued in the past that proper disposal of human faeces would not play an important role in the control of *S. japonicum*, owing to the fact that the infection is one in which several domestic animals act as transmitters of the disease to man. On the one hand, the importance of the animal reservoirs is heightened by the fact that no strain differences of the parasite have been established, but of the other hand their relative role is small compared to that of man. This would obviously point to the importance of sanitary disposal of human excreta in the control of transmission. It is however, believed that the need for a wider programme of rural sanitation as part of a bread-based rural development project aimed at raising general economy, education and living standards is necessary and until this is done and the use of latrines becomes a habit of life, the role of latrines will continue to be of doubtful value.

In the transmission of all human schistosomes, the universal epidemiological factor of the affinity between children and any body of water must also be considered. The juvenile section of the population has the highest prevalence rates, in the least cooperative in any programme for the disposal of excreta, and is the major source of the infection in the snails. Unless safe washing, bathing and

recreation facilities can be provided to entice children away from snail habitats, conditions will persist which more or less ensure the continuance of transmission.

Case Finding and Mass Treatment

Treatment

It is generally recognized that there is a need for carrying out critical and comparative assessment of various anti-schistosome drugs. This is very necessary in view of the vital role of treatment in the control of schistosomiasis and to observe thus far of an effective and non-toxic drug against schistosomiasis suitable for mass treatment. With the increasing need for a truly adequate and non-toxic drug against schistosomiasis, a number of antimonial as well as non-antimonial preparations have came out in the past few years. Among the preparations tried are the following:

Antimonial Preparations

1. Stibophen – recent trials using 1 cc per 10 kg body weight with maximum of 5 cc showed that a course of 15 injections given every other day except the first 3 doses which is given daily in increasing amounts (1/3, 2/3 and full dose) gave a stool negative conversion rate of 48.6% and an egg count reduction rate of 96.2% six months after treatment. Reactions commonly observed are nausea, vomiting, body weakness and anorexia. Deaths due to the drug were encountered.

2. Astiban – This is sodium antimoney-a, a'-dimercaptosuccinate. Giving of the drug at 10 mg/kg body weight for 4 days gave stool negative conversion rate of 69% on the 6th month. After treatment, severe reactions were however encountered even with a lower dosage.

3. Sodium Antimony dimethylcysteine tartrate (NaP) – Results are encouraging which is similar or better than Astiban. However, cardiac ischemia were observed even with a low dosage of 3 mg/kg body weight given for 5 days.

Non-Antimonial Preparations

1. Dehydroemetine – Preliminary trials using 2 to 2.5 mg per kg per day for 20 days shows some activity in the form of egg suppression. This, however, similar to antimonial preparation showed cardiac-toxic reactions.

2. Hycanthone – A dose of 3 mg per kg body weight per day repeated twice at intervals of 2 weeks showed a poor effect on schistosome egg output. Trials were discontinued due to its side effects.

3. Pararosaniline Pamoate – This was administered orally at 35 to 40 mg per

kg per day for two 14 day treatment periods separated by a 7 day rest period in between. The reactions were less but the long course of treatment and relatively bigger size of the capsules vitiated its beneficial effects.

4. Niridazole – This drug given at 20 to 25 mg/kg body weight for 10 to 14 days produced encouraging results but with side reactions in the form of nausea and vomiting. Temporary hallucinations were observed in some of the patients treated. Reducing the dose to 15 mg/kg/day for 24 days gave a stool negative conversion rate of 79.5%. This dose is presently being recommended though it is still far from satisfactory. A 10-day suppressive treatment is suggested should the patient be not able to continue with the 24 day treatment course. With this treatment regiven, egg output were suppressed from 68.8 to 93.5% for a duration of 6 months after treatment. Stool negative conversion rate was 28.6 one month after treatment, becoming zero 6 months after. Ten-day treatment repeated at monthly interval is still in progress.

Health Education

It should not be overlooked that the successful application of any control measures requires informed cooperation from the human population. The sympathies of the people should be enlisted from the outset by a suitable campaign to make them aware of the nature of the problem, and of the reasons which underlie the action being taken. It may be mentioned here that while very modern method (cine-film, especially of the cartoon type, distribution of pamphlets and posters, lectures, radio and television broadcasts, demonstration) should be employed, experience has shown that informal talks to small group by sympathetic individuals trained in this aspect of health educations which provide the opportunity for question and answer, are most effective in the long run.

TECHNICAL AIDS AND PROCEDURES

Definitions and General Considerations

1. An area is endemic if:

   1) Snails (*O. quadrasi*) with *S. japonicum* infection are found.

   2) Indigenous cases are present.

2. A case is an individual who is passing *Schistosoma* ova or positive for circum-oval precipitin test (COPT).

3. Infected individuals react differently to the infection and may or may not suffer from the disease so that this infection may be classified under any of the following groups:

   1) Asymptomatic cases – infected with schistosomiasis but do not manifest the disease. The only abnormality is a positive stool or a positive COPT.

   2) Symptomatic cases – those who have evidence of both the infection and the disease.

      Classification –

      a) Mild –
      ocassional abdominal pains, occassional diarrhea and dysentery, no absence from work.

      b) Moderate –
      includes (1) above with anemia (Hb less than 10 gm per 100 ml using Haden-Jausser hemoglobinometer), or weakness, inability to do hard work.

      c) Severe –
      includes (2) above with recurring attacks of diarrhea and dysentery. Frequent absence from work.

      d) Very severe –
      includes (3) above with ascitis and/or emaciation. Total absence form work.

      The following classification described by Hackett (1944) was employed to determine the degree of splenic enlargement:

Class of spleen	Description
0	Normal spleen, not palpable even on deep inspiration
1	Spleen palpable only on deep or at least more than normal inspiration
2	Spleen palpable on normal breathing but not projected below a horizontal line half-way between the costal margin and the umbilicus, measures along a line dropped vertically from the left nipple.
3	Spleen with lowest palpable point projected more than half-way to the umbilicus but not below a line drawn horizontally through it.
4	Spleen with lowest palpable point below the umbilical level but not projected more than half-way towards a horizontal line through the symphysis pubis
5	Spleen with lowest palpable point below the lower limit of class 4

Diagnostic Procedures

Detection of cases can be done through stool examination of the population, through the use of circumoval precipitin test or skin test:

1) Stool examination technics include:

a. Direct fecal smear (DFS) examination:

Procedure –

i. Examine grossly for mucus, blood, parasites, etc.

ii. Make 2 cover glass preparations on one slide by comminuting 1 to 2 gms of feces in 1 to 2 drops of normal salt solution and examine under a low and high power objectives of the microscope.

Normal Saline Solution:

1. Sodium chloride – 8.50 grams
2. Distilled water to make 1,000 cc

b. Glycerine sedimentation & egg hatching technic:

Procedure –

i. Measure 5 cc of feces by displacement in 15 cc of tap water in a conical sedimentation flask and comminute with glass stirring rod.

ii. Add 0.5% solution of glycerine in tap water strring at the same time. Allow sediment to settle for 15 – 20 minutes. Pour supernatant fluid.

iii. Repeat procedure three to four times or until supernatant fluid is clear.

iv. Strain sediment in 4 layers of ordinary surgical gauze preliminary soaked in tap water over a funnel.

v. Examine sediment under the microscope.

vi. Wash sediment into Erlenmeyer flask full of water and examine it for free swimming miracidia after 2 or more hours.

c. Merthiolate-Iodine-Formaldehyde Concentration (MIFC) technic:

Procedure –

i. Stock of 'MF' solution:
250 cc distilled water
250 cc tincture No. 99 merthiolate (1 : 1,000) Lilly
25 cc solution of formaldehyde USP
5 cc glycerine

ii. Lugol's solution (5%):
Iodine – 5.0 grams
Potassium iodine – 10 grams
Water – 100 cc

Procedure –

i. Pour 9,4 cc of 'MF' stock solution into test tube containing 0.6 cc of Lugol's solution.

ii. Place 1 cc of feces by displacement into the tube and stir thoroughly with an applicator stick; place a stopper, then shake, vigorously for 5 seconds.

iii. Strain through 2 layers of ordinary surgical gauze in a 15 ml centrifuge tube then add 4 cc of refrigerated ether, insert again a stopper and shake vigorously. (If ether remains on top after shaking, add 1 cc of tap water and reshake). Remove stopper and let it stand for one minute.

iv. Centrifuge for 1 minute at 1,600 rpm. Four layers will appear: 1) Ether on top; 2) Fecal layer; 3) MIF layer; and 4) Sediment which contains ova.

v. Loosen fecal plug with applicator stick.

vi. Pour off quickly and carefully all except the bottom layer containing the sediment.

vii. Examine sediment under the microscope.

Stool examination can be done by any one of the technics enumerated above. It should be added that often a single stool examination will not demonstrate an infection so that in highly suspected cases several examinations are recommneded.

2) Circumoval Precipitin Test (COPT)

When stool examination fail to show eggs and schistosomiasis is strongly suspected; COPT can be resorted to. This serologic test is both sensitive and specific and the filter paper method can be employed for epidemiological surveys. This is carried out as follows:

a. Cut area of filter paper absorbing blood into 6 pieces.

b. Soak in PBS (0.2 ml) for 60 minutes at room temperature.

c. Remove filter paper afer 1 hour soaking.

d. Place about 200 mature lyophilized eggs on a glass slide.

e. Place 1 drop of extracted solution with capillary pipette on lyophilized eggs. (Plasma is used in place of extracted solution.)

f. Rim coverglass with vaseline and place over mixture.

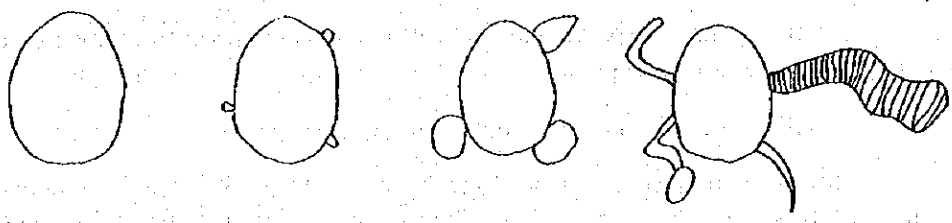
g. Incubate at 37°C for 24 – 72 hours in a moist chamber.

h. Examine reactions under the microscope.

Interpretation of Results:

Precipitates may form as early as within 24 hours or late as 72 hours. Every 24 hours of incubation, the reaction is examined microscopically under low power. Proper illumination is important in recognizing precipitate with a lighter color than the egg content and occurred only in mature egg. When no precipitate is seen, the preparation is again incubated for another 24 hours. Reaction is declared negative only if no precipitate is observed after 72 hours.

The degree of precipitation may be classified into bleb-like and segmented projection from the egg shell. The bleb may be small (SB), medium (MB) or large (LB). Likewise, the segmented precipitate may be small (SS), medium (MS) or large (LS) as illustrated below:



Type 0 | Type I (doubtful) | Type II | Type III

Fig. 5 Classification according to Yokogawa *et al.*, 1967

Yogore *et al.* (1968) designates as positive COPT only when segmented precipitate is seen in at least one egg, ignoring as negative those with only bleblike precipitates. For our purpose, medium and large blebs are also considered positive COPT reactions and the small blebs are ignored as doubtful. The small bleb is difficult to recognize by untrained technicians.

Ordinarily, the result of the COPT is recorded as either negative (0) or positive (+). However, if the average degree of precipitation and percentage of eggs with precipitate are desired for research purposes, the following sample data are entered and computed based on 50 mature eggs counted/observed:

	Type of Reaction			%	Index
0	I	II	III		
30	5	9	6	40	27.3

Positive rate = Sum of the number of eggs with precipitate under Type I, II, & III multiplied by 2.

= $(6 + 9 + 5) \times 2 = 40\%$

100 eggs were supposed to be examined

50 eggs were actually counted

$$\text{Index} = \frac{\Sigma ni}{N} \times \frac{100}{3}$$

where i = degree of precipitation from 0-Type III

ni = frequency of eggs with i type of precipitation

Computation:

Type III: $6 \times 3 = 18$

Type II: $9 \times 2 = 18$

Type I: $5 \times 1 = 5$

Type 0: $30 \times 0 = 0$

$50 \quad 41$

$$\text{Index} = \frac{\Sigma ni}{N} \times \frac{100}{3}$$

$$= \frac{41}{50} \times \frac{100}{3}$$

$$= \frac{82}{3}$$

$$= 27.3$$

Quantitative Thick Smear Method (as adapted by Katz & Col., 1972):

Material required:

a) Glass slides for microscopy.

b) Cover slip of wettable cellophane paper (24 x 30) of average thickness (40 – 50 micra), previously immersed, for 24 hours, in a solution containing 100 ml glycerin, 100 ml water and 1 ml aqueous solution of green malachite at 3%. Before using the cover slip of paper, let the excess imbibing solution flow away.

c) Wire net (IBRAS-Sao Bernardo do Campo-No. 120-threads-warp and woof.: 0.09 mm)

d) Rectangular card (3 x 4 x 1.37 cm) with a central hole 6 mm in diameter.

e) Toothpick with one rectangular extremity.

f) Absorbable paper.

Technique:

a) Place the feces sample on absorvable paper.

b) Press the upper part of the sample with the wire net.

c) Withdraw the feces that passed through the net and convey them to the central hole of the card which must be lying over the glass slide.

d) After filling up the central hole, carefuly withdraw the card, thus leaving the feces on the glass slide.

e) Cover the feces with the appropriate cover slip and press the slide, after

having inverted it, against a sheet of absorbing paper.

f) Examine under microscope, 1 – 2 hours later.

g) The number of eggs present in the fecal sample multiplied by 23, will give the number of eggs per gram of feces.

3) Skin Test

The skin test is very sensitive but not adequately specific and its indicated use is a principally as a screening procedure. A positive reaction should be considered only as an indication for carrying out other diagnostic procedures.

Procedures --

a) Inject intradermally 0.03 to 0.05 cc (Ave. 0.04) of 1 : 10,000 antigen (adult whole worm extract) on the volar surface of the forearm.

b) Inject intradermally on equal amount of N.S.S. on the same forearm a few inches below.

c) Make a reading after 15 – 20 minutes and note area of wheal.

d) An increase by 5 sq. mm in area or more means a positive skin reaction.

Snail Sampling Methods

The purpose of sampling is to determine the characteristic of a universe or population group without examining all the individuals. One of the characteristics is density and to be correctly estimated, areas of differing density must be represented proportionally among the samples. Hence, the wider the distribution of the samples in the area, the more correct average figures are obtained.

1) Ring Method

This method involves the use of a brass ring cut in 2 inches thickness from a metal tube or shell 13.5 cms in diameter. A starting point is marked on the ground in the small habitat close to the water line (in case of a habitat with water) and without previous inspection the metal ring is dropped and all the snails inside it are collected with the aid of a pair of forceps. The snails are placed in small paper envelope which are identified with an appropriate numbering system. The samples are spaced 5 meters.

A minimum of 30 samples are collected before the average density is computed. In cases where a long series of negative samples are obtained, the limits of the snail habitats are presumed to have been crossed and the sampling is terminated. The ring number with last snail is taken as the last sample and

subsequent samplings to be done in the future should stop at that point, unless there is an evidence of complete transfer of the snail colony due to flushing out effects of floods, etc., in which case a new sampling route is planned.

In case of snail habitats where in the water at the time of initial sampling is absent, a properly marked place in the moist bank where the snails are found may be designated as starting point of the sampling and there on may proceed along the line left by the maximum water level.

The snails are then transferred to the laboratory and sorted as to size, counted, crushed and examined, as to sex and presence of infection under a low power magnification. The results are then entered in the appropriate forms.

The most important thing to guard against in this method of sampling is the temptation to ensure the inclusion of snails as the ring is dropped on the ground or to collect the snails seen in the immediate vicinity but outside the ring.

The latter is likely to be true especially in areas where no snails were collected in the "ring". If objectivity is maintained throughout the sampling and the subsequent ones afterwards, then a sufficiently correct estimate of the snail population is obtained and becomes valid for purposes of evaluation and comparison later.

2) Tube Sampling

In snail habitats that are under water, the ring sampling is not practical and so the tube sampling is used. The sampler has the same diameter as the ring used in the ring sampling. The tube is driven into the place where snail population is to be determined until it is about 4 – 6 inches deep into the soil and with the help of a shovel the whole soil sample is transferred to a pail. This soil sample is passed through a series of sieves and the snails are recovered and counted.

3) Attractants such as filter paper (20 x 20 cm) can be used to have an estimate of the snail distribution and to measure the snail density with a limited reliability. The method can be used efficiently in waters where tube or ring samplers are of no use (Tanaka *et al.*, 1975).

It may be stated here that the tube sampling is the only method yielding appreciable numbers of dead snails. Against this advantage must be balanced the extra time involved in the tube method. This is preferable where depth of water of dense grasses make ring collecting or filter paper method impossible, and where the age structure of the population is of primary importance.

4) Simple methods for determination of the necessary sample size and transformation of the survey data for comparative population study of unevenly distributed animals.

Determination of the population size is necessary in the control of intermediate host or vector animals and the effect of control activity is often determined by comparing the significant difference of population densities at two or more different time intervals or among different conditions by means of *t*-test or analysis of variance. However, most animals are distributed unevenly in the field and thus make the statistical analysis difficult.

Even though statistical methodology has been developed and many theoretical methods were proposed, these methods have not been utilized in the practice of control activities because of the complexity of the mathematical manipulations which were not understood by most staff members. These methods, therefore, were of limited practical application in control activities.

Recently, the distribution of *Oncomelania quadrasi*, the intermediate host snail of *Schistosoma japonicum*, was extensively studied for the type of distribution, determination of necessary sample size and methods of transformation based upon the statistical theories on a large number of sampling data.

From the analyses, the simplest method following the theoretical rules was selected so that anyone can calculate the necessary sample size for collection and compare the population densities by the simplest method of transformation in a short time using a handy calculator. For the calculation of the necessary sample size, no calculating machine is necessary and for that of transformation, a simple calculator with a function key of logarithm is sufficient.

The following methods to process the survey data established from the population study of snails can be applicable to the survey of mosquito larvae or other animals which are distributed unevenly. The application of these methods is expected to be utilized widely in the routine survey of animals and in the evaluation of control of animals as well as other ecological studies (Makiya *et al.* 1979).

Theoretical Background

The step by step procedure of the method is described in this chapter. The data used are only for illustrative purpose.

a) Determiation of sample size

The minimal necessary sample size (q) was determined by the following formula using the dispersion index (Id) by Morisita (1962);

$$q = \frac{t^2}{E^2}\left(Id - 1 + \frac{1}{\bar{x}}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where t is the Student's t value, $\bar{x}$ arithmetic mean of snail count per sample and E the relative error calculated by $E = t.SE/\bar{x}$, where SE is the standard error of x.

The dispersion index Id is defined as in the following formula:

$$Id = n \frac{\Sigma x (x-1)}{T(T-1)} \quad \ldots\ldots\ldots\ldots (2)$$

where n is the number of samples, x snail count per sample and T the total number of snails collected.

For usual field surveys, 30% of the relative error ($E = 0.3$) and $t = 1$ are permissible. Then formula (1) becomes much simpler with the additional arrangement for the convenience of the calculator manipulation

$$q = (\frac{1}{\bar{x}} + Id - 1) \times 11.11 \quad \ldots\ldots\ldots (3)$$

where q is the minimal necessary sample size.

b) Method of transformation

For the comparative study of the different population densities, the normalization of the data following any appropriate formula is necessary. There have been many methods of transformation proposed so far. Since most of the formulas were derived from mathematical theories and needed parameters which governed the uneven distribution, they were rarely used by practical ecologists because of their complexity. The case of the transformation by different formulas was examined in a large number of survey data of the snails. The formulas examined were the following 9 and it was concluded that the transformation by $log\ (x + 0.01)$ was simple and efficient, often more efficient than the theoretically derived complicated formulas.

$y = \log (x + 1)$	(Williams, 1937)
$y = k^{-1/2} \sin h^{-1}\sqrt{kx}$	(Beall, 1942)
$y = k^{-1/2} \sin h^{-1}\sqrt{k (x + 1/2)}$	(Bartlett, 1947)
$y = \log (x + k/2)$	(Anscombe, 1949)
$y = \sin h^{-1}\sqrt{(x + 0.375) (k - 0.75)}$	(Anscombe, 1949)
$y = x^{1-c/2}$	(Taylor, 1961)
$y = \sin h^{-1}\sqrt{x (b-1)/(a+1)}$	(Iwao and Kuno, 1968)

The following two formulas were added to evaluate the many negative (0 count) samples in this study.

$y = \log (x + 0.1)$
$y = \log (x + 0.01)$

From the results of our study, the use of transformation by $log\ (x + 0.01)$ is recommended. Moreover, the formula is nonparametric, simple to understand, and can be calculated by anyone irrespective of the degree of uneven distribution of animals.

Practical Application

The method of calculation is shown for determination of the minimal necessary samples and for the comparative study of densities in two surveyed populations by *t*-test after transformation.

a) The minimal necessary sample size

At first, a survey area is determined. Then 30 samples are taken preliminarily at random or systematically in a given area. Examples of collection in the populations I and II are shown in Fig. 1 (In these cases, the systematic samping was made in a grid way).

At the next step, the dispersion index *Id* is calculated according to the procedures shown in Table 1.

Fig. 1 Example of sampling in 2 populations

POPULATION I						POPULATION II					
0	1	0	0	0	4	0	0	0	0	0	0
0	2	4	0	1	11	0	0	0	0	0	0
16	19	1	1	5	16	10	8	0	0	0	0
46	5	0	7	18	18	73	19	5	0	0	0
10	7	36	6	1	15	57	36	28	8	6	0

Table 1 Calculation of necessary values for obtaining the dispersion index

Population I			Population II		
snail/sample x	$x-1$	$x(x-1)$	snail/sample x	$x-1$	$x(x-1)$
0	-1	0	0	-1	0
0	-1	0	0	-1	0
0	-1	0	0	-1	0
0	-1	0	0	-1	0
0	-1	0	0	-1	0
0	-1	0	0	-1	0
0	-1	0	0	-1	0
1	0	0	0	-1	0
1	0	0	0	-1	0
1	0	0	0	-1	0
1	0	0	0	-1	0
1	0	0	0	-1	0
2	1	2	0	-1	0
4	3	12	0	-1	0
4	3	12	0	-1	0
5	4	20	0	-1	0
5	4	20	0	-1	0
6	5	30	0	-1	0
7	6	42	0	-1	0
7	6	42	0	-1	0
10	9	90	5	4	20
11	10	110	6	5	30
15	14	210	8	7	56
16	15	240	8	7	56
16	15	240	10	9	90
18	17	306	19	18	342
18	17	306	28	27	756
19	18	342	36	35	1,260
36	35	1,260	57	56	3,192
46	45	2,070	73	72	5,256
250		5,354	250		11,058
No. of samples n =		30			30
Total No. T =		250			250
Mean $\bar{x} = T/n$ =		8.333			8.333
$\Sigma x(x-1)$ =		5,354			11,058

In the table, x stands for snail count per sample, i. e., 0, 1, 2, . . ., 36 and 46 in the population I. Therefore, $x\ (x-1)$ indicates 0 (0 – 1), 1 (1 – 1), 2 (2 – 1), . . ., 36 (36 – 1) and 46 (46 – 1), resulting 0, 0, 2, . . ., 1,260 and 2,070. The notation $\Sigma x\ (x-1)$ means the summation of all values of $x\ (x-1)$, i.e., 0 + 0 + 2 + . . . + 1,260 + 2,070 = 5,354 as shown in Table 1.

The number of samples n is 30, the total number of snails T is 250 and $T\ (T-1)$ is 62,250 in the population I. By substituting these values into the formula (2), the dispersion index Id will be $30 \times 5{,}354/62{,}250 = 2.5802$.

When Id is larger than 1.0, the distribution of the population is regarded as uneven. And the larger the index, the more unevenly the population is distributed.

In the same way, the Id value for the population II is calculated to be 5.3292. Both snail populations are known to be distributed unevenly, the degree of clustering being larger in the population II than in I.

By substituting the obtained values of Id and x in the formula (3), the necessary sample size q for the population I is obtained in the following calculation:

$$q = \left(\frac{1}{8.333} + 2.5802 - 1\right) \times 11.11 = 18.89 = 19$$

This indicates that 19 samples are necessary and still be within the 30% relative error in this snail population. In the same way, the minimal necessary sample in the population II is 50 as shown in Table 2. The more aggregatively the population is distirbuted, the more samples are necessary even when the mean densities are the same as in this example.

Table 2. Mean, dispersion index and necessary sample size

Population	$\bar{x}$	Id	q	n	n/q
I	8.333	2.5802	19	30	1.58
II	8.333	5.3292	50	30	0.60

$\bar{x}$: mean Id: dispersion index

q : necessary sample size n: no. of samples collected

($t = 1$, $E = 0.3$)

In Table 2, the final conclusion is that 30 samples taken in the population I are quite enough as the number is over the necessary sample size but in the population II, 30 samples are less than the minimal requirement of 50 and 20 additional samples or more should be taken.

Following the calculation of the dispersion index *Id* and mean density $\bar{x}$, a nomograph, prepared for various degrees of *Id* and $\bar{x}$ (Fig. 2), is referred to for a quick decision on the minimal sample size necessary. If a given snail population

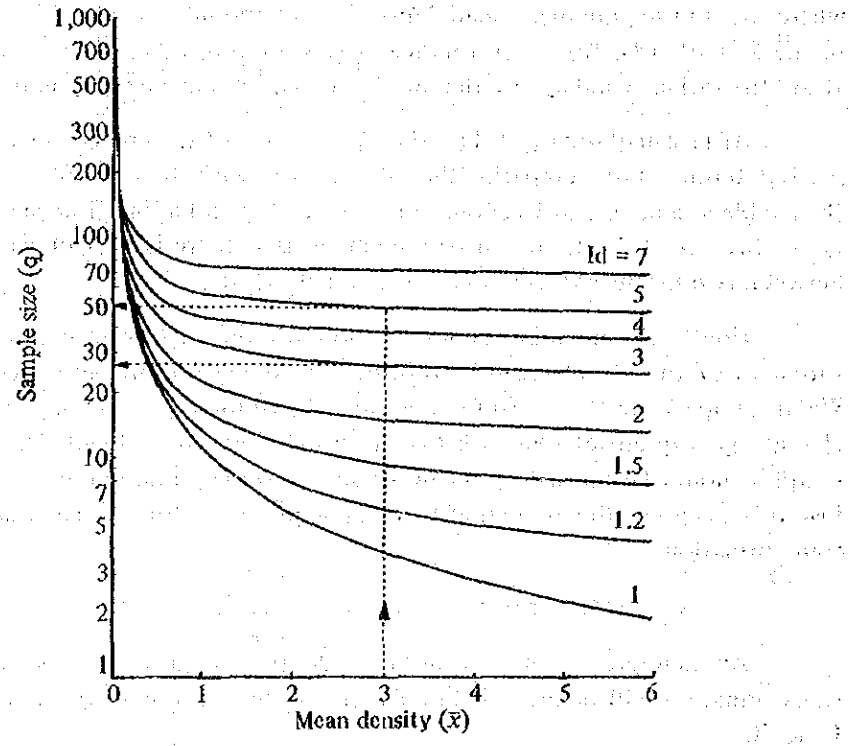


Fig. 2 Nomograph to obtain the minimal necessary sample size (q) based upon the mean density ($\bar{x}$) and dispersion index (Id)

has a degree of dispersion $Id = 3$, it can be read in the nomograph that 30 samples are necessary for the mean density of 3.0 and if $Id = 5$ with the same mean density, about 50 samples are necessary. The graphical reading of the above-mentioned 2 cases is shown by the dotted lines in Fig. 2.

b) Transformation for *t*-test

In order to evaluate a snail control measure, it is necessary to compare the mean density between two snail populations, for instance, before and after the control measure or between molluscicide-treated and non-treated populations. Application of transformation will be shown in an example of the *t*-test.

For such purpose, *t*-test is utilized by calculating t_0-value according to the following formula:

$$t_0 = \frac{|m_1 - m_2|}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \times \frac{n_1 + n_2}{n_1 n_2}}} \quad \ldots\ldots\ldots (4)$$

where m_1 and m_2 are mean snail densities in the population 1 and 2, respectively. In the formula (4), $|m_1 - m_2|$ indicates the absolute value of $(m_1 - m_2)$, s_1^2 and s_2^2 are the variance and n_1, n_2 the number of samples in each population.

After calculating t_0-value, the significance of difference between m_1 and m_2 is determined by comparing the calculated t_0 with the value of t referred from the t-table at a degree of freedom of $(n_1 + n_2 - 2)$ and a significance level of α. If $t_0 > t\,(n_1 + n_2 - 2, \alpha)$, it can be concluded that there is a significant difference between two means at a $100 \times (1 - \alpha)\%$ reliable level.

However, one important condition for this significance test is that the variances of the samples can be regarded as almost uniform in different groups. When samples are taken from unevenly distributed populations, the variance changes greatly correlating with the mean density. Accordingly, the variance of samples should be unified by means of an appropriate method of transformation. For this purpose, the following formula is proposed for a reliable and practical transformation;

$$y = \log (x + 0.01) \quad \ldots\ldots\ldots (5)$$

An example of comparison of the density by means of t-test utilizing data transformation will be given below for the aforementioned two snail populations I and II.

To calculate the t_0 value using the transformation formula (5), snail counts (x) at each sample in the population I, i.e., 0, 1, 2, ... , 36 and 46, are transformed by *log* (0 + 0.01), *log* (1 + 0.01), *log* (2 + 0.01), ... , *log* (36 + 0.01) and *log* (46 + 0.01) into the respective y values, i.e., -2.00, 0.00, 1.30, ..., 1.56 and 1.66 describing up to the second decimal point as shown in Table 3. The transformation of data is given for all samples calculated in the same way for population II (Table 3).

Table 3 Calculation of transformed values

Population I			Population II		
Snail/sample x	$x+0.01$	$y = log\ (x+0.01)$	Snail/sample x	$x+0.01$	$y = log\ (x+0.01)$
0	0.01	-2.00	0	0.01	-2.00
0	0.01	-2.00	0	0.01	-2.00
0	0.01	-2.00	0	0.01	-2.00
0	0.01	-2.00	0	0.01	-2.00
0	0.01	-2.00	0	0.01	-2.00
0	0.01	-2.00	0	0.01	-2.00
0	0.01	-2.00	0	0.01	-2.00
1	1.01	0.00	0	0.01	-2.00
1	1.01	0.00	0	0.01	-2.00
1	1.01	0.00	0	0.01	-2.00
1	1.01	0.00	0	0.01	-2.00
1	1.01	0.00	0	0.01	-2.00
2	2.01	0.30	0	0.01	-2.00
4	4.01	0.60	0	0.01	-2.00
4	4.01	0.60	0	0.01	-2.00
5	5.01	0.70	0	0.01	-2.00
5	5.01	0.70	0	0.01	-2.00
6	6.01	0.78	0	0.01	-2.00
7	7.01	0.85	0	0.01	-2.00
7	7.01	0.85	0	0.01	-2.00
10	10.01	1.00	5	5.01	0.70
11	11.01	1.04	6	6.01	0.78
15	15.01	1.18	8	8.01	0.90
16	16.01	1.20	8	8.01	0.90
16	16.01	1.20	10	10.01	1.00
18	18.01	1.26	19	19.01	1.28
18	18.01	1.26	28	28.01	1.45
19	19.01	1.28	36	36.01	1.56
36	36.01	1.56	57	57.01	1.76
46	46.01	1.66	73	73.01	1.86
250		4.02	250		-27.81

No. of samples n = 30 30

Mean $\bar{y} = \Sigma y/n = 4.02/30 = 0.134$ $-27.81/30 = -0.927$

Σy^2 = 48.2002 96.4501

Variance $s^2 = (\Sigma y^2 - (\Sigma y)^2/n)/(n-1)$

$= (48.2002 - (4.02)^2/30)/29$ $(96.4501 - (-27.81)^2/30)/29$

= 1.644 = 2.436

After transforming all the snail counts, statistical values of m (mean density = $\bar{y}$), s^2 (variance) and n (the number of samples) are calculated separately in both populations as in Table 3. In case of using a calculator having a function to calculate the standard deviation (s), variance (s^2) is available by the square of s based on n.

For comparison of two means of snail density between the populations I and II, the t_0 value and the degree of freedom are obtained as follows;

$$t_0 = \frac{|0.134 - (-0.924)|}{\sqrt{\frac{(30-1) \times 1.644 + (30-1) \times 2.436}{30+30-2} \times \frac{30+30}{30 \times 30}}} = 2.877$$

$$df = n_1 + n_2 - 2 = 30 + 30 - 2 = 58$$

In the t-table, t-value at 60 degree of freedom and 1% significance level ($\alpha = 0.01$ or 99% reliability) is found to be 2.660. Since the calculated $t_0 = 2.877$ is larger than the referred $t = 2.660$ from the table, a conclusion is made with 99% reliability that the mean snail density in the population II is significantly less than that in the population I (Table 4).

Table 4 Result of t-test to campare the difference of density in 2 populations

Population	n	Transformed data		Non-transformed data	
		m (= $\bar{y}$)	s^2	m (= $\bar{x}$)	s^2
I	30	0.134	1.644	8.333	121.40
II	30	-0.927	2.436	8.333	318.09
Between populations		t_0	Conclusion	t_0	Conclusion
I - II		2.877*	I > II	0	I = II

* Significant at 1% level

It can be generally said that if t_0 is larger than the t-value at 5% (0.05) significance level, the difference is regarded to be significant; and if t_0 is larger than the t-value at 1% (0.01) level, the difference is regarded as highly significant.

In this example, if the difference is examined in the original data, both means are 8.333 as shown in the right column in Table 6. There is no difference of density between the populations I and II, since $t_0 = 0$. Where two populations have significantly different variances, i.e., 121.40 and 318.09, as in this example, t-test should not be applied to this kind of comparison.

Comparative study of densities in two populations by *t*-test often leads to a wrong conclusion if the uneven distribution of the animals and a big difference of variances are disregarded and non-transformed original data are used.

For the study of analysis of variance, the same method of transformation can be uitlized. After the original counts per sample are transformed by *log* $(x + 0.01)$, analysis of variance can be performed based upon each transformed value.

Laboratory Procedure for Infecting Snails

The method employed for infecting snails in the laboratory consists of putting the snails and miracidia together in a drop of water on a watchglass. The disappearance of the miracidia after a lapse of time, usually 20 -- 30 minutes, is taken as an indication that penetration was successful. As a speedy process for infecting a large series of snails, this procedure may be practicable, although some accuracy is sacrificed.

Fresh miracidia hatched from the eggs are collected individually with the aid of micropipette and transferred with as little water as possible to a watch-glass containing the snail. All that happens to the miracidia and snails thereafter is watched continuously under a dissecting microscope and observations are recorded.

It will be observed that various organs of the snails will be attacked by the miracidia -- namely, the foot region, just above the eyebrow, proboscis, tentacles, etc. and often times they would swim straight into the shell and disappear. Occasionally they would appear to be stimulated by some substance emanating from the snail. Some will be noted to attach and detach themselves from the snail many times until they become weak and die.

Specific Chemotherapy

Up to the present, there is no highly effective drug against schistosomiasis which can kill the parasite without giving toxic effect on the host. Antimonial compounds are now limited to cases confined in the hospitals and for cerebral cases. For outpatients, ambilhar or niridazole is given with the following guidelies:

Ambillhar for the treatment of schistosomiasis japonica.

Dosage:

The average dosage is 15 mg per kilogram body weight per day for 24 days. The daily dosage should be taken in two fractional doses (morning and evening) either during of after meals.

If the patient can not tolerate the drug, the duration of treatment can be shortened to 10 days which can be repeated every 2 to 3 months should the patient still be suffering from schistosomiasis or has a recurrence of the disese.

If the daily dose of 15 mg per kilogram body weight for 10 days cannot be tolerated, reduction in the daily dose to as low as 10 mg/kg/day for as long as 35 days may be used for its suppressive effect on the egg-laying of the flukes.

Side Effects:

Side effects during treatment may be due to either directly to the drug itself or indirectly to its antiparasitic action.

Gastro-intestinal disturbances such as loss of appetite, nausea and vomiting may occur. These are only temporary in nature and if not so severe, drug administration may still be continued.

In rare cases, particularly in dosage higher than 15 mg/kg/day central nervous disorders (excitability, anxiety, confusional states, hallucination and in exceptional instances, convulsions) may be encountered. In such cases, *Ambilhar should be withdrawn at once.* Symptomatic treatment of reactions is advised whenever necessary otherwise the patient may be left untreated since these reactions are only transient in nature.

Patients with hepatosplenic form of schistosomiasis require particularly careful surveillance.

Destruction of the parasite leads to the release of foreign proteins which may act as antigens and react with antibodies that are already present; the resultant manifestations consist chiefly of tiredness in the muscles and limbs. Symptomatic relief can be afforded by administering anthihistamines.

Contraindications:

1. Ambilhar should not be used for ambulant therapy in the following condition.
   a) Severe liver damage
   b) Portacaval anastomoses
   c) Patients in poor general condition with severe malnutrition, protein deficiency, advanced anemia.
   d) Patients with a history of psychotic disorders, and epilepsy.
2. Ambilhar should not be administered with Isonicotinic Acid Hydrozide

(INH) due to their synergestic action on the nervous system.

Note: The urine of patient under treatment with Ambilhar may become deep brown in color. This discoloration is no cause for alarm and quickly disappears once treatment has been completed.

Standard Method for Bioassaying Molluscicides

Medical malacologists are often called upon to test the effectiveness of existing and new molluscicides. Standard methods are desirable in order to provide a common basis for the comparison of results obtained by workers in various parts of the world against different species of intermediate hosts and under various conditions. The following procedures which are established at NIH Tokyo and are recommended by WHO are being used for amphibious *Oncomelania* snails (Yasuraoka, 1970).

Snails to be used:

Laboratory-reared and/or freshly collected uninfected snails.

Container:

The container employed should be a Petri dish of glass (12 cm in diameter and 3 cm in height) with a cover. A vinyl net stretched on a plastic frame is to be put in the inside of the dish to prevent the snails from crawling out of the test solution.

Number of Replicates:

A minimum of three containers at each concentration with ten snails each should be used.

Water:

Distilled water should be used to make up the test solutions and for the controls.

Concentration of Molluscicide:

A twofold dilution (solution, suspension, emulsion, etc.) series of 0.1, 1.0, 10, and 100 ppm of the candidate molluscicide is first employed. Futhermore, a twofold series is used after the critical range is determined.

Temperature:

Temperature is an important ambient factor. The recommended temperature is 25°C ± 1°C for those species that harbor the human-infecting schistosomes.

Exposure Period:

An exposure period of 48 hours is adequate.

Recovery Period:

After the snails have been exposed to the candidate mollusicide, they are washed in running water for several minutes to remove traces of the molluscicide from their shells and transferred to a dish containing distilled or aged tap water for recovery. The recovery period is 48 hours.

Criteria of Death:

Snails that had been killed during the recovery period can be recognized by discoloration, absence of muscle contraction, and/or crushing in order to examine for signs of life, such as muscle contractions and so on. Snails that are obviously dead in the recovery dishes should be removed to prevent fouling.

LC_{50} and LC_{90} Determination:

LC_{50} and LC_{90} values should be computed by the method of Litchfield and Wilcoxon (1949).

Controls:

A minimum of three identical containers containing ten snails in 100 ml of water should be used to ascertain the mortality rate among nonexposed snails.

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