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Monogenetic trematodes (Dactylogyridae: Ancyrocephalinae)
on the gills of tilapia (a warm-water cultured fish) with
special reference to Cichlidoqyrus sclerosus Paperna and
Thurston 1969

by

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DECLARATION

I declare that this work is entirely of my own undertaking and whatever help I have received is in the acknowledgement.

Faiyaz

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PREFACE

This work was undertaken because of the extreme paucity of knowledge of Monogenea, especially those on the gills of warm-water cultured fishes.

In South-East Asia and other third world countries, pond fish culture is growing in importance. Inadequate knowledge of fish diseases and fish parasitism has led to massive outbreaks of fish parasites, resulting in high mortalities of fish fry and fingerlings, especially as a result of monogenetic infections.

The monogenetic trematodes of tilapia were chosen for purposes of study in order to propagate the pioneering efforts of Paperma and Thurston (1969) and also to enhance further knowledge on the diseases of tilapias, which are important food fishes in the developing countries of Africa and Asia.

CONTENTS

	<u>Page</u>
List of Abbreviations, units and symbols	(i)
List of Chemical names	(v)
List of Tables	(vi)
List of Figures	(viii)
Abstract	(xi)
Chapter 1.1. General Introduction	1
1.2. Literature Review	4
1.2.1. Taxonomy	4
1.2.2. Reproductive Biology	4
1.2.3. Host-specificity	9
1.2.4. Site-specificity	10
1.2.5. Effect of density	11
1.2.6. Host-defense mechanism	13
Chapter 2. The taxonomy of some Monogenea from the gills of tilapia, a warm-water cultured fish	15
2.1. Introduction	15
2.2. Materials and methods	17
2.3. Results	18
2.3.1. Description of <u>Cichlidogyrus sclerosus</u>	18
2.3.2. Description of <u>Cichlidogyrus sclerosus</u> <u>minutus</u>	27
2.3.3. Description of <u>Cichlidogyrus tilapiae</u>	32
Chapter 3. The reproductive biology of <u>Cichlidogyrus</u> <u>sclerosus</u>	37
3.1. Introduction	37
3.2. Materials and methods	39
3.2.1. Preliminary experiments to locate the eggs of <u>C. sclerosus</u>	39

	<u>Page</u>
3.2.2. Observations on the egg-laying behaviour of <u>C. sclerosus</u> , the formation of eggs and time intervals in the formation of an egg	40
3.2.3. Egg development and egg hatching of <u>C. sclerosus</u>	41
3.2.4. Experiments on the effect of host mucus, pH and urea on the egg development and hatching of <u>C. sclerosus</u>	41
3.3. Results	43
3.3.1. Behaviour of <u>C. sclerosus</u> during egg-laying ...	43
3.3.2. Time intervals in the formation of an egg	43
3.3.3. Egg development and egg hatching of <u>C. sclerosus</u>	46
3.3.4. Description of the oncomiracidium	48
3.3.5. Behaviour of the oncomiracidium	48
3.3.6. The egg production of <u>C. sclerosus</u> on excised gill arches	50
3.3.7. The results of the effect of host mucus, urea and pH on egg development and egg hatching of <u>C. sclerosus</u>	51
3.3.8. The effect of different concentrations of urea on egg development and egg hatching of <u>C. sclerosus</u>	54
3.3.9. Egg development at different pHs	55
3.3.10. Effect of higher pH levels on egg development and hatching of <u>C. sclerosus</u>	56
3.4. Discussion	58
Chapter 4. Host-specificity and site-specificity of <u>Cichlidoqyrus sclerosus</u> on the gills of tilapia	66
4.1. Introduction	66
4.2. Materials and methods	68
4.2.1. Host-specificity experiment	68
4.2.2. Site-specificity experiment	70

	<u>Page</u>
4.3. Results	73
4.3.1. Host-specificity experiment	73
4.4. Site-specificity experiment	80
4.4.1. Spatial distribution of <u>C. sclerosus</u> adults	80
4.4.2. Analysis of data for adult <u>C. sclerosus</u> distribution on the right gill arches	82
4.4.3. Analysis of data comparing right and left gill arches for <u>C. sclerosus</u> distribution	84
4.4.4. The spatial distribution of juvenile <u>C. sclerosus</u> on the gills	86
4.4.5. Analysis of data for juvenile <u>C. sclerosus</u> distribution on the right gill arches.....	87
4.4.6. Analysis of data comparing right and left gill arches for <u>C. sclerosus</u> juvenile distribution	89
4.4.7. The spatial distribution of <u>C. t. minutus</u> ...	91
4.4.8. Analysis of data for <u>C. t. minutus</u> on the right gill arches	92
4.4.9. Analysis of data comparing right and left gill arches for <u>C. t. minutus</u>	94
4.4.10. Summation of result for site-specificity experiment	96
4.5. The relationship between lengths of fish and number of parasites	97
4.5.1. Linear regression analysis	97
4.6. Discussion	100
Chapter 5. The effect of density of tilapia, <u>Sarotherodon mossambicus</u> , and the effect of hydrocortisone on the build-up of <u>Cichlidogyrus sclerosus</u> infections under controlled conditions	110
5.1. Introduction	110
5.2. Materials and methods	112

	<u>Page</u>
5.2.1. Experiments to determine the effect of density of tilapia on the build-up of <u>C. sclerosus</u> infections	112
5.2.2. Hydrocortisone experiment	114
5.3. Results	116
5.3.1. Results of the experiments on the effect of density of tilapia on the build-up of <u>C. sclerosus</u>	116
5.3.2. Results of Poisson Distribution and Negative Binomial Distribution fitted to data from all tanks at weekly intervals	129
5.3.3. Results of hydrocortisone experiment.....	145
5.4. Discussion	149
Chapter 6. General Conclusions	156
Appendices	165
References	238

ABBREVIATIONS, UNITS AND SYMBOLS

$<$	smaller than
$>$	greater than
min	minutes
μ	microns
cm	centimetre
No.	number
Σ	summation of
r_{12}	correlation coefficient
t	students t-distribution
s^2	variance
\bar{x} or \bar{y}	arithmetic mean
n	number of data
Us	Mann-Whitney Statistic
%	percentage
/	per
ANOVA	Analysis of Variance
df	degrees of freedom
ss	sum of squares
ms	mean square
F	variance ratio
log	logarithmic
μ moles/ml	micromoles per millilitre
μ gm/ml	microgram per millilitre
$\sqrt{\quad}$	square root
Rp	least significant difference
p	treatments
fe	error degree of freedom

$b_y x$	regression coefficient
$\sum x^2$	sum of square value
$\sum x$	sum of value
$\sum xy$	sum of products
s_b	standard error of the regression coefficient
$p)$	
$q)$	parameters of the Negative Binomial Distribution
$k)$	
Δ	heat change
χ^2	Chi-square test for goodness of fit
χ_{*}^2	Chi-square* test for goodness of fit based on Fisher (1950)
f	observed frequency
ϕ	expected frequency
A_x	accumulated frequency
T.S.	transverse section

ABBREVIATIONS, UNITS AND SYMBOLS

<u>A. lobianchi</u>	<u>Acanthocotyle lobianchi</u>
<u>A. rupestris</u>	<u>Ambloplites rupestris</u>
<u>C.</u>	<u>Cichlidogyrus</u>
<u>C. bifurcatus</u>	<u>Cichlidogyrus bifurcatus</u>
<u>C. haplochromii</u>	<u>Cichlidogyrus haplochromii</u>
<u>C. tilapiae</u>	<u>Cichlidogyrus tilapiae</u>
<u>C. t. minutus</u>	<u>Cichlidogyrus tubicirrus minutus</u>
<u>D. anchoratus</u>	<u>Dactylogyrus anchoratus</u>
<u>D. coeliaca</u>	<u>Dactylocotyle coeliaca</u>
<u>D. extensus</u>	<u>Dactylogyrus extensus</u>
<u>D. solidus</u>	<u>Dactylogyrus solidus</u>
<u>D. vastator</u>	<u>Dactylogyrus vastator</u>
<u>D. denticulata</u>	<u>Diclidophora denticulata</u>
<u>D. esmarkii</u>	<u>Diclidophora esmarkii</u>
<u>D. luscae</u>	<u>Diclidophora luscae</u>
<u>D. merlangi</u>	<u>Diclidophora merlangi</u>
<u>D. paradoxum</u>	<u>Diplozoon paradoxum</u>
<u>E. hippoglossi</u>	<u>Entobdella hippoglossi</u>
<u>E. soleae</u>	<u>Entobdella soleae</u>
<u>E. melleni</u>	<u>Epibdella melleni</u>
<u>G. aphae</u>	<u>Gyrodactylus aphae</u>
<u>G. elegans</u>	<u>Gyrodactylus elegans</u>
<u>G. medius</u>	<u>Gyrodactylus medius</u>
<u>G. pannonicus</u>	<u>Gyrodactylus pannonicus</u>
<u>L. gibbosus</u>	<u>Lepomis gibbosus</u>
<u>P. xenopodis</u>	<u>Protopolystoma xenopodis</u>

<u>R. emarginata</u>	<u>Raia emarginata</u>
<u>S.</u>	<u>Sarotherodon</u>
<u>S. aureus</u>	<u>Sarotherodon aureus</u>
<u>S. homorum</u>	<u>Sarotherodon homorum</u>
<u>S. mossambicus</u>	<u>Sarotherodon mossambicus</u>
<u>S. niloticus</u>	<u>Sarotherodon niloticus</u>
<u>S. spiluris</u>	<u>Sarotherodon spiluris</u>
<u>S. zilli</u>	<u>Sarotherodon zilli</u>
<u>T.</u>	<u>Tilapia</u>
<u>T. leucosticta</u>	<u>Tilapia leucosticta</u>
<u>U. adpectus</u>	<u>Urocleidus adpectus</u>

LIST OF CHEMICAL NAMES

NaHCO_3	sodium bicarbonate
Na_3PO_4	sodium phosphate
Na_2HPO_4	sodium hydrogen phosphate
NH_3	ammonia
PGDH	phosphogluconate dehydrogenase
NADP	nicotinamide adenine dinucleotide phosphate
MgCl_2	magnesium chloride
PMS	phenazine methosulphate
HCl	hydrochloric acid
PGI	phospho glucose isomerase
F6P	fructose 6 phosphate
MTT	3 - (4, 5 - Dimethylthiazole - 2) - 2, 5 - diphenyltetrazolium bromide
G6PDH	glucose 6 phosphate dehydrogenase
H and E	Hematoxylin and Eosin
MSB	Martius-Scarlet Blue
ACTH	adrenocorticotrophic hormone

LIST OF TABLES

<u>Table Number</u>		<u>Page</u>
I	The measurements of <u>C. sclerosus</u> by different authors. Measurements are in microns	26
II	Measurements of <u>C. t. minutus</u> on gills of <u>Haplochromis</u> species and <u>C. t. minutus</u> on gills of <u>Sarotherodon mossambicus</u>	31
III	The measurements of <u>C. tilapiae</u>	35
IV	The time intervals in minutes between egg formation and egg laying of five specimens of <u>C. sclerosus</u> observed <u>in situ</u>	45
V	A summary of the results of egg production of <u>C. sclerosus</u> on excised gill arches of <u>S. spiluris</u> and <u>S. mossambicus</u> at 25°C.....	50
VI	Effect of host mucus on development of eggs of <u>C. sclerosus</u>	53
VII	The effect of different concentrations of urea on egg development and egg hatching of <u>C. sclerosus</u>	54
VIII	The effect of higher pH levels on egg development and egg hatching	57
IX	The infection level of <u>C. sclerosus</u> on two hosts, <u>S. spiluris</u> and <u>S. mossambicus</u> , in three tanks. (In Tank 9 there was no intermingling of the fish as the barrier was intact till the end of the experiment)...	74
X	Results of pooled data from all tanks	76
XI	The spatial distribution of <u>C. sclerosus</u> adults	80
XII	Square-root transformation of data for adult <u>C. sclerosus</u> distribution	82
XIII	Spatial distribution of juvenile <u>C. sclerosus</u>	86
XIV	Square-root transformation of data for juvenile <u>C. sclerosus</u> distribution	87
XV	Spatial distribution of <u>C. t. minutus</u>	91
XVI	Square-root transformation of data ($\sqrt{y + \frac{1}{2}}$) for <u>C. t. minutus</u> distribution	92

<u>Table Number</u>	<u>Page</u>
XVII	The level of infection of <u>C. sclerosus</u> on <u>S. mossambicus</u> at weekly intervals for two different densities of fish 117
XVII	Results of preliminary experiment on the effect of density of tilapia on the build-up of <u>C. sclerosus</u> 118
XIX	The mean number of <u>C. sclerosus</u> on <u>S. mossambicus</u> at weekly intervals. The results are from Density A group 121
XX	The mean number of <u>C. sclerosus</u> on <u>S. mossambicus</u> at weekly intervals 122
XXI	Statistics of original distribution and fitted Negative Binomial Distribution 130
XXII	The level of <u>C. sclerosus</u> infections on <u>S. aureus</u> in the experiment with hydrocortisone 146
XXIII	The level of <u>C. sclerosus</u> infections on <u>S. mossambicus</u> in the experiment with hydrocortisone 147

LIST OF FIGURES

<u>Figure Number</u>		<u>Page</u>
1	Generalized diagram of a Monopisthocotylean monogenetic trematode	19
2	Drawing of <u>Cichlidoqyrus sclerosus</u> from gills of tilapia	21
3	Opisthaptor armature of <u>C. sclerosus</u>	22
4	Reproductive structure of <u>C. sclerosus</u>	23
5	Drawing of <u>Cichlidoqyrus tubicirrus minutus</u> from gills of tilapia	28
6	Drawing of <u>Cichlidoqyrus tilapiae</u> , with details of opisthaptor armature, from gills of tilapia	33
7	Behaviour of <u>C. sclerosus</u> during egg laying	44
8	Diagrams showing the development of the egg of <u>C. sclerosus</u> , from the first day of incubation to the hatching of the oncomiracidium	47
9	Scattergram for numbers of <u>C. sclerosus</u> on excised gill arches, against number of eggs	52
10	Diagram of gill arch and microbranchio-spines	
11	Prevalence of <u>C. sclerosus</u> on <u>S. spiluris</u> and <u>S. mossambicus</u> , from individual tanks ...	77
12	Prevalence of <u>C. sclerosus</u> on <u>S. spiluris</u> and <u>S. mossambicus</u> , from all tanks	79
13	Spatial distribution of <u>C. sclerosus</u> , its juveniles and <u>C. t. minutus</u> on the gill arches of <u>S. spiluris</u> and <u>S. mossambicus</u>	81
14	Linear regression of numbers of <u>C. sclerosus</u> on lengths of tilapia	99
15	Graph of numbers of <u>C. sclerosus</u> per sample, against interval in weeks, for two densities of <u>S. mossambicus</u>	119

<u>Figure Number</u>	<u>Page</u>
16	Graph of mean <u>C. sclerosus</u> per fish, against interval in weeks, for two densities of <u>S. mossambicus</u> 120
17	Graph of numbers of <u>C. sclerosus</u> per sample, against interval in days, for Density A tanks 123
18	Graph of mean intensity of <u>C. sclerosus</u> per fish, against interval in days, for Density A tanks 124
19	Graph of numbers of <u>C. sclerosus</u> per sample, against interval in days, for Density B tanks 125
20	Graph of mean intensity of <u>C. sclerosus</u> per fish, against interval in days, for Density B tanks 126
21	Average weekly level of infection of <u>C. sclerosus</u> on tilapia 127
22	Observed frequency distributions for week 5 and fitted Poisson and Negative Binomials for combined results 131
23	Observed frequency distributions for week 6 and fitted Poisson and Negative Binomials for combined results 132
24	Observed frequency distributions for week 7 and combined results with fitted Poisson and Negative Binomial Distributions 133
25	Observed frequency distributions for week 8 and combined results with fitted Poisson and Negative Binomial Distributions 134
26	Observed frequency distributions for week 9 and combined results with fitted Poisson and Negative Binomial Distributions 135
27	Observed frequency distributions for week 10 and combined results with fitted Poisson and Negative Binomial Distributions 136
28	Observed frequency distributions for week 11/12 and fitted Poisson and Negative Binomial Distributions for combined results. 137

<u>Figure Number</u>	<u>Page</u>
29	Total observed frequency distributions and combined 'A' results showing fitted Poisson and Negative Binomial Distributions 139
30	Total observed frequency distributions and combined 'B' results showing fitted Poisson and Negative Binomial Distributions 140
31	Relationships between the statistics of the observed distributions and parameter p of the fitted Negative Binomials 142
32	Relationship between the statistics of the observed distributions and parameter k of the fitted Negative Binomials 143
33	Relationship between the variance of the Negative Binomial Distribution (pkq) and the variance of the observed distribution (s ²) 144
34 a.	The level of <u>C. sclerosus</u> infection for hydrocortisone-treated, and control, <u>S. aureus</u> 148
34 b.	The level of <u>C. sclerosus</u> infection for hydrocortisone-treated, and control, <u>S. mossambicus</u> 148

ABSTRACT

Monogenetic trematodes (Dactylogyridae : Ancyrocephalinae) on the gills of tilapia (a warm-water cultured fish) with special reference to Cichlidogyrus sclerosus Paperna and Thurston 1969.

The brief descriptions of Cichlidogyrus sclerosus, C. t. minutus and C. tilapiae by Paperna and Thurston (1969) have prompted further taxonomic studies of these species of monogenetic trematodes on the gills of tilapia. There are few morphological variants between the species described with the archetypes of Paperna and Thurston (1969) except for differences in measurements.

The pioneering efforts of Paperna and Thurston (1969) have been complemented with further studies on some aspects of the biology of C. sclerosus. In this study the fecundity of C. sclerosus on excised gill arches is approximately 3; the period of incubation is 4 days at 25°C; host mucus and urea are good hatching inducers; pH 7 is found to be most favourable for development and hatching of the eggs.

C. sclerosus, highly specific to the gills of tilapia, shows a definite preference for the anterior medial sections of hemibranchs of the first gill arches. C. t. minutus also shows preference for similar sites while juveniles of C. sclerosus are found randomly on the gills. The preference for the first gill arch could be attributed to the lack of microbranchiospines, which prevent passage of oncomiracidia over the gills.

There is also a significant linear regression between lengths of fish and number of parasites. As the fish gets older the number of parasites will increase.

The density experiments show the overdispersed distribution of C. sclerosus on S. mossambicus. The parasite numbers also appeared to build up after two weeks and decline after two weeks. This could be related to the life span of C. sclerosus and also a host defense mechanism. This is verified by the huge build-up of C. sclerosus after injection of host tilapia with hydrocortisone. Further elucidation of this host defense mechanism would be of great significance in the field of monogenetic trematode biology.

CHAPTER ONE

1.1 INTRODUCTION

Fish culture is becoming of increasing importance in third world countries as (a) available wild stocks become over-exploited, (b) the demand for fish protein increases, and (c) the capability of farming, by means of controlled reproduction, artificial feeding and managed water supply, to greatly enhance yields in a sustainable and self-renewing way, is recognised.

However, the ideal methods for aquaculture in equatorial regions are not yet fully defined and, currently, poor husbandry practices, resulting in excessive densities of fish stocked under poor conditions of aeration, encourages high levels of parasitic infections, notably with protozoans and monogenetic trematodes as confirmed by Sarig (1968; 1971).

The monogenetic trematodes of freshwater fishes are widely distributed and are, not infrequently, very pathogenic parasites. They are, as a rule, ectoparasitic. Although there are likely to be others, to date only two highly pathogenic monogenetic trematodes have been described from freshwater teleosts. These are Dactylogyrus vastator and D. anchoratus. Considerable importance is attached to these two pathogens and major studies have been carried out by Wunder (1929), Ljalman (1951), Iziunova (1956), Paperna (1963a, 1963b), Prost (1963), Kollman (1972) and Molnar (1976).

With regard to less pathogenic monogeneans in freshwater fish, the abundant literature includes Bauer and Nikolskaya (1954), Paperna (1964a, 1964b), Musselius and Ptashuk (1970), Molnar (1971a), Musselius et al. (1973), Hanek et al. (1974), Kritsky and

Thatcher (1974a), Molnar et al. (1974), Imada et al. (1976), Ogawa and Egusa (1977a; 1977b), Nakatsugawa and Muroga (1977).

All of the works detailed above are from temperate countries. With regard to the study of monogenetic trematodes from warm-water cultured fish of the tropics, where the year-round high temperatures and seasonal rainfall characteristics produce very different conditions, the literature is very much more scanty. The most significant contributor has been Paperna (1959; 1960a; 1961; 1963a; 1963b; 1964a; 1964b; 1964c; 1965; 1968; 1969; 1973) and his co-worker Thurston (Paperna and Thurston, 1968; 1969). Their major contributions related to the definition of the main monogenetic trematodes from East and Central Africa.

Although the biology, ecology, population dynamics and geographical distribution of monogenetic trematodes from warm-water cultured fish all require study, there is a particular need for taxonomic data on the species from tropical regions. Knowledge of the identity of the species being studied is the basis for all parasitological investigations and, currently, there is very little detailed information available to serve as a basis for more biological studies.

The present study was carried out to provide a baseline of biological information on one particular monogenean of tropical pond fish - Cichlidogyrus sclerosus. This species lent itself particularly well to such a study because it is consistently found on the gills of tilapias - important food fish in Africa and Asia - and supplies of such fish were readily available for research purposes at the Institute of Aquaculture, of the University of Stirling. Very little was known of the parasite but it had been

described by Paperna and Thurston in 1969. In the present study, aspects of its biology are defined under the following headings:-

1) Taxonomy:- The monogenean is described with two other species, Cichlidogyrus tubicirrus minutus and Cichlidogyrus tilapiae, which are also observed on the gills of the tilapias studied and could represent a source of possible confusion.

2) (a) Reproductive biology

Egg-laying, development and hatching.

The behaviour of C. sclerosus during egg-laying, the development of the egg from the time of egg-laying to the hatching of the oncomiracidium, the behaviour of the oncomiracidium after hatching and mode of attachment to the fish gills, are all described.

(b) The fecundity of the parasite, as observed from excised gill arch preparations.

(c) The effect of pH and urea on the egg-development, egg-hatching and survival of C. sclerosus.

3) The degree of host and site-specificity of C. sclerosus in aquarium conditions.

4) The effect of host density and of host treatment with hydrocortisone on the build-up of C. sclerosus.

1.2 Literature review

1.2.1. Taxonomy

Although, as indicated above, there is a relatively extensive amount of literature available on infestation of temperate fish with monogenean parasites, information on the infection of warm-water fish species with such parasites is much more limited. Indeed, most of the entire literature is critically confined to the studies of Paperna in Israel and Central and East Africa (Paperna, 1959; 1960a; 1960b; 1961; 1963a; 1963b; 1964a; 1964b; 1964c). Paperna (1960a) studied the monogenetic trematodes of cichlids and assigned a new genus, Cichlidoqyrus, which seemed to be relatively specific for cichlids.

From India taxonomic work on Monogenea mainly results from the efforts of Jain (1952-1958). From South-East Asia the taxonomic work is scarce, with sporadic reports from North Vietnam (Ha Ky, 1968; 1971) and from Malaysia (Hanek and Furtado, 1973). Ha Ky (1971) described several new species of Dactylogyrus while Hanek and Furtado (1973) described a new species of Gyrodactylus from a Malaysian catfish, Clarias batrachus.

1.2.2. Reproductive biology

This aspect of study of the Monogenea is concentrated mainly on a few individuals of the Dactylogyrus and Gyrodactylus genera (Iziumova, 1956; 1958; Paperna, 1963a; 1963b; Prost, 1963; Khalil, 1964; Molnar, 1971a; Lester and Adams 1974a; 1974b; Imada et al., 1976). One of the most extensive of such studies on Dactylogyrus is that of Prost (1963) who investigated the development and the pathogenicity of D. anchoratus and D. extensus for breeding carps. Among her findings of significance for the

management of infections in ponds were that dactylogyrids were susceptible to freezing and drying and that there was a strict correlation between the number of eggs laid and temperature.

Kearn (1970), in his work on the marine monogenean Dictyocotyle coeliaca on Raja naevus, showed that egg-development was favoured by high pressure and low temperature. In addition to these physical factors affecting reproduction, there is also a suggestion of a special host or other specific factor, directly influencing hatching. Euzet and Raibaut (1960), who looked at the development of the post larvae of Squalonchocotyle torpedinis, were the first to suggest such a component, possibly in host mucus. Host mucus was also shown to elicit hatching in Acanthocotyle lobiianchi by Macdonald (1974) and Entobdella solea by Kearn (1974a). Kearn and Macdonald (1976) also found that fully developed eggs of E. soleae hatched when treated with dilute solution of urea, or ammonium chloride, in sea water. The eggs did not respond to sea-water solution containing trimethylamine oxide or glutamine though there was, apparently, some response to arginine.

This is in contrast to the study of Kearn (1974b) in which he found that there was no daily hatching rhythm nor was mucus an effective hatching stimulant for Entobdella hippoglossi, a skin parasite of the halibut Hippoglossus hippoglossus.

The effect of temperature on the oviposition of monogenetic trematodes was studied by Iziunova (1956) and Prost (1963). Iziunova (1956) showed that D. vastator normally deposited from 4-10 eggs in 24 hours but, under unfavourable conditions or at a higher temperature, the number of eggs deposited increased. This was similar to the findings of Imada and Muroga (1978) who found that

Pseudodactylogyryrus microrchis on cultured eels increased their oviposition rate from 1.2 at 10°C to 9.6 (4.9 - 19.0) at 20°C but fell to 7.7 (3.0 - 9.8) at 28°C respectively.

Bauer et al. (1969), cited by Lucky (1973), looked at the bionomy of monogenetic trematode on gills of Aristichthys nobilis (bighead carp). He found that intense reproductive activity of Dactylogyryrus aristichthys occurred at 22-25°C. Paperna (1963a) is the only person who has done extensive study of D. vastator under tropical conditions in Israel. He found that there was no great difference between the local D. vastator in Israel and that from Eastern Europe. He found that, in both cases, the response to salinity was the same, optimal temperature was 22-24°C and optimal development of the egg occurred at 28°C. There is still a huge gap in current knowledge of the biology of other monogenetic trematodes from warm-water fish. It is known that egg production of monogenetic trematodes increases when the environment is adverse (Iziumova, 1958). Hence higher temperature and low oxygen content will lead to greater egg production, but this has not yet been elucidated in the tropics.

Oxygen has also been shown to be an important environmental parameter which can affect the biology of Monogenea. Iziumova (1958) found that, as the oxygen content in the water decreased, general depression was observed in the worms and they changed their place of attachment on the gills of the fish, moving to the ends of the gill lamellae where, presumably, they could obtain greater oxygen exposure, while the number of eggs deposited was increased. With regard to D. vastator, the influence of oxygen was not so obvious as this parasite remained in the same place of attachment and fed normally, even under conditions of considerable

deficiency of oxygen.

Houlihan and Macdonald (1979) compared the egg production and oxygen consumption, at different oxygen partial pressures, of Diclidophora merlangi and E. soleae. In D. merlangi the respiratory rate declined sharply as the oxygen pressure in the water fell and egg production almost ceased at a PO_2 of little below air saturation. D. merlangi lives on the gills of pelagic fish and there is a likelihood that the parasite has saturated air passing over it. Both respiratory rate and egg production in E. soleae were independent of ambient PO_2 . E. soleae lives on the undersurface of a flatfish which spends part of its time buried in sandy mud, where PO_2 could well be low for significant periods. These studies have all been carried out on marine monogeneans. So far there are no parallel recorded observations on a freshwater monogenean.

The timing of the occurrence of the hatching process in monogenean parasites plays an important part in their location of the host. Egg hatching can be induced by factors, such as chemical substances from the host (Kearn and Macdonald, 1976), mechanical substances (Ktari, 1969) and by light (Bychowsky, 1957).

Kearn (1973) has also shown that endogeneous hatching rhythms could be controlled by light/dark regimes and Kearn (1982) also showed that rapid hatching occurs in the monogenean, Entobdella diadema, when the light is extinguished. According to Kearn (1982) there is a need for detailed studies of responses to total and partial light intensity reduction, using light levels comparable with those of the natural environment.

Bychowsky (1957) characterised monogeneans into two

groups, according to the fate of the eggs, viz. those whose eggs fall directly to the bottom after oviposition and those whose eggs are attached to the gills. In the latter group his example was Nitzschia sturionis. In this species eggs were found attached to the buccal cavity of the sturgeon. In the case of freshwater monogeneans, Bovet (1967), having studied extensively several aspects of the biology of Diplozoon paradoxum, concluded that the presence of eggs in the gill chamber of the host was not of normal occurrence. Llewellyn (1972) stated that he examined hundreds of specimens of Kuhnia scombri, from Scomber scombrus, at Plymouth but never found their eggs on the gills. Hence most authors are agreed that, in the vast majority of monogeneans, the eggs are released into open water and are dense enough to sediment to the bottom of lakes, ponds and seas (Kearn, 1967a). Thus, it is to be expected that the oncomiracidia will normally infect the host when the latter is stationary at the bottom, in those areas where the eggs have come to rest. There is evidence that some oncomiracidia have the ability to perceive water currents (Llewellyn, 1972). Kearn (1976) suggested that further attention should be paid to possible behavioural adaptation of this kind, which may promote proximity of host and infective stages of parasite.

The only known account of an intermediate host being required for a monogenean is the work of Bychowsky and Nagibina (1967). Their observations showed that the intermediate hosts in question were various small pelagic fish from different regions of the South-eastern Pacific. They found numerous larvae of Pricea and Gotocotyla species located at the ends of the gill filaments, in peculiar swellings, and all reached the same stage of development and

stopped, awaiting capture by the definitive final host.

1.2.3. Host-specificity

Most monogeneans are host-specific, as is vouchsafed by examples in the literature (Bychowsky, 1933; Hargis, 1953c; Llewellyn, 1957). In particular, Bychowsky (1957) carried out a thorough review of the literature and pointed out that 711 out of the 957 species of Monogenea known at that time were each confined to a single species of a host. Iziunova (1953a) discussed at length the methods of studying the specificity of monogenetic trematodes. Kearn (1967b), who carried out experiments on host-finding and host-specificity in *E. soleae*, speculated that it was host-finding by chemoreception which gave rise to the phenomenon of host-specificity in Monogenea. Each monogenean parasite is apparently attracted only by the specific odour of the host. The specific substance or substances which permits the chemosensitive oncomiracidia to recognise its host has not yet been investigated.

Price and McMahon (1967) stated that host-specificity is currently receiving a considerable amount of attention. There is a lot of information yet to be discovered, but it is a well-established fact that the basic mechanism is chemical in nature. Llewellyn (1957) suggested that blood-borne antibodies may contribute towards the mechanism of host-specificity in the Monogenea. Little experimental work has been done with regard to this.

Rohde (1978a) found that host-specificity in marine Monogenea did not vary with latitude. He also stated that the higher host-specificity of Monogenea, compared to Digenea, was the direct result of the tendency of Monogenea to follow what he called

a "K-strategy". K-strategic species tend to produce fewer offspring, but these are highly advanced morphologically, developing into complex adults. Hence the fewer offspring means that survival chances are lessened. Therefore, by being host-specific, the host range is restricted and less energy will be dissipated in locating a specific host. Rohde (1980) also proposed 3 indices which describe host-specificity of a parasite, which can also be applied to any association between organisms. Price (1968) stated that Cichlidoqyrus is an outstanding example of a parasite which exhibits a great degree of specificity for its hosts. This narrow specificity could have arisen as a result of the host and parasite evolving and speciating together (Shulman, 1958; Cameron, 1964). Cichlidoqyrus is also located at certain specific sites on the particular host and hence also exhibits site-specificity.

1.2.4. Site-specificity

Most monogeneans are site-specific (Llewellyn, 1956; Frankland, 1955; Wiles, 1968; Suydam, 1971; Wooten, 1974). Llewellyn (1956) found that Diclidophora merlangi occurred most frequently on the outermost gill arch of Gadus merlangus. Similarly, Frankland (1955) indicated that Diclidophora denticulata was more prevalent on the same individual gill arch of Gadus virens. Wiles (1968) found that a similar situation occurred in freshwater species, with Diplozoon paradoxum occurring most often on the first two gill arches of Abramis brama. According to several authors (Suydam, 1971; Wooten, 1974) the precise location of the monogenean can be directly influenced by the direction of the gill-ventilating current. Suydam (1971) discussed only some of the factors which seem to influence site-specificity. In order to give a complete

picture of this host-parasite relationship there is a need for further physiological, behavioural and ecological studies.

Iziumova (1953a) was particularly interested in possible functional or morphological difference in gill arches, which might explain site-specificity. In her studies of the gills of carp and crucian carp, Carassius carassius, she examined the differences in dimensions in the number of gill lamellae. She also studied the direction of the water current with Indian ink and carmine powder. Her results indicated there was no apparent functional nor morphological difference which could affect infection of either carp, or crucian carp, gills by D. vastator.

Several authors (Frankland, 1955; Llewellyn, 1956; Kearn, 1968a) have assumed that differential distribution is due to factors operating at the time of infestation of the larvae. The assumption is that the larvae is passively taken into the host's mouth and subsequently effects attachment when being passed over the gills. One of the factors for site selection could be due to the age of the host rather than the age of the parasite. In an older host the gill lamellae have a larger surface area. The parasites therefore have to be aggregated in a particular site to ensure ease of fertilization and mating in those individuals which undergo copulation. Molnar (1971b) also confirmed that site selection of Dactylogyrus is due to the age of the host, rather than the age of the parasite.

1.2.5. Effect of density

The density of both infra-populations (all individuals of a single parasite species within an individual host) and supra-populations (all individuals of a given parasite species in all

stages of development within all hosts of an ecosystem) of parasites will change when there is a change in the density of the host population, whether due to stress or not (Esch et al. 1975).

Overcrowding of fish favours the appearance of many parasitic diseases. The density of fish in natural waters is never as high as in ponds. The risk of epizootics is thus especially great in a crowded population of a density of hundreds of thousands of fish per hectare (Bauer et al. 1973).

There is very little information with regard to the effect of fish density on the build-up of dactylogyrid numbers although, for instance, Paperma (1960b) stated that artificial fish ponds are conducive to the build-up of monogenetic trematodes, especially those species of Monogenea which are susceptible to low oxygen content, densely crowded fish, still water, and higher temperature.

Molnar (1971b) in his studies of Dactylogyryus lamellatus on grass carp, Ctenopharyngodon idella, found that, in a medium stocking density of ninety individuals per square metre, infestation remained low (30-100 parasites per host) but at a higher population density (200 individuals per square metre) some of the fishes developed clinical dactylogyrosis during the six week nursery period. In the viviparous monogenetic trematode Gyrodactylus the intensity of infection also increases with density of fish. Average intensity of infection with Gyrodactylus elegans and G. medius in underyearling carp, in aquarium conditions, increase 3.5 and 1.2 times respectively, if the density of the fish was increased 3 times. Similar results were obtained in ponds. At a density of 50,000 fry per hectare the rate of infection, with G. elegans, in a pond was 53.3%, compared

with 40% at a density of 30,000 fry per hectare (Bauer, et al. 1973). Of interest is the effect of host density on infection dynamics, as carried out by Keymer (1982). She found that the mean parasite burden of the flour beetle Tribolium confusum, exposed to known densities of the cestode Hymenolepis diminuta eggs, was found to decrease exponentially with increasing host density.

The regulation of host-parasite population is usually attributed to density-independent processes. With density-dependent processes there are few examples, the most notable being the work of Mills (1979) who showed that the survival and reproduction of Transversotrema patialense decreased at high population density. Immune responses could also regulate parasite numbers in a density-dependent manner (Bradley, 1974). Though the densities of the fish populations will affect numerical occurrence of the parasites they have not been reported as causing modification of the maturation cycles of the parasites, especially the trematodes (Chubb, 1979).

1.2.6. Host-defence mechanisms

Although there is a considerable amount of literature, with regard to the defence mechanisms of parasites to their hosts, there is also some literature regarding the defence mechanisms of hosts to their parasites. Wunder (1929) and Paperna (1964c), for instance, reported that proliferation of gill epithelial tissue contributed to the decrease of parasite numbers by rendering the surface less favourable. There are also speculations about the presence of antigens in mucus, which could result in immune responses. This has been shown by Nigrelli and Breder (1934) who found that Epibdella survive longer in mucus from susceptible fish

than in mucus from "immune" fish. Immunoglobulins have been found to be present in the serum and mucus of the plaice, Pleuronectes platessa (Fletcher and Grant, 1969). Fletcher and White (1973a, 1973b) also indicated lysozyme activity and antibody production in the plaice, P. platessa. Harris (1973) and Hines and Spira (1974) also showed that an epidermal immune response is evident in fish. The only work so far that considered humoral antibody activity as a means of rejection of the parasite is that of Vladimirov (1971). He showed that decrease of D. vastator coincided with high levels of antibody.

Mulligan (1968) defined "self-cure" as the elimination of a burden of parasites, as a result of an immune response on the part of the host. This term was introduced by Stoll (Stoll, 1929), but it was not until twenty years later (Stewart, 1950a; 1950b; 1950c; 1953 and 1955) that the term was used with any degree of precision. This term was also referred to by Paperna (1960a). He stated that cichlid fish have an acquired resistance to Cichlidogyrus species. He found that, in the wild, the cichlid fish often lost its infection. He also found that it was difficult to maintain levels of infection under aquarium conditions. These findings, however, only represent a beginning to the investigation of this interesting and important aspect of trematode biology and it would be a particularly valuable area for further study.

CHAPTER TWO

The taxonomy of some Monogenea from the gills of tilapia, a warm-water cultured fish.

2.1. INTRODUCTION

The Class Monogenoidea was first established by Van Beneden in 1858, according to Bychowsky (1957). The order, Monogenea, was established by Carus in 1863.

Most of the early studies on Monogenea are taxonomic (Hargis, 1953b; Mizelle and Price, 1964; Price, 1967; Price and Arai, 1967; Price and McMahon, 1967; Price et al. 1969; Hanek and Furtado, 1973; Lucky, 1973; Mays and Miller, 1973; Kritsky and Thatcher, 1974a; Rizvi, 1974; Mamayev and Parukhin, 1975; Mattison, 1976; Ogawa and Egusa, 1977a; 1977b). However, with regard to Monogenea on the gills of warm-water cultured fish, the studies are few and far between. Most of the work has been done by Paperna (1959-1973), the majority of it based on the cichlid fishes of Africa. Other works include Price and McClellan (1969) and Price and Pike (1969). Gussev (1973) also looked at a collection of Monogenea from thirty-seven species of freshwater fish in India. He discussed taxonomic criteria of dactylogyrids, the relationship between monogenean characters, life cycle and host age, ancestral monogeneans, the functional morphology of the organ of attachment and the comparative morphology, zoogeography, evolution and history of freshwater monogeneans.

The genus Cichlidogyrus was first described by Paperna in 1960 from the cichlid fishes of Israel. The characteristic features were: three to five pairs of head organs;

opisthaptor separated from the rest of the body by a narrow constriction; the four anchor hooks similar or dissimilar; two supporting bars, a single piece bent in the middle - the v-shaped bar and the compound bar which consists of three articulated pieces, forming a cruciform appearance. Paperna considered Cichlidogyrus as intermediate between Ancyrocephalus and Actinocleidus species. Only ten years later, in 1969, was the monogenetic trematode from the gills of cichlid fish from Africa looked at. The cichlid fish were taken from Lake Victoria at Jinja and Kazi (near Kampala), from Lake Albert at Butiaba and from Lake George and they were examined for monogenetic trematodes. C. sclerosus was found on Haplochromis species from Lake George, Tilapia leucostica from Jinja, T. mossambica from Kajansi, and T. zilli from Jinja. C. t. minutus were found on Haplochromis and T. leucostica.

The only two other recent works on C. sclerosus are Duncan (1973), who looked at C. sclerosus from the gills of T. mossambica from Alligator Lake and Sampalo Lake in the Philippines, and Kritsky and Thatcher (1974b), who described the parasite from T. mossambica from Rio Cauca Puerto de la Torre, Cali, Valle, Colombia in South America.

The description given by Paperna and Thurston (1969) was rather brief, hence the author decided to look at the morphology of C. sclerosus in detail. The author also studied the morphology of C. t. minutus and C. tilapiae. The morphology was first studied of species from fish kept in the aquarium tanks at the University of Stirling. These were later compared to specimens obtained from hybrid species of tilapia from the ponds

at the Universiti Pertanian Malaysia.

2.2 MATERIALS AND METHODS

The parasites were obtained from freshly-excised gills of fish kept in aquarium tanks at the Institute of Aquaculture, University of Stirling and also from the gills of fish netted from the ponds of Universiti Pertanian Malaysia, Serdang. They were teased out from the gill filaments using two finely curved suture needles. These parasites were examined alive. They were placed on a glass slide and covered with a coverslip for examination. This proved a satisfactory method for looking at the internal organs. As the water evaporated from the slide and the parasite gradually flattened, it became more transparent and the visceral organs were rendered clearly visible.

For making semi-permanent mounts the parasites were relaxed in a solution of 2 gms of chlorotone in 500 ml of water. They were fixed in 5% formalin and mounted in glycerine alcohol. This technique was adapted from Hargis (1953a).

The author also carried out a sectioning method in order to see the relationship of the internal organs clearly. The technique used was that of the Agar Method, Kimball and Purdue Modification 1962 (Stone and Cameron, 1964). A small amount of melted agar was put into a short length of glass tubing, sealed at one end. The agar was chilled to harden it. Some parasites were pipetted on top of the agar and a fixative added. The fixative was drawn off after the parasites had settled. More agar mixed with eosin was added and chilled. Water was pipetted down the sides of the agar, and under it, and this loosened the block out of the tubing. The block of agar was dehydrated,

cleared and blocked, like a piece of tissue. When the tissue was sectioned the parasites were found at the junction of the coloured layer with the uncoloured layer. The author found that this method was not satisfactory as the parasites were orientated at random and it was difficult to get sections through the reproductive structures. Sometimes the agar disintegrated after sectioning.

Precise measurements for taxonomical purposes were made on fixed mounted specimens. These parasites were relaxed, either in hot water (70°F) or chlorotone solution, fixed in formalin, then mounted in glycerine jelly on a glass slide. As the opisthaptor sclerites were quite evident, it was not found necessary to stain them by the method of Žďárská (1976).

2.3. RESULTS

The terminologies used in the descriptions of the monogenetic trematodes are as shown in Figure 1, which is a slight modification of the generalized diagram by Price and McMahon (1967). The author has also used the term, "anchor", for the larger attaching hooks of the haptor, according to Mueller (1936). The smaller attaching devices of the haptor are termed "hooks", according to Price and McMahon (1967).

2.3.1. Suborder Monopisthocotylea Odhner, 1912

Superfamily Dactylogyroidea Yamaguti, 1963

Family Dactylogyridae Bychowsky, 1933

Subfamily Ancyrocephalinae Bychowsky, 1937

Genus Cichlidogyrus Paperna, 1960.

The following description of C. sclerosus was based

GENERALISED DIAGRAM OF A MONOPISTHOCOTYLEAN MONOGENETIC TREMATODE

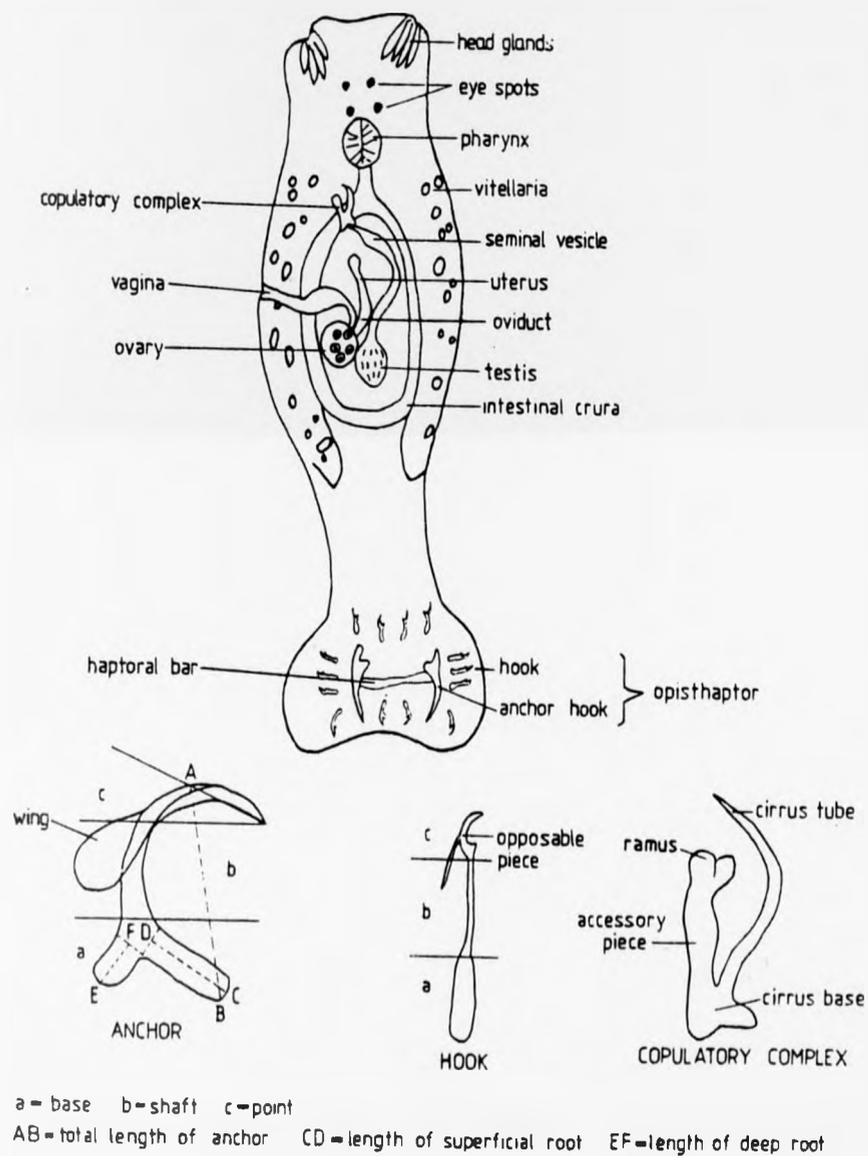


Fig. 1. Generalised diagram of a Monopisthocotylean monogenetic trematode.

GENERALISED DIAGRAM OF A MONOPISTHOCOTYLEAN MONOGENETIC TREMATODE

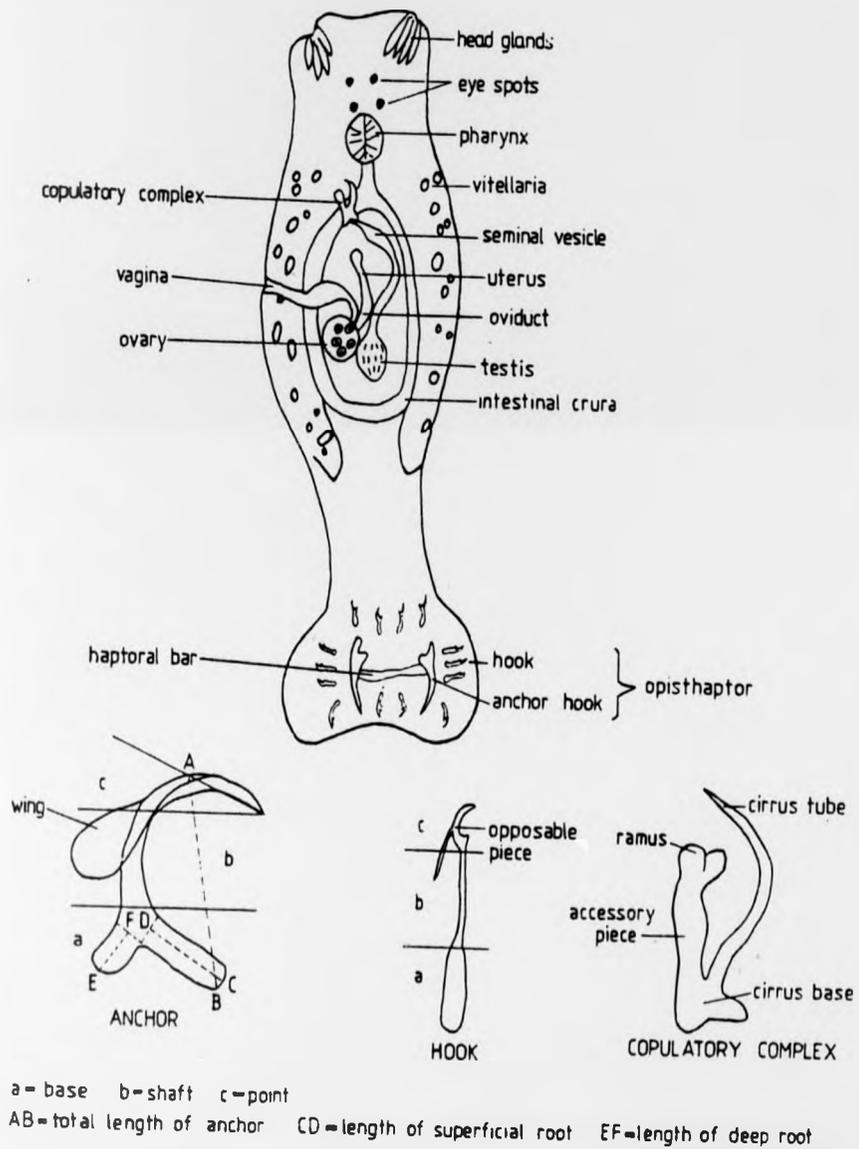


Fig. 1. Generalised diagram of a Monopisthocotylean monogenetic trematode.

on ten specimens and units of measurements were in μ .

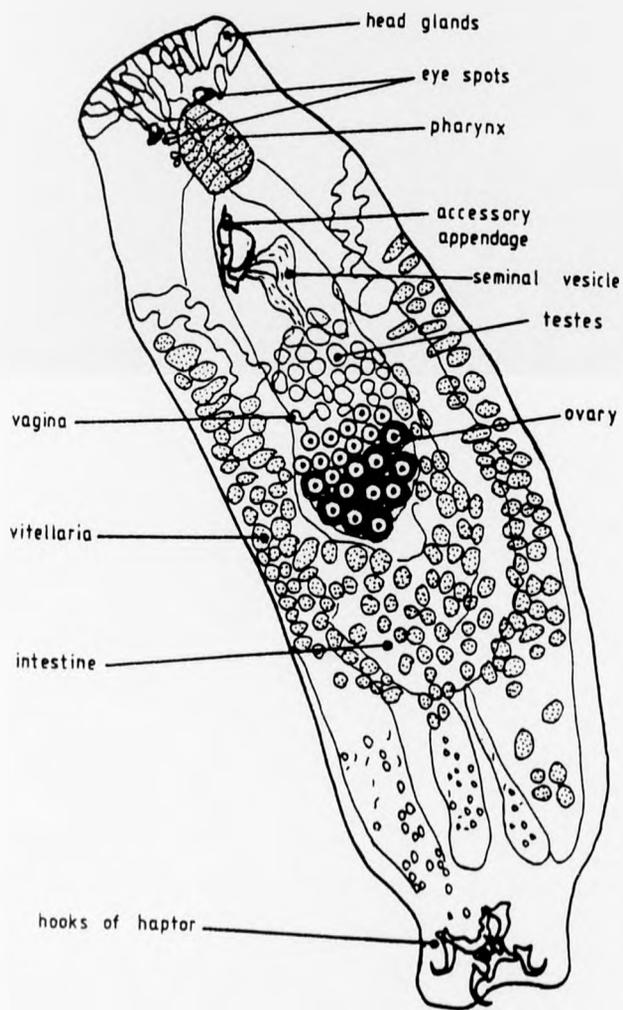
It was a large monogenean belonging to subfamily Ancyrocephalinae, with a smooth cuticle. The length measured 631 (500-800) μ and the width was 143 (103-210) μ . It had one pair of eyespots. The pharynx was almost circular (32.9 x 46.2) μ and the intestinal crura was joined posteriorly. The vitellaria was well developed, especially along the course of the intestines and beyond the ovary (Fig. 2).

The anchors had a solid perforated base. The dorsal anchors (anchor x) were the anchors bounding the dorsal compound bar. Length of anchor x was 29.9 (23-37) μ . The dorsal compound bar had a shallow v-shaped basal piece with two accessory pieces present, giving it a cruciform appearance. Length of bar x was 30.9 (26-36) μ . The ventral anchors (anchor v) was 28.1 (23-37) μ . It bounded the v-shaped ventral bar 41.4 (35-45) μ (Fig. 3).

There were 14 hooks surrounding the anchors, though some of the hooks were difficult to see because of their orientation. The first central pair of hooks measured 11.4 (11-12) μ , the second central pair measured 14.4 (14-15) μ and the distal pair measured 18.1 (17-19) μ .

The copulatory organ was very distinctive. The cirrus tube, or ejaculator, had an oval-mouthed funnel at one end, on one side of which was a flat, striated plate with serrations at one edge. The other end of the cirrus tube opened into a long sickle-shaped structure with a pointed end. Length of cirrus tube was 47.2 (37-62) μ measured along its whole length (Fig. 4).

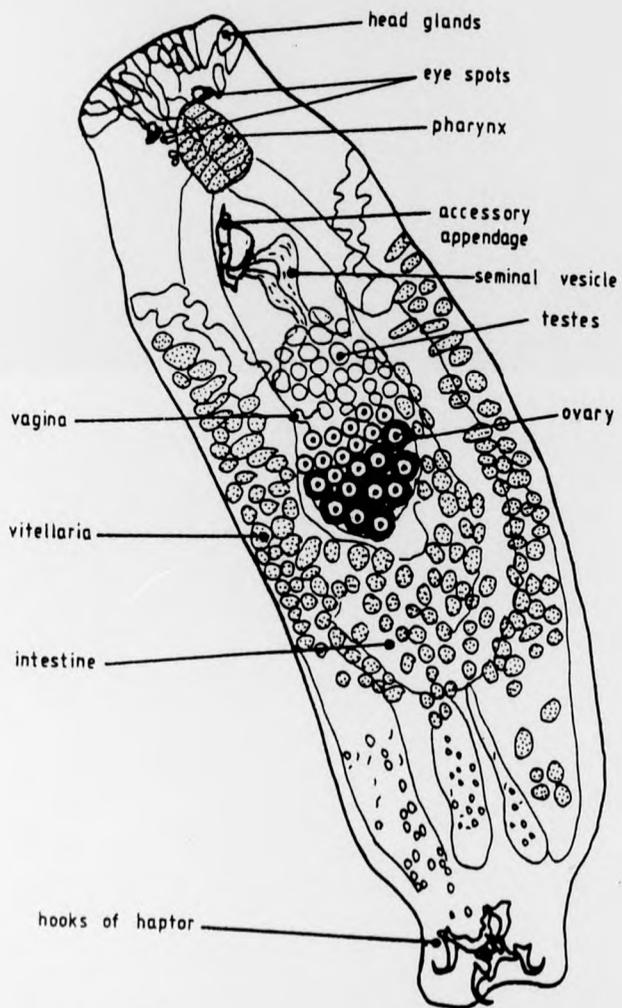
The accessory piece was large, robust and was attached to the base of the funnel of the cirrus tube. The other



Cichlidogyrus sclerosus

82 μ

Fig. 2. Drawing of *Cichlidogyrus sclerosus* from gills of tilapia.



Cichlidogyrus sclerosus

82 μ

Fig. 2. Drawing of *Cichlidogyrus sclerosus* from gills of tilapia.



Fig. 3. Opisthaptoral armature of *C. sclerosus*.



Fig. 3. Opisthaptoral armature of *C. sclerosus*.

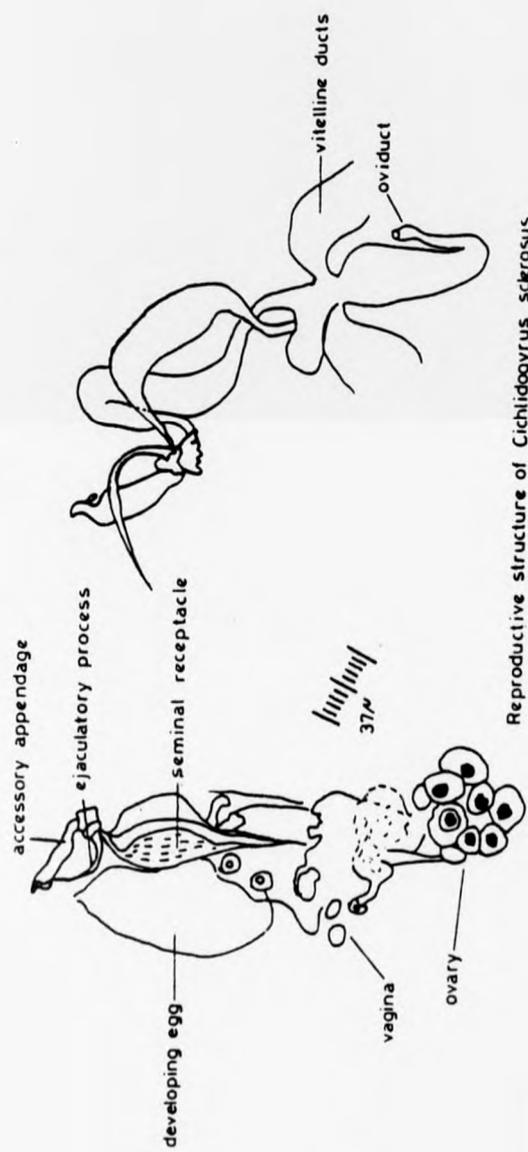


Fig. 4. Reproductive structure of C. sclerosus.

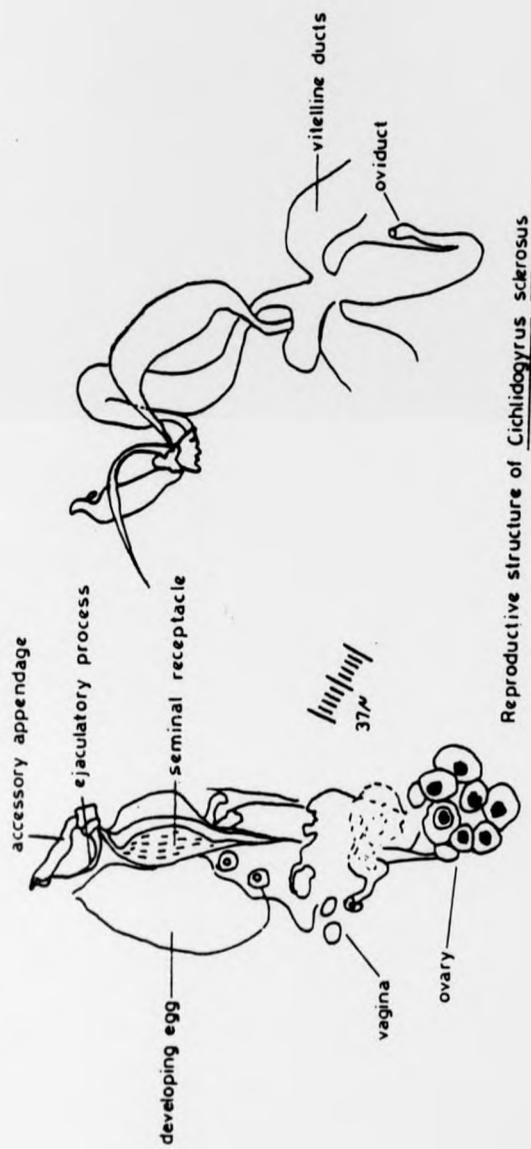


Fig. 4. Reproductive structure of *C. sclerosus*.

end of the accessory piece gave rise to two finger-like projections (rami). The length of the accessory piece was 48.17 (44-51) μ . The female genital system consisted of an ovary lying in the posterior intestinal space. It was irregularly ovoid and was made up of large cells with prominent nuclei. The vagina, positioned at the anterior end of the parasite, had a thickened appearance. The seminal receptacle was seen arising from the anterior part of the ovary. The male genital system consisted principally of the testes, which lay adjacent to the ovary. The vas deferens opened into the funnel of the cirrus tube. Behind it was a wide sac - the seminal vesicle. When the vitellaria were squeezed out of the specimens, the junction of the oviduct and vitelline ducts could be seen.

Host:- Sarotherodon mossambicus

Host locality:- Aquarium tanks, Institute of Aquaculture, University of Stirling.

Location:- gill filaments.

COMMENTS AND REMARKS

There was very little morphological difference between the C. sclerosus described here and the archetype described by Paperna and Thurston (1969) and Duncan (1973); the differences being confined to minor variations in measurements. The anchors were not perforated in the archetype of Paperna and Thurston (1969), but these were seen in the specimens described by Duncan (1973) and the specimens of C. sclerosus examined from the fish kept at the Institute of Aquaculture, University of Stirling. The perforations were not seen in the anchors of the parasites

obtained from Malaysian fish.

Paperna and Thurston (1969) found that the lengths of the worms differed in different host species, being largest in T. leucostica. The sclerotinoid organs were, however, of similar size in all the species examined. They did not observe the vaginal prop in any of the specimens though Duncan (1973), Kritsky and Thatcher (1974b) and the present author did observe the vaginal prop in the species they examined. This could be due to intraspecific variation. The measurements for C. sclerosus are shown in Table I.

TABLE I. The measurements of C. sclerosus by different authors. Measurements are in microns.

Dimensions of body and chitin parts	C. sclerosus Paperna and Thurston (1969)	C. sclerosus Duncan (1973)	C. sclerosus Kritsky et al. (1974)	C. sclerosus Own material from Stirling	C. sclerosus Own material from Malaysia
Total body length	650 - 700	535 - 707	520 - 720	500 - 800	425 - 840
Width	100 - 200	111 - 131	80 - 120	103 - 210	100 - 175
Copulatory complex					
Cirrus tube	50 - 60	28 - 61	57 - 66	37 - 62	54 - 61
Accessory piece	39 - 50	42 - 51	45 - 61	44 - 51	50 - 56
Anchor v	29 - 37	33 - 36	31 - 34	23 - 37	24 - 36
Anchor x	26 - 27	33 - 36	30 - 31	23 - 37	33 - 35
Bar v	42 - 53	33 - 39	36 - 44	35 - 45	50 - 65
Bar x	37 - 40	35 - 48	33 - 46	26 - 36	40
Hook 1st central pair	6 - 7	12)		11 - 12	11 - 14
2nd central pair	6 - 7	15)	14 - 18	14 - 15	14 - 15
distal pair	10 - 14	17)		17 - 19	16 - 18

- 2.3.2. Suborder Monopisthocotylea Odhner, 1912
 Superfamily Dactylogyridae Yamaguti, 1963
 Family Dactylogyridae Bychowsky, 1933
 Subfamily Ancrycephalinae Bychowsky, 1937
 Genus Cichlidogyrus Paperna, 1960

Paperna and Thurston (1969) also described the new species, Cichlidogyrus tubicirrus minutus. Their distinguishing characteristics were a tubiform cirrus tube and a single accessory piece, ending in a spike-like or finger-like projection. They noted 3 species which differed from each other in the cirrus tube of the copulatory organ. These were Cichlidogyrus tubicirrus longipenis, C. t. magnus and C. t. minutus. The author, during routine examination of the gills of tilapia, found a few of these small monogeneans with four eyespots. At first they were thought to be juveniles of C. sclerosus but, on detailed study, they were found to have a complex reproductive apparatus and laid eggs which were very much smaller than the eggs of C. sclerosus. They were identified as C. t. minutus and the following measurements (in μ) were based on six specimens.

The average length was 317 (132-349) μ and 81 (34-151) μ wide. The pharynx was 38 x 42 μ . There were 4 eyespots and 4 pairs of head glands (Fig. 5).

The copulatory organ had two parts. The tubiform cirrus tube was slender and was 43 (31-51) μ long. Paperna and Thurston (1969) described the cirrus tube, or ejaculator, of C. t. minutus as ending in a thin-walled vesicle-like dilation. Here the author had also observed that the tip of the cirrus tube did appear to fade out into a thin, transparent, triangular-shaped

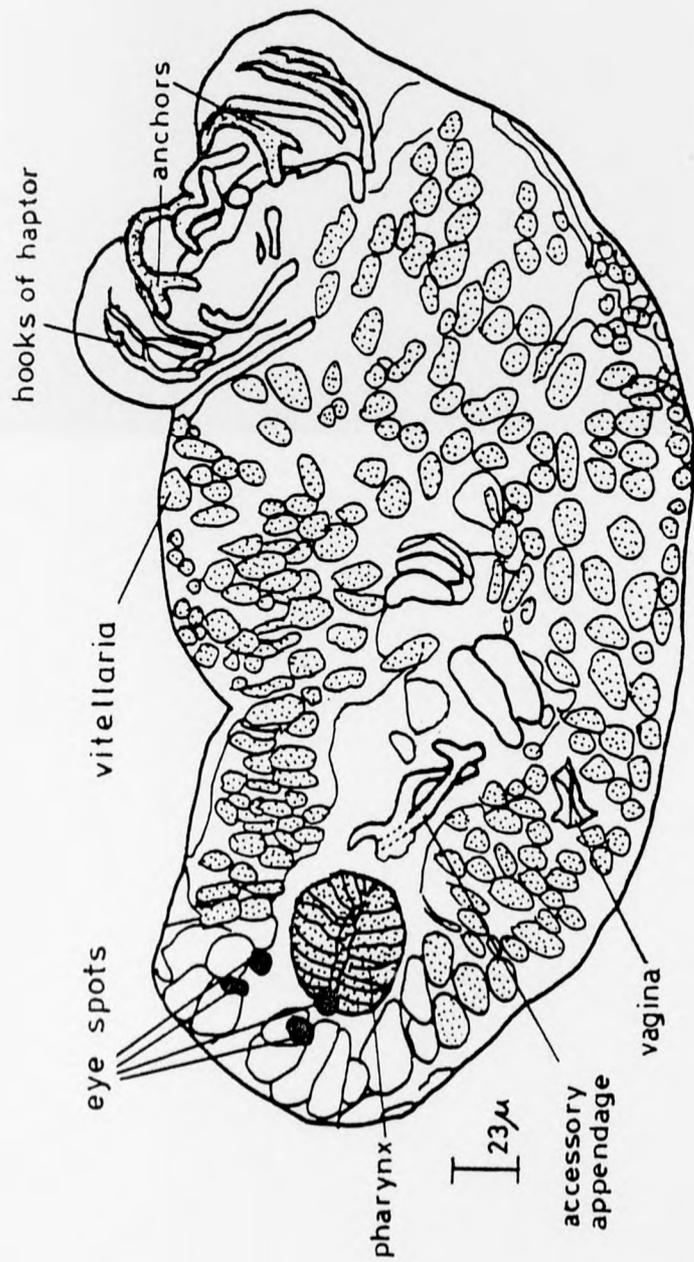


Fig. 5. Drawing of Cichlidogyrus tubicirrus minutus from gills of tilapia.

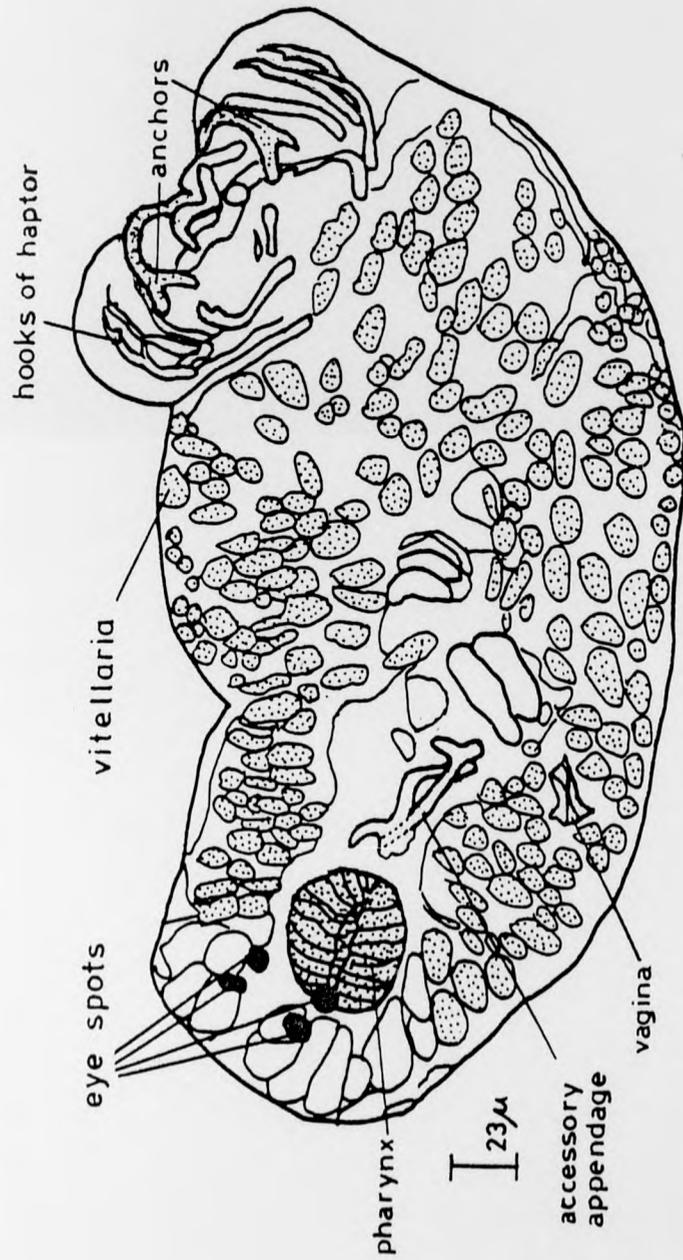


Fig. 5. Drawing of Cichlidogyrus tubicirrus minutus from gills of tilapia.

structure in the space between the finger-like projections of the accessory piece. The rest of the reproductive structures - the ovaries, testes, seminal vesicles and vas deferens - were not very clearly distinguishable as they were covered with a dense vitellaria. The vagina was rather distinctive as a short, robust, chitinous organ. Paperna and Thurston (1969) mentioned the fact that in C. tubicirrus, C. haplochromi and C. bifurcatus, the accessory piece of the copulatory organ has a similar structure. The copulatory organ of C. tilapiae is slightly different but, if distorted during mounting, it would be difficult to distinguish it from the other species.

The opisthaptor had two pairs of anchor hooks of equal size. Anchor x was 23-30 μ and anchor v was 27.5 (23-30) μ . The bar x was 30 (23-33) μ and bar v was 51 (42-58) μ . The hooks were very large and rudimentary. They measured from 15-44 μ . The hooks, therefore, do differ from the C. t. minutus described by Paperna and Thurston (1969). This difference could be due to differences in host species. These C. t. minutus were obtained from the gills of Sarotherodon mossambicus, whereas those in the original description were from Tilapia leucostica and Haplochromis quiarti. Table II gives the range of measurements for C. t. minutus on Haplochromis species, described by Paperna and Thurston in 1969, and C. t. minutus from S. mossambicus described by the author.

Host:- S. mossambicus, S. nilotica

Locality:- Aquarium tanks, Institute of Aquaculture, University of Stirling.

Location:- Gills.

REMARKS

The C. t. minutus described here has a distinctive, short, robust, vaginal prop which was not observed by Paperna and Thurston (1969). The length of C. t. minutus was also much smaller than the ones described by Paperna and Thurston (1969), but the rudimentary marginal hooks were much longer. The differences in size could be due to intraspecific variation, or it could be due to different rates of growth of hooks, or the hard parts of the opisthaptor, at different temperatures. Ergens (1975) found that the hard parts of the opisthaptor of Gyrodactylus aphae and G. pannonicus decreased in accordance with an increase in water temperature and vice-versa.

TABLE II. Measurements of C. t. minutus on gills of Haplochromis species and C. t. minutus on gills of Sarotherodon mossambicus.

Host	<u>Haplochromis</u> spp. <u>C. t. minutus</u> Paperna and Thurston (1969)	<u>S. mossambicus</u> <u>C. t. minutus</u> Own material
Dimensions of body and chitin parts		
Total body length	500 - 700	132 - 349
Width	50 - 120	34 - 151
Number of eyes	2 (-4)	4
Copulatory complex		
Cirrus tube	46 - 55	31 - 51
Accessory piece	37 - 43	
Anchor v	22 - 32	23 - 30
Anchor x	26 - 33	23 - 30
Bar v	59 - 73	42 - 58
Bar x	29 - 43	23 - 33
Hook 1st central pair	3 - 11	11 - 15
2nd central pair	12 - 13	25 - 32
distal pair	20 - 26	32 - 44
Number of specimens measured	5	6

- 2.3.3. Suborder Monopisthocotylea Odhner, 1912
 Superfamily Dactylogyridae Yamaguti, 1963
 Family Dactylogyridae Bychowsky, 1933
 Genus Cichlidogyrus Paperna, 1960

Cichlidogyrus tilapiae was first identified by Paperna (1960a). The parasite appears to be somewhat rare as, according to Paperna and Thurston (1969), they also had difficulty finding only one specimen of C. tilapiae on S. mossambicus in their extensive studies.

The length of these specimens of C. tilapiae was 431 (362-500) μ , width 122 μ . This was an average of two specimens. The body was long and slender, the oval opisthaptor was clearly demarcated from the body. Only two eyespots were seen (Fig. 6).

The body was covered with a dense vitellaria, so it was difficult to distinguish the reproductive system clearly. The structure of the copulatory complex was quite evident. The copulatory organ consisted of a long cirrus tube, slightly bent at the tip. Length of cirrus tube was 31 (30-31) μ and it had a funnel-shaped base. The accessory piece, 27 (25-29) μ , consisted of a long, thin plate with folded rims, which arose from the funnel-shaped base, and ended in a curved point.

The basal piece of the cross-shaped bar, or compound bar, was more elongated and divided into three constrictions where the other two appendages were attached, giving the appearance of the x. The v-shaped bar was also slightly higher. The large dorsal anchor hooks measured 32 (29-35) μ and the ventral anchor hooks measured 29 μ . The length of bar x was 26 μ and the length of bar v was 42 μ . The first central pair of hooks was 12 (11-

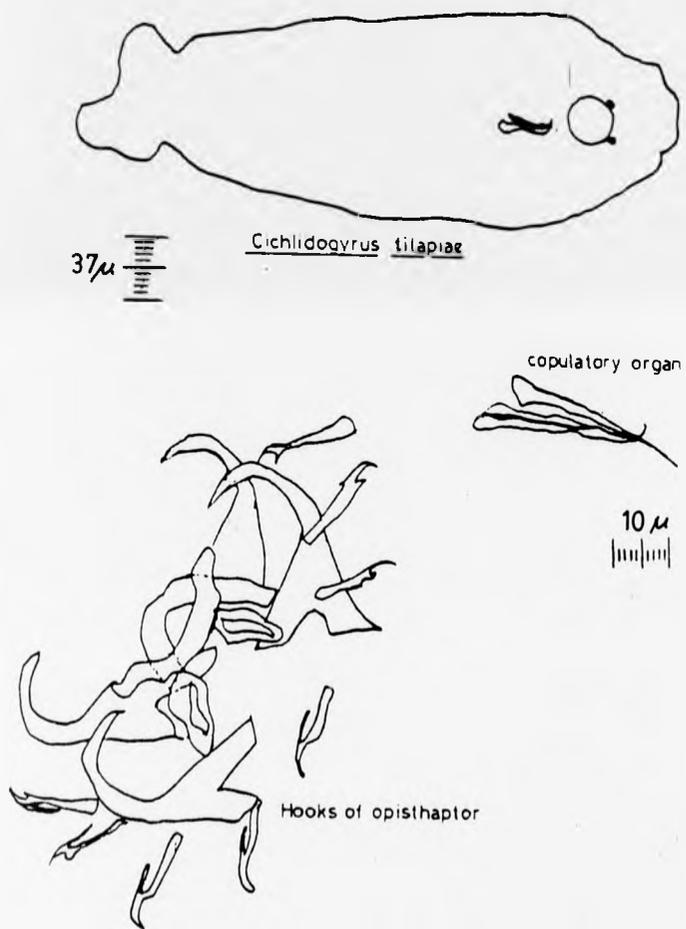


Fig. 6. Drawing of *Cichlidoqyrus tilapiae*, with detail of opisthaptor armature, from gills of tilapia.

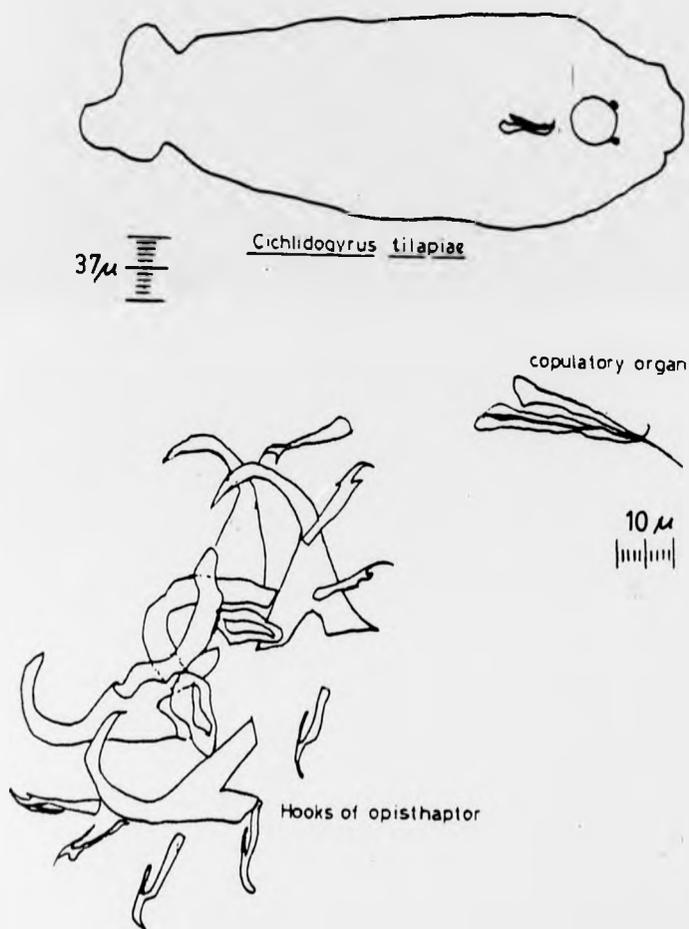


Fig. 6. Drawing of Cichlidoqyrus tilapiae, with detail of opisthaptor armature, from gills of tilapia.

13) μ ; the second central pair measured 13.8 (13.8-13.9) μ , and the distal pair measured 18 (17.5-18.6) μ . Table III shows the range of measurements of C. tilapiae described by Paperna (1960a) and those of the present author.

Host:- S. mossambicus, S. nilotica

Locality:- Aquarium tanks, Institute of Aquaculture, University of Stirling.

Location:- Gills.

REMARKS

The C. tilapiae described here differed from C. tilapiae described by Paperna (1960a) in that the author did not observe the fine sensory hairs extending from the lobes of the prohypostome. Another difference was the absence of symmetrical, tooth-like projections on the internal surface of the v-shaped bar which was present in the archetype described by Paperna in 1960. The host species of C. tilapiae, as described by Paperna (1960a), are Tilapia nilotica, T. gallilea and Tristramella sacra. The fish were located at the Fish Culture Research Station at Dor and the Fisheries Field Station at Ginossar, on the shores of the sea of Gallilee, in Israel.

TABLE III. The measurements of *C. tilapiae*.

Dimensions of body and chitin parts of trematodes	<i>C. tilapiae</i> Paperna 1960	<i>C. tilapiae</i> Kritsky and Thatcher 1974	<i>C. tilapiae</i> Own material
Body length	160 - 509	380	362 - 500
Width	30 - 142	90	122
Number of eyes	4		2
Copulatory complex			
Cirrus tube	19 - 37	29	30 - 31
Accessory piece	22 - 33	31	25 - 29
Anchor v	26 - 33	31	29 - 35
Anchor x	26 - 40	40	29
Bar v	34 - 98	57	42
Bar x	18 - 38	28	26
Hooks 1st central pair	7 - 17)		11 - 13
2nd central pair	13 - 17)	15	13.8 - 13.9
Distal pair	21)		17.5 - 18.6

2.4. Summary

Three species of Cichlidogyrus species have been described from the gills of tilapia kept in aquarium tanks at the University of Stirling. These were Cichlidogyrus sclerosus, C. t. minutus and C. tilapiae. Of these C. sclerosus was the most common species found and C. tilapiae was the most rare. The main differences between the C. sclerosus described by the author and the archetype of Paperma and Thurston (1969) were the presence of the perforation of the anchors and a vaginal prop in the species described by the author as compared to the species described by Paperma and Thurston (1969).

In the case of C. t. minutus, a short distinctive vaginal prop was observed by the author but was not seen by Paperma and Thurston (1969). The absence of fine sensory hairs from the lobes of the prohaptor and the absence of tooth-like projections on the internal surface of the v-shaped bar distinguished the C. tilapiae described by the author from that of Paperma (1960a).

CHAPTER THREE

The reproductive biology of Cichlidoqyrus sclerosus.

3.1. INTRODUCTION

There is only one reference available on the egg-laying, egg development and egg hatching of the freshwater Ancyrocephaline. This is by Cone (1979) who described the hatching of Urocleidus adspectus on yellow perch (Perca flavescens). Cone found that eggs of U. adspectus hatched in 5-6 days at 20°C. Emergence of the oncomiracidium was by mechanical dislodgement of the operculum and swelling of the fluid-filled sacs, the lateral and median head glands playing no role in the hatching process. There was no increase in the size of the eggs during incubation and perch mucus had no effect on egg hatching, which always took place during the hours of darkness.

These findings in the study on a freshwater species contrasted with the study of Macdonald (1974) who showed that host mucus induced hatching of the eggs of the marine monogenean Acanthocotyle lobianchi. Other hatching factors were urea and ammonium chloride (Kearn and Macdonald, 1976). They found that the highest levels of hatching of the eggs of E. soleae generally occurred at urea concentrations of 0.003 to 0.005 μ moles/ml and the threshold for ammonium chloride was between 0.1 and 0.2 μ moles ammonium chloride per ml. of sea water.

With regard to the structure of the oncomiracidia, extensive studies on marine monogenean have been done by Kingston et al. (1969). They studied the morphology of ten species of Monogenea from sixteen species of fish from the Chesapeake Bay area. They showed that, in the Monopisthocotylean oncomiracidium,

there were four pigmented eyespots, the opisthaptor al armature had fourteen marginal hooks and the cilia were situated in three zones: the anterior, equatorial and posterior cone.

The difference in specific morphology of eggs and larvae could also be of value in taxonomic investigations. Khotenovskii (1975) showed by silver impregnation that the position of ciliated cells and sensills of larvae appeared to differ from species to species of the freshwater Diplozoon he studied.

Apart from this, most studies on the reproductive biology of monogeneans have been either on marine species (Kearn, 1963; 1965; 1967a; 1974b) or mainly on freshwater dactylogyrids, typically D. vastator (Paperna, 1963a), D. anchoratus, and D. extensus (Prost, 1963). In all the latter studies it was found that temperature was an important factor in egg laying and egg development. Prost (1963) found that Dactylogyrus eggs were more susceptible to dessication and freezing, a point of great practical importance in the management of fish ponds (although the latter feature is unlikely to have much relevance in the tropics). In Israel, Paperna (1963a) found that eggs of D. vastator hatch quickly, that is within 2-3 days, and that optimal proliferation of the parasite occurred at 22^o to 24^oC, the rate of egg development of the parasites being linear with temperature.

Ammonia and oxygen concentration are two parameters which do not seem to affect propagation of D. vastator (Paperna, 1963a) although, in the case of the marine Dicliphoran, D. merlangi, its egg production decreased when pressure of oxygen in the surrounding environment fell (Houlihan and Macdonald, 1979). This is probably because D. merlangi lives on the gills of a

pelagic fish in which the gills are always well saturated with oxygen.

Since there are very few studies on the reproductive biology of freshwater monogeneans and, as the biology of C. sclerosus has not been studied, the following study was carried out. As there is no information as to the size and structure of the egg, the author had to carry out a series of experiments to locate the eggs. Once these were located it was relatively easy to carry out the series of observations described below, which enabled its reproductive biology to be elucidated.

3.2. MATERIALS AND METHODS

3.2.1. Preliminary experiments to locate the eggs of C. sclerosus.

Initially, water was siphoned out of tanks containing infected fish, the water centrifuged till only a small portion was left, and the deposit examined under a dissection microscope. However, large amounts of detrital material rendered differentiation and enumeration of the eggs of the monogenean difficult and the technique was abandoned.

The next method to be investigated was the zinc sulphate flotation method (see Appendix 1A). The water from tanks containing infected fish was centrifuged, ether was added, it was centrifuged again and the supernatant liquid decanted. Water was added to the deposit and it was centrifuged a third time. The supernatant liquid was again decanted. The deposit was transferred to another centrifuge tube, zinc sulphate added till the surface formed a convex meniscus at the mouth of the tube, and it was allowed to stand for 10 minutes. A coverslip was placed on the surface, to remove the floating particles, and then placed on a

slide with a drop of Lugol's iodine. This method was fairly successful in locating the eggs and allowing their identification, as no faecal material was present.

Another method employed was the Formol-ether technique (see Appendix 1B). Ether was added to the water siphoned out of infected tanks. This was centrifuged, the fatty layer at the interface was loosened, and the supernatant liquid was poured away till only the deposit remained. The deposit was mixed, placed on a slide, and examined under the low-power objective of a microscope. The eggs were also easily located using this method. There was, however, a major disadvantage with these two methods in that the presence of the chemicals appeared to affect the viability of the eggs. None of the eggs collected by these two methods showed any development during incubation.

Finally, it was decided to place surgically-excised, infected gills in Petri dishes and determine whether the C. sclerosus on them could lay eggs. This was the most successful method of all, for the C. sclerosus not only laid the eggs, but these were viable and the method made the observation of live eggs and their hatching mechanism possible.

3.2.2. Observation on the egg-laying behaviour of C. sclerosus, the formation of eggs and time intervals in the formation of an egg.

The behaviour of C. sclerosus, during egg laying, was studied thus: The observations were made on 5 parasites. These parasites were arbitrarily assigned letters A, B, C, D and E. These worms were observed laying eggs on excised gill arches in Petri dishes, with constant illumination from above. The dishes

were placed on a heated mat, which maintained the temperature of the water in the Petri dish at 25°C. The time intervals from the formation of the egg to its extrusion from the worm's body was also determined.

3.2.3. Egg development and egg hatching of C. sclerosus.

The structure of the egg of C. sclerosus, its embryonic development and hatching at 25°C under laboratory conditions, the general features of the oncomiracidium and its behaviour after hatching, were all observed and described.

3.2.4. Experiments on the fecundity of C. sclerosus on excised gill arches of S. mossambicus and S. spiluris in Petri dishes.

Infected gill arches 1-4, excised from each side of the fish's head, were placed in Petri dishes containing copper-free water. The Petri dishes were placed on a bench and illuminated. The temperature for each dish was 25°C. The gill arches were observed for up to 7.5 hours.

3.2.5. Experiments on the effect of host mucus, pH and urea on the egg development and hatching of C. sclerosus.

In these experiments, batches of eggs were obtained from C. sclerosus which were allowed to lay eggs on excised gill arches. These eggs were collected using thin Pasteur pipettes, and placed in embryo dishes. Since each individual of C. sclerosus laid its eggs over a limited period of time, it was difficult to collect large batches of eggs at any one time.

The eggs were incubated in a dessicator jar, held at 25°C in an open water bath. A light, fitted with a time switch

to give a twelve hour photoperiod, illuminated the incubator system.

Preliminary experiments were first carried out to see which medium was best for egg development. The eggs were put into embryo dishes containing distilled water, artificial spring water (see Appendix 1C for its constituents) and physiological saline. On the following day, host mucus and associated epithelial cells were obtained by scraping lightly the dorsal surface of the bodies of five fish.

Ten eggs were placed in embryo dishes containing either artificial spring water, or copper-free water, with and without host mucus. Each embryo dish contained 10 ml. of the medium. 0.5 ml. of host mucus was added.

In all these experiments, the temperature of the incubator was kept constant at 25°C. This temperature was chosen because it was the temperature of the tanks in the tropical aquarium, where the stock fish and infected fish were kept. It was found, incidentally, that, on one occasion, when the incubator broke down and temperatures fell to 18°C, there was no further development in the eggs.

In the next group of experiments, urea was placed in the embryo dishes and these were incubated at 25°C and subjected to alternating 12 hour periods of light and darkness. The urea concentrations ranged from 0.01 to 0.1 μ mole/ml.

Finally, copper-free water at different pHs was used. The pH was altered by mixing acetic acid buffers and phosphate buffers (refer Appendix 1D for concentrations used). These were also incubated at 25°C, again in a twelve hour photoperiod.

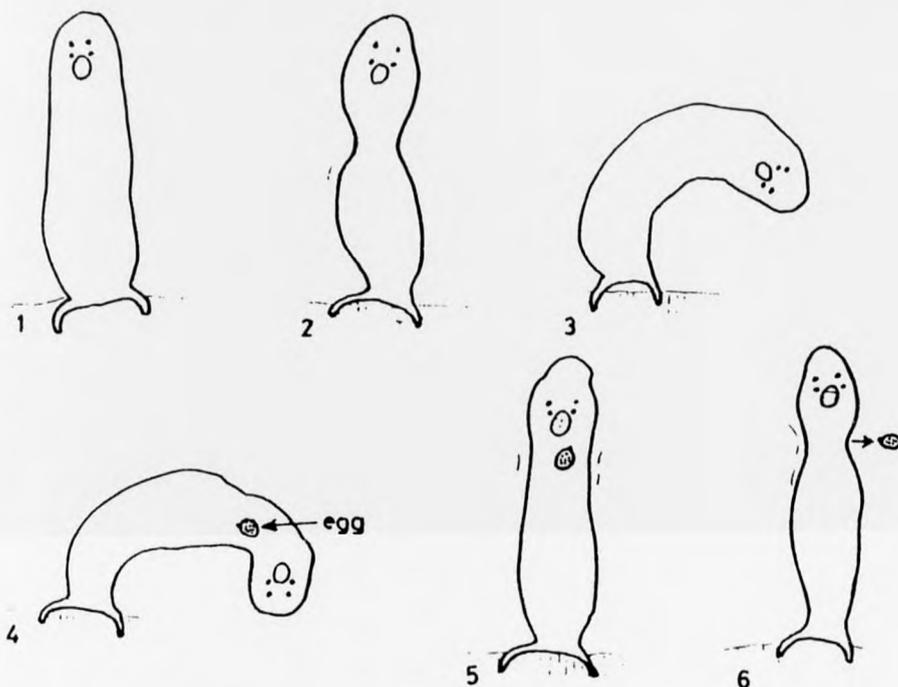
3.3. RESULTS

3.3.1. Behaviour of C. sclerosus during egg laying (Fig. 7)

The following observations were made on ten specimens of C. sclerosus. When a parasite was examined on a gill arch it exhibited a series of random searching movements. Occasionally it exhibited a series of contractile movements. After these series of contractile movements the parasite bent its body over, till the anterior end touched the gill filaments. If examined under the low-powered microscope (20X) the yolk cells are seen moving down the vitelline duct to the common vitelline duct. They then pass to the ootype where they combine with an ovum to form a colourless ovoid structure. In the ootype the series of peristaltic movements send the egg forwards and backwards while the shell is formed. From the ootype it is pushed into the uterus. Here the egg appears tanned with a short stalk or filament at one end. After another series of rapid contractile movements the parasite straightened itself and continued behaving normally, that is it exhibited random searching movements and feeding on mucus. After several minutes it elongated, straightened its body and the egg appeared at the genital pore. The tip of the cirrus tube of the accessory piece could also be seen projecting at this opening. The egg was released when the worm again contracted and elongated its body suddenly.

3.3.2. Time intervals in the formation of an egg.

The body of C. sclerosus was very transparent and it was possible to observe the egg being formed in the uterus and to see it being released by the parasite, after specific time intervals as shown below. Here the parasite had been removed from the gills

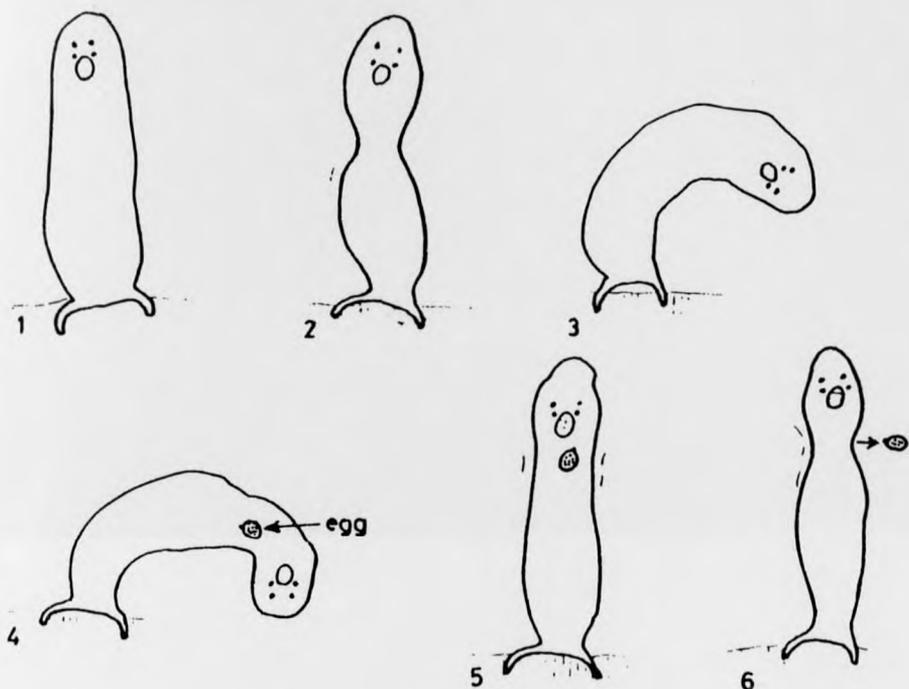


Behaviour of C. sclerosus during egg-laying.

Fig. 7. Behaviour of C. sclerosus during egg laying.

Legend for Fig. 7

1. C. sclerosus at the start of contractile movements.
2. Rapid series of contractile movements.
3. C. sclerosus bending over and anterior end touching the gill filaments.
4. The egg appearing as a colourless ovoid structure.
5. C. sclerosus straightening itself; egg becoming more tanned and appearing at the genital pore.
6. Expulsion of egg after sudden contraction and elongation of the body of C. sclerosus.



Behaviour of C. sclerosus during egg-laying.

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5. C. sclerosus straightening itself; egg becoming more tanned and appearing at the genital pore.
6. Expulsion of egg after sudden contraction and elongation of the body of C. sclerosus.

and observed on a slide under the microscope.

The following table refers to 25 observations made on 5 worms arbitrarily called A, B, C, D, E.

For this experiment, the mean average time taken for production of each egg was forty-five minutes. However, the results for individual parasites seemed to indicate they varied as regards the time of their egg laying. The shortest time an individual parasite required to lay one egg was 15 minutes and the maximum time was more than an hour.

TABLE IV. The time intervals, in minutes, between egg formation and egg laying of five specimens of C. sclerosus observed in situ.

Period of observation	Specimens of <u>C. sclerosus</u>					Average time (in minutes)
	A	B	C	D	E	
1st	15 min	48 min	50 min	85 min	55 min	50.6
2nd	30 min	25 min	nil	nil	35 min	30.0
3rd	62 min	nil	nil	nil	nil	62.0
4th	28 min	27 min	25 min	nil	40 min	30.0
5th	60 min	45 min	63 min	35 min	50 min	50.6
Total	195 min	145 min	138 min	120 min	180 min	

3.3.3. Egg development and egg hatching of C. sclerosus.

The egg of C. sclerosus, when first laid, was operculate, yellowish-brown in colour, ovoid and measured 62μ long and 50μ wide, with a thin stalk-like appendage at one end. The freshly-laid egg contained a lot of vitelline material. As the embryo developed, the length of egg increased so much so that, by the time of hatching, it had reached 86μ long.

When first laid, the egg had a dense mass of vitelline cells, which seemed to be slightly separated from the edge of the egg shell (Fig. 8 diagram a). On the second day of incubation, the embryo appeared as an aggregation of cells (diagram b). These cells appeared to be in constant motion. Later, on the second day, the mass of cells began to take the shape of the oncomiracidium. At three days the size of the eggs averaged 76μ long and 60μ wide and 4 eye spots were apparent on the embryo (diagram c). The hooks and cilia were hardly discernible at this stage. The vitelline cells were also very much reduced but a large clear sac was visible, between the opisthaptor and the anterior end of the parasite (diagram e). The rhythmic beating of the cilia pushed the vitelline cells towards the periphery, such that they appeared to line the operculum (diagram f). The elongation and contraction of the embryo appeared to take place every 5 minutes or so. Occasionally, it would move very violently. The cilia appeared to move in alternate directions quite frequently.

It was observed that, prior to hatching, the oncomiracidium appeared to thrust its anterior end towards the opercular pole. The anterior end was constricted and whether, at this time, there were secretions from the head glands was not

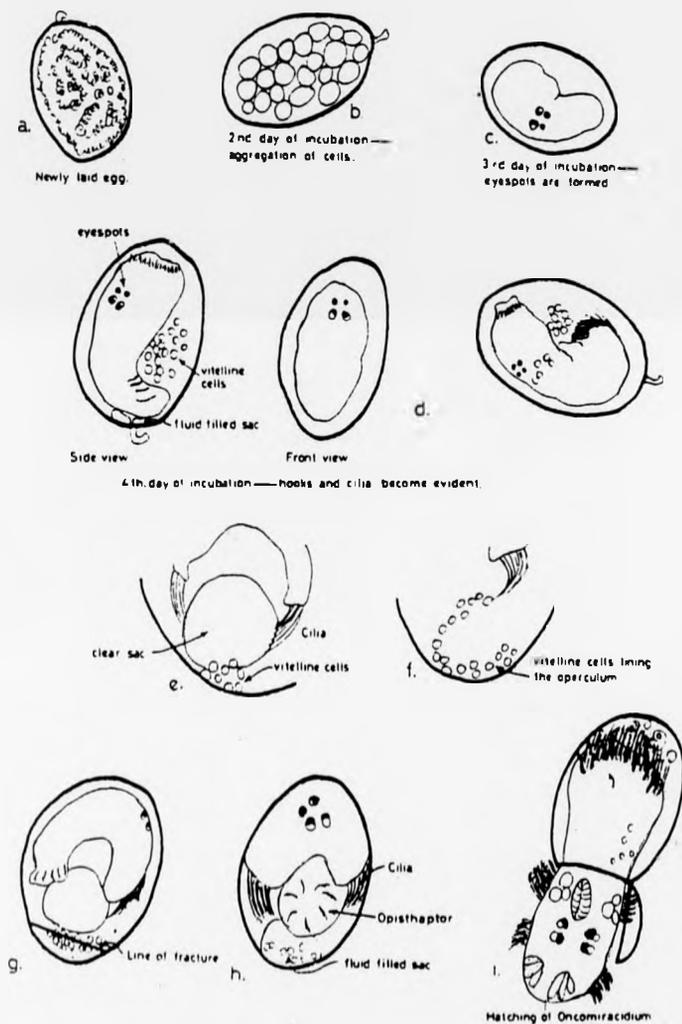


Fig. 8. Diagrams showing the development of the egg of *C. sclerosus*, from the first day of incubation to the hatching of the oncomiracidium. (Not to scale)

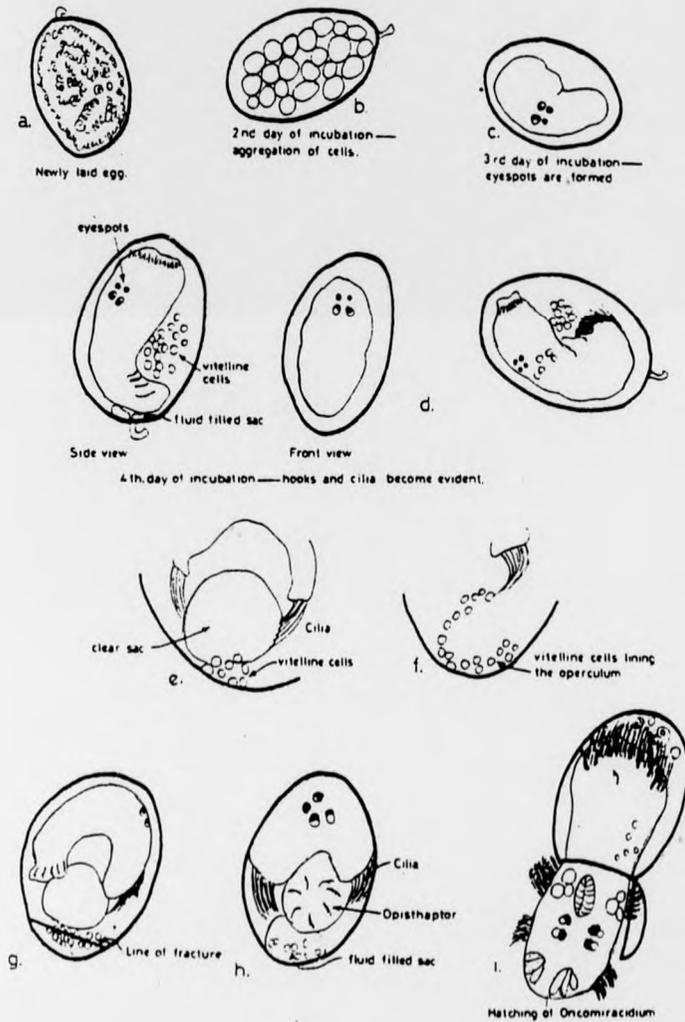


Fig. 8. Diagrams showing the development of the egg of *C. sclerosus*, from the first day of incubation to the hatching of the oncomiracidium. (Not to scale)

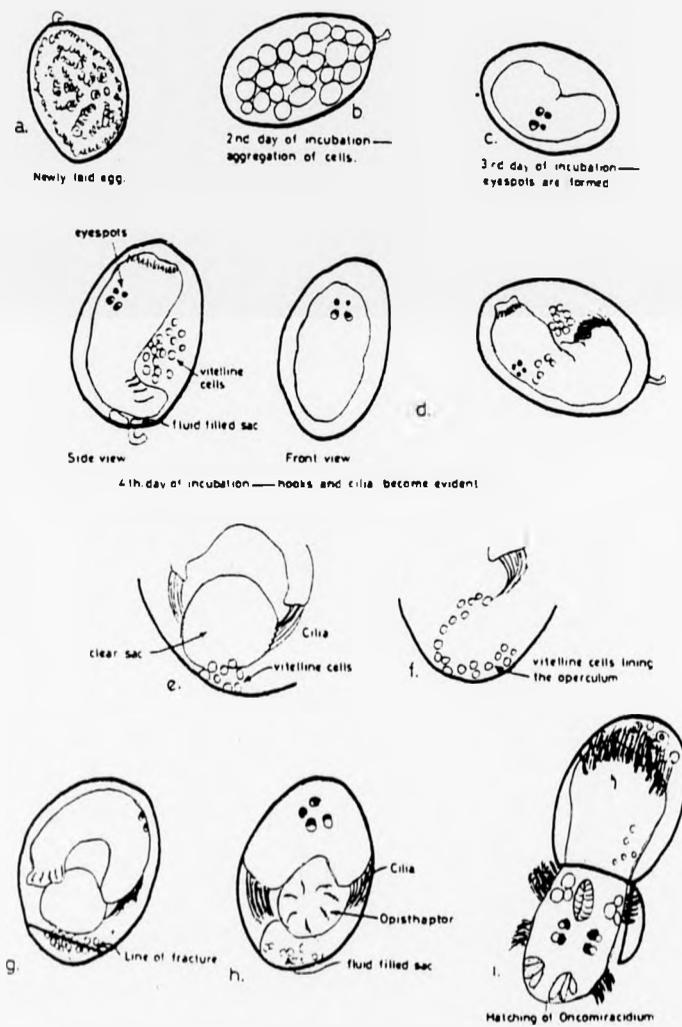


Fig. 8. Diagrams showing the development of the egg of *C. sclerosus*, from the first day of incubation to the hatching of the oncomiracidium. (Not to scale)

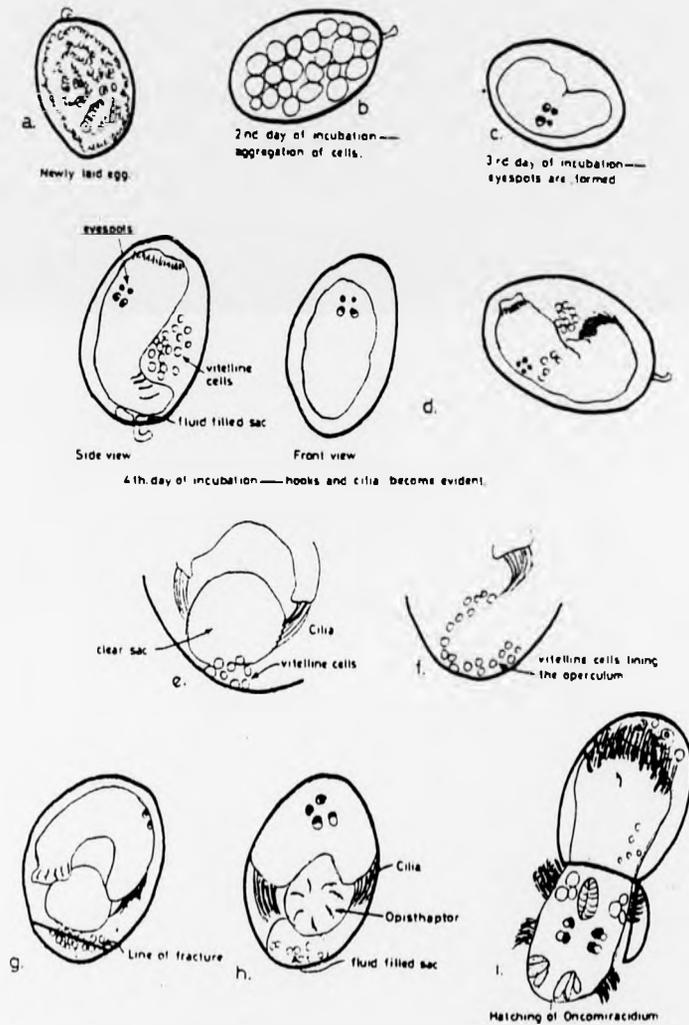


Fig. 8. Diagrams showing the development of the egg of *C. sclerosus*, from the first day of incubation to the hatching of the oncomiracidium. (Not to scale)

known.

The cilia of the oncomiracidium beat rapidly and continuously, its body elongating and contracting rapidly; the line of fracture appearing more and more evident, till eventually the operculum hinged open. The oncomiracidium squeezed its anterior through the opercular opening, the rest of its body wriggled through and the swelling of the large vitelline sacs pushed the oncomiracidium out. The period of hatching was very short and many times the actual hatching movement was missed, even after hours of waiting.

3.3.4. Description of the oncomiracidium.

The oncomiracidium was elongated and cylindrical to almost pyriform in shape. It averaged 158.1μ long and 55.8μ wide and was slightly narrower in the mid-region.

There were four pigmented eyespots. Each eyespot had a crystalline lens. The eyespots were found anterior to the pharynx, which averaged 23.25μ long and 13.95μ wide. On either side of the pharynx were groups of cells, which could well represent the flame cells of the excretory system. At the anterior end were ducts of the head glands.

Tufts of cilia were found at the anterior end, the mid-region and the posterior end of the oncomiracidium. Fourteen large hooks were seen in the opisthaptoral region, the two small hooks being in the centre.

3.3.5. Behaviour of the oncomiracidium.

The following observations were made on ten oncomiracidia:

After emerging from the egg shell, the oncomiracidium swam quickly in a straight line and it appeared to be photopositive, initially. When it was examined in an embryo dish, using the stereo microscope, it appeared to exhibit gyrating movements upwards, whilst swimming around in a spiral.

When an S. zilli fry was introduced in an embryo dish, containing a free-swimming oncomiracidium, the latter stopped swimming, and only started moving again after 5 minutes. It started swimming again and, as the tilapia fry started beating its pectoral fins, a water current was created. The fry also made swallowing movements, which created a water current going into its buccal cavity. The oncomiracidium, in the course of its swimming, appeared to follow, or be swept along by, the water current. Once in the flow of the water the cilia stopped beating and the oncomiracidium was engulfed in the water current flowing into the buccal cavity of the fish. This could well be the method of infection of the gills. The life of the oncomiracidium was not very long. Between 45 minutes to an hour the oncomiracidium usually stopped swimming and descended to the bottom of the Petri dish where it lay motionless, in a moribund state.

In a few cases, the oncomiracidium descended to the bottom of the dish and shed its cilia. It then made looping movements in the dish. This was similar to the behaviour of D. paradoxum larvae as described by Bovet (1967).

The juvenile of C. sclerosus also appeared to exhibit looping movements on the gill filaments. The juveniles were small in size, less than 230 μ , and did not have any visible reproductive structures. The vitellaria appeared rather faint and scattered.

The juveniles were found on the outside edge of the gill filaments. On several occasions, the author observed them moving very quickly and usually migrating towards the basal area of the filaments, near to the gill arch.

However, in the case of C. sclerosus, the embryo dish examination did not enable the author to observe development of the oncomiracidium to the juvenile stage, as the non-ciliated larva did not survive very long in the dish. It succumbed after approximately forty-five minutes.

3.3.6. The egg production of C. sclerosus on excised gill arches.

The raw data for this experiment is shown in Appendix 2. The overall results were as follows:-

TABLE V. A summary of the results of egg production of C. sclerosus on excised gill arches of S. spiluris and S. mossambicus at 25°C.

Fish species providing gills	Time intervals in hours	Number of <u>C. sclerosus</u>	Number of eggs	Mean egg per <u>C. sclerosus</u> per hour	Number of eggs per 24 hours per <u>C. sclerosus</u>	Standard deviation	Fecundity	Standard deviation
<u>S. spiluris</u>	5.50	36	128	0.64	15.27	6.17	3.50	1.41
<u>S. spiluris</u>	5.50	31	74	0.50	11.97	9.88	2.74	2.26
<u>S. mossambicus</u>	7.50	3	5	0.30	7.20	7.92	2.25	2.47
<u>S. mossambicus</u>	4.25	6	17	0.78	18.64	10.49	3.30	1.86
					Average	Average	Average	Average
					13.27	8.62	2.95	2.00

The fecundity here is defined as the ratio of the number of eggs to the number of egg laying parasites, or the number of eggs laid by one parasite in a particular period of examination. The fecundity results in the above table have been obtained from Appendix 2.

The results show that the average fecundity of C. sclerosus was 2.95 ± 2.00 . Average egg production per 24 hours was 13.27 ± 8.82 , a very wide range.

A correlation analysis, carried out to determine the intensity of association between the number of parasites and the number of eggs collected, gave a significant value ($r_{12} = 0.757$; $P < 0.001$). The scattergram is shown in Figure 9.

3.3.7. The results of the effect of host mucus, urea and pH on egg development and egg hatching of C. sclerosus.

In the preliminary experiments, carried out to determine the best medium for the development of eggs, after five days there was no development in the eggs kept in distilled water, artificial spring water, physiological saline or copper-free water. One day after addition of host mucus, there was a slight change in eggs kept in artificial spring water and copper-free water. There was rapid embryonic development with eggs kept in artificial spring water and copper-free water with mucus added to both. After nine days of incubation the embryos were fully formed, in artificial spring water with host mucus and copper-free water with host mucus. There was 100% hatching in the copper-free water and only 60% hatching from the artificial spring water.

In a subsequent experiment, batches of ten eggs each were incubated in the embryo dishes, containing artificial spring

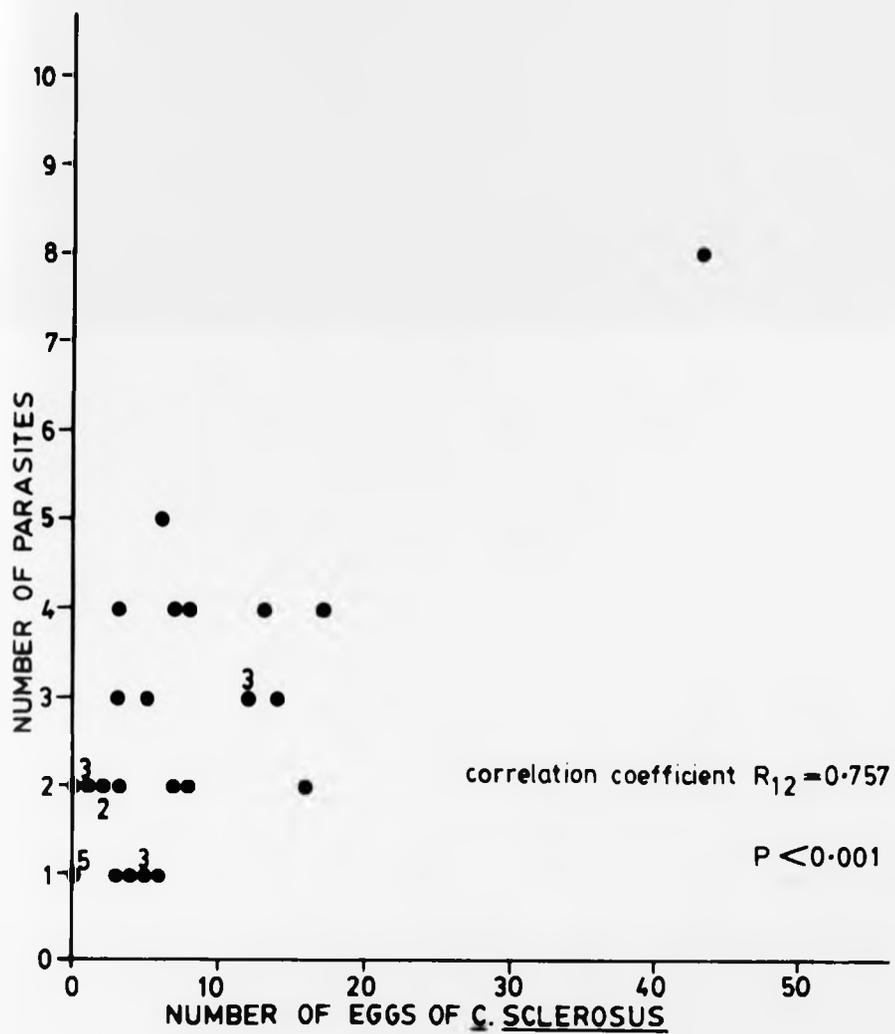


Fig. 9. Scattergram for number of *C. sclerosus*, on excised gill arches, against number of eggs.

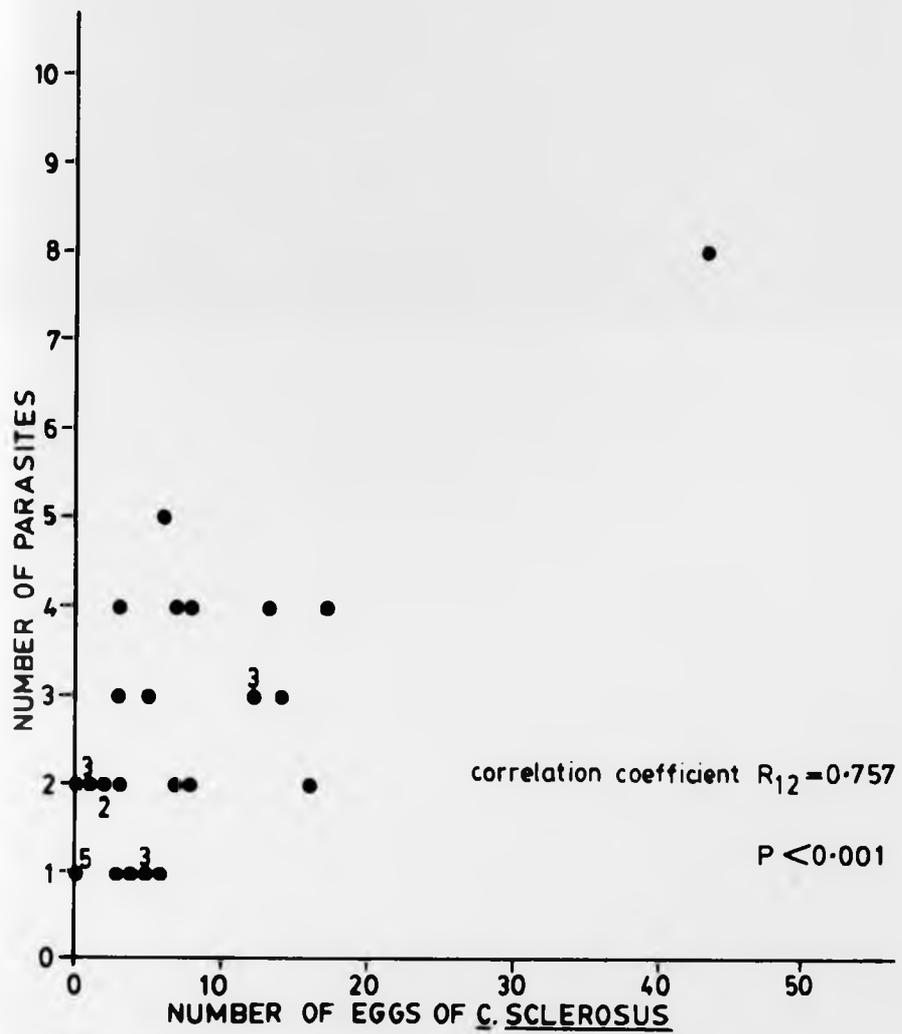


Fig. 9. Scattergram for number of *C. sclerosus*, on excised gill arches, against number of eggs.

water and host mucus and copper-free water and host mucus. After four days, eyespots were formed in one-half of the eggs kept in artificial spring water and host mucus, while all eggs kept in copper-free water and host mucus had fully developed eyespots. After nine days all eggs hatched out in copper-free water, whereas only seven hatched out of artificial spring water. There was no development in the controls which consisted of artificial spring water and copper-free water but with no mucus added. The results are shown in Table VI.

TABLE VI. Effects of host mucus on development of eggs of C. sclerosus.

Medium	Interval in days	Number of eggs	Stage of development
Artificial spring water and host mucus	4	10	Eyespots formed in 5 eggs.
Copper-free water and host mucus	4	10	Eyespots formed in all eggs
Artificial spring water and host mucus	9	10	Larva fully developed. Only 7 hatched out.
Copper-free water and host mucus	9	10	All larva hatched out.

3.3.8. The effect of different concentrations of urea on egg development and egg hatching of C. sclerosus.

In a preliminary experiment, out of twenty eggs incubated in copper-free water, only six (30%) hatched out after six days. Urea was then introduced and, on the following day, fourteen (70%) hatched out. Hence, in a subsequent experiment, different concentrations of urea were used to see if there was a threshold at which most eggs would hatch out. The results are shown in the following table.

TABLE VII. The effect of different concentrations of urea on egg development and egg hatching of C. sclerosus.

Concentration of urea in μ moles/ ml	Interval in days	Number of eggs incubated	Numbers hatched	% hatching
0.010	5	17	11	64.71
0.025	5	6	3	50.00
0.033	5	10	6	60.00
0.050	5	15	12	80.00
0.100	5	20	12	60.00

In this experiment the number of eggs varied. This was because of the difficulty of obtaining the eggs individually. The eggs normally were entangled in the mucus of the excised gill arches. Each egg was picked up using the drawn out end of a dropper pipette. When the eggs were entangled in the mucus, it was difficult to pick out single eggs. Hence the tendency was to pick them up in batches, entangled in a blob of mucus, and put

into each embryo dish.

Another experiment was carried out, whereby a batch of five eggs were placed in each of a series of cavity blocks, using different concentrations of urea. The medium was aerated copper-free water. The concentrations of urea used were 0.01, 0.03, 0.05, 0.075 and 0.1 μ mole/ml. The average results for the three days were as follows:-

Concentration of urea in μ moles/ml.	% fully developed larvae	% hatching
0.01	20.0	20
0.03	40.0	35
0.05	20.0	40
0.75	40.6	no results
0.10	55.0	50

3.3.9. Egg development at different pHs.

Eggs were placed in water of varying pH in cavity slides. The different acidity levels were obtained by means of acetic acid buffers and phosphate buffers (refer Appendix 1D). As the cavity slide was small, only one egg was kept in each cavity slide. The pH of the media ranged from 4 to 7.6 at 0.2 intervals. Hence there was a total of 19 eggs in one experiment. Three groups of experiments were carried out. The eggs only developed in pH 5.6 to 7.6 but, though developed, they did not survive to hatching.

The effect of pH was also studied in a second experiment. For three days, 3 batches of twelve eggs each were kept in pH 4 to 7.6. All eggs were partially developed in pH 7.6. After four days, pH 7 had one (8.3%) fully developed larva and eight (66.6%) were still developing. Therefore pH 7 - 7.6 were

found to be favourable for development of the eggs.

3.3.10. Effect of higher pH levels on egg development and hatching of C. sclerosus at 25°C.

For higher pH levels, only sodium bicarbonate buffer and sodium phosphate buffer could be used. A batch of fifteen eggs incubated in copper-free water at pH 7, was examined after five days. It had ten (66.6%) fully developed larvae.

In the medium of distilled water and sodium bicarbonate buffer at pH 7.6, eleven (73.3%) had fully developed larvae, but of these, only two hatched out. In distilled water and sodium phosphate buffer giving a pH of 7.6, four out of fifteen were fully developed (26.6%) but none hatched out.

The effect of higher pH levels was determined in a second experiment. Batches of fifteen eggs were placed in copper-free water pH 7, sodium bicarbonate and distilled water pH 8, and sodium phosphate and distilled water pH 8. At the end of five days, the medium of sodium bicarbonate and distilled water had fourteen fully developed larvae, whereas copper-free water and sodium phosphate medium had twelve fully developed larvae. The results are shown in Table VIII.

TABLE VIII. The effect of higher pH levels on egg development and egg hatching.

Medium	Copper-free water	Na HCO ₃ + distilled water	Na PO ₄ + distilled water
pH	7	8	8
Number of eggs	15	15	15
Number of fully developed larvae	12	14	12
Numbers hatched	12	13	6
Percentage of hatching	80	86.6	40

3.4. DISCUSSION

The process of egg formation of C. sclerosus is similar to that of Microcotyle spinicirrus described by Remley (1942). The only difference is the absence of a genito-intestinal canal in C. sclerosus, hence the yolk cells and ovum do not combine in the genito-intestinal canal but in the common vitelline duct before moving into the ootype. The elongation and constriction of the body of C. sclerosus is equivalent to the rapid peristaltic movements of Microcotyle spinicirrus. At this stage the yolk cells are being pushed into the developing egg in the ootype. It is possible that, when enough vitelline cells have been pushed into the developing egg, the opening is sealed by a short stalk or polar filament.

Llewellyn (1957) suggested that the presence of polar filaments could be the result of accumulating capsules in the uterus of the parent. There is no evidence to indicate that the polar filament is used for attachment of eggs to the fish gills. Over 900 fish were examined (in the site-specificity experiment) and no eggs were found to be actually attached to the gills.

The average time taken for C. sclerosus to lay an egg was forty-five minutes. This time is much greater than D. vastator which, according to Ljajman (1951), takes about 11.6 minutes. But the number of eggs laid by D. vastator depends on temperature and on the age of the worm. D. vastator 8 to 9 days old produces, on the average, from 1 to 3 eggs/day. The rate of egg production reaches its peak in worms 11 to 13 days old, which produce 5-15 eggs. After that age the egg deposition gradually diminishes but there is one strong increase, just prior

to the death of the worm. Unfavourable conditions, such as low oxygen level, rise in temperature and death of hosts result in increased egg deposition. This, according to Iziunova (1953b) is a typical feature of parasites which reacts to unfavourable conditions by increasing fertility and ensuring the survival of the species.

In the fecundity experiments, the fecundity level of C. sclerosus obtained from observations on parasites laying eggs on excised gill arches, gave an average value of 2.95. As the parasites were still feeding on the excised gill arches, this could approximate conditions close to a natural situation. Increased egg production occurred when the parasite was removed from the gills and placed on a glass slide under a coverslip, which constituted an artificial environment (Ljaiman, 1951).

Ljaiman (1951) showed that D. vastator laid 5.17 eggs/hour at 28°C. However, he measured the egg production rate of parasites removed completely from the gills, that is, under completely artificial conditions. Hence the egg production rate he obtained was much higher than that obtained by Paperna (1963a), who obtained for D. vastator an oviposition rate of 1.68/hour at room temperature of 28°C. Paperna carried out his experiments under natural conditions, that is, from the gills of live fish kept in little beakers. He based his experiments on the work of Iziunova (1953b) who showed that oviposition in natural conditions was slower than if the parasite was removed from the gills.

There are also contradictions about the effect of O_2 concentration in the water on egg production of some monogenetic trematodes. Iziunova (1958) found that the egg production of D.

vastator was not affected by the oxygen concentration in the water. Houlihan and Macdonald (1979), on the other hand, showed that the respiration rate of D. merlangi decreased as the oxygen concentration of the water declined, and the egg production virtually ceased as the oxygen pressure fell below saturation. D. merlangi lives on the gills of a pelagic fish (Merlangus merlangus) and, therefore, the parasite normally has water saturated with air passing over it.

The parasites may themselves be regulating their rate of egg production. It was observed that C. sclerosus was very active at egg laying time. These active movements call for the expenditure of energy and increased respiration. The oxygen usage of the parasite could be safely increased where higher oxygen levels occur to allow greater energy for egg production.

Embryonic development of the eggs of C. sclerosus is similar to that described by Cone (1979) for the Ancyrocephalid U. adspectus, the only difference being that eggs of C. sclerosus increased in size considerably from time of egg laying to time of hatching, whereas the eggs of U. adspectus remained constant in dimensions. Paperna (1963a) reported that eggs of D. vastator also undergo swelling. This could well be due to an osmotic hatching mechanism (Davis, 1968). This is said to be the case in the digenean, Fasciola hepatica (Wilson, 1968). He found that the viscous cushion increased in size with increase in salinity. The expansion of the cushion must therefore produce considerable expansion within the egg. There would thus be a pressure build-up prior to hatching and this build-up could be due to the slow expansion of the sacs, due to water entering from the external medium, or to slow expansion of the cushion, due to the same cause. The hatching mechanism is activated by the receipt of an

external stimulus, such as light, and the opercular seal gradually weakened by a proteolytic hatching enzyme (Rowan, 1956).

An alternative hypothesis to explain hatching has been offered by Wilson (1968), who speculated that the mechanism is as follows:

- (a) the miracidium is stimulated into activity by light;
- (b) the movements of the miracidium changes the internal surface of the viscous cushion so it becomes more permeable to the fluid egg contents and becomes hydrated; the increased internal pressure finally breaks open the operculum. This could possibly be the mechanism of hatching of C. sclerosus.

It was observed that eggs placed in copper-free water, without host mucus, did not develop, but eggs placed in the same medium containing host mucus, or which had been exposed to the host, do develop. In the presence of host mucus, the oncomiracidium appears to be more vigorous. Hence host mucus could stimulate the oncomiracidium into vigorous activity. According to Macdonald (1974) the host mucus could dissolve the opercular cement or activate the larva which, in turn, secretes a hatching enzyme, capable of softening the opercular cement, or cause the larvae to extend its body and push off the operculum.

It is noted that monogenean eggs seem to hatch much faster at a higher temperature of incubation. U. adspectus hatched after 5-6 days incubation at 20°C (Cone, 1979) whereas C. sclerosus took only 4 days at 25°C. Prost (1963) found that the development of larvae of D. anchoratus eggs lasted 7-8 days at 15.5 to 17°C and 3-4 days at 19-20°C. Paperma (1963a) found that D. vastator hatched 2-3 days following oviposition when

conditions were optimal.

On the contrary, hatching can also be inhibited by osmotic pressure, darkness and high body temperature. In the digenean, Schistosomatidae, hatching is inhibited by an osmotic pressure over about $\Delta = 5^{\circ}\text{C}$ and by the absence of light. When the infected faeces are diluted with water, hatching rapidly occurs. Osmotic pressure has an even more marked inhibitory effect. Hatching of eggs of Schistosoma mansoni is almost completely inhibited by 0.6% NaCl. Extensive hatching occurs only until dilution of 0.1% is reached. Hence, eggs in blood, gut contents or urine will only hatch on reaching water. The mechanism of inhibition is not known.

Of interest in this respect is the miracidia of Opistorchis felineus, a digenean which has an asymmetrical internal organization and is fully developed when the egg is laid. Hatching does not occur in the water but only after ingestion by certain snails. This is probably to ensure that hatching occurs in the right intermediate host, hence ensuring the survival of the species (Smith, 1976).

In some monogeneans it has been shown that the free-swimming larva invades the new host by means of an intermediate host. Bychowsky and Nagibina (1967) have found that two narrowly specific Gastrocotylids, Pricea multae and Gotocotyle species, were found in abundance on the gills of their respective fish hosts, Scomberomorus commersoni and Sawara nipponica. The juvenile worms were not found but the young gastrocotylids were found on a variety of small fish (29 species belonging to 16 families) throughout the year. All the young parasites had the same

structure and similar haptors. The smaller parasites moved actively over the gills but become less mobile as they grew older and settled at the distal end of the primary lamella. The host tissue grows around the parasite, leaving the anterior third of the body projecting. There was no degenerating larvae nor empty craters. Hence it was supposed that the juveniles live for some time embedded in the gills and are in a state of suspended development. These larvae are the juveniles of Pricea and Gotocotylea which live on large predatory fish when no young juveniles are found.

The behaviour of the oncomiracidium within the egg, prior to hatching, was similar to that described by Cone (1979). The oncomiracidium expanded and contracted its body, with the anterior end thrusting towards the operculum. Many authors have reported mechanical dislodgement of the operculum by thrusting movements of the oncomiracidium (Frankland, 1955; Bychowsky, 1957; Bovet, 1967; Owen, 1970; Molnar, 1971a and Macdonald, 1974).

The oncomiracidium occasionally rotated on its axis as was observed by Kearn (1975) in his study of E. soleae. Kearn also described the presence of head glands which disappeared soon after the larva hatched.

In the case of C. sclerosus, it was not evident whether head glands helped in hatching. The glands were seen in the newly-hatched oncomiracidium and while it was still in the egg, during the periods of elongation and contraction, the anterior end of the worm did constrict (Fig. 8d). Whether, at this time, the glandular secretion was exuded is not known.

The oncomiracidium of C. sclerosus was photopositive

at first, similar to those monogeneans described in the literature (Bychowsky, 1957; Llewellyn, 1972; Cone, 1979; and Kearn, 1980). The period of swimming in a straight line probably occurred once it had detected a host and was swimming towards it. Once in the flow of currents going into the buccal cavity of the fish, the larva stopped swimming and was gently drawn in, similar to the monogenean larvae described by Bovet (1967). Using a stereomicroscope he observed that Diplozoon paradoxum larvae increased its speed of swimming on reaching the respiratory current of its host fish Rutilus rutilus and Abramis brama. Once in the gill-ventilating current it stopped swimming and was gently drawn into the buccal cavity. He never found the larvae occupying the skin of the hosts. Kearn (1968a) has suggested that the oncomiracidia and juveniles of some diplectanid, tetraonchid and dactylogyrid gill parasite migrate from the body surface to the gills of the host fish. Prost (1963) found two routes of invasion for D. anchoratus invading young carp. In young fish up to a month old, 10 mm long, invasion was by way of the mouth and usually the operculum. After random swimming the larva attaches itself to the skin. Later it migrates and aggregates around the base of the pectoral fins, before entering the ventral ends of the opercular openings. In older fish the passive mode of entry into the buccal cavity was the common means of invasion. In the case of C. sclerosus, the author has not come across any oncomiracidia, juveniles or adults of C. sclerosus attached to the skin and it is unlikely that this is the common mode of invasion of the gills.

With regard to the effect of urea, no definite threshold for development and hatching was defined in the study,

though in one experiment 80% hatched out in urea concentration of 0.5μ moles/ml. Kearm and Macdonald (1976) have shown that eggs of E. soleae and A. lobianchi hatch out in greater numbers at urea concentration of 0.1μ mole/ml and ammonium chloride at 1.0μ mole/ml. Urea could add to the alkalinity of the medium and, as the eggs have shown to develop faster in a higher alkaline environment, this may explain how urea can act as an inducer to development and hatching. Urea is contained in the faeces and urine of the host. As the fish hosts tend to settle at the bottom of the tanks or ponds at night, the defaecation and urination of the host could influence the development of the egg and also stimulate hatching at a time when potential hosts are available in the vicinity.

Egg development, being favoured in an alkaline environment, also agrees with the relationship of C. sclerosus to cichlid fish. Cichlid fish are indigenous to African lakes which, according to Fryer and Iles (1972), have a high pH (around pH 8 to 9). Hence it is not surprising that, at such pHs, the parasites, which have evolved with the cichlid fish, should also be particularly active.

CHAPTER FOUR

Host-specificity and site-specificity of
Cichlidoqyrus sclerosus on the gills of tilapia.

4.1. INTRODUCTION

There is a considerable amount of literature on the host-specificity of the order Monogenea (Hargis, 1953c, 1957; Llewellyn, 1956; 1957; Price and McMahon, 1967; Price and Henderson, 1969; Glaeser, 1974; Rohde, 1978a). There have also been a few reports on site-specificity of the group, particularly in relation to the branchial arches of fish (Suydam, 1971; Wooten, 1974; Hanek and Fernando, 1978a; 1978b; 1978c; Llewellyn and Macdonald, 1980).

According to Llewellyn (1957), most Monogenea appear to have very specific positions on the gills of their host. Gastrocotyle trachuri is found about midway along the lengths of the primary lamella, but Pseudaxine trachuri are attached nearer to the distal tips of the lamella on the fish host, Trachurus trachurus. There is also a well-defined preference for gill arches two, three, one and four (in that order) by monogeneans of the host fish, Lepomis gibbosus and Ambloplites rupestris (Hanek and Fernando, 1978a). They also found that the monogeneans studied had a definite preference for the anterior medial and posterior medial section of the hemibranchs. In the same year they also carried out an extensive study on spatial distribution of Urocleidus ferox, the dominant parasite on L. gibbosus (Hanek and Fernando, 1978b). Rohde (1978b) also showed that only one monogenean species is scattered over the filaments of all four gills.

Cichlidoqyrus has been described extensively by

Paperna and Thurston (1969) and these authors have pointed out that this group of Monogenea is specific to the gills of cichlid fish. They also mentioned the possibility of each Cichlidogyrus species occupying a different niche on the gills, according to their physico-chemical needs, and that this has not yet been investigated. Price (1968) indicates that Cichlidogyrus exhibits high host-specificity. All of these statements are qualitative and there has been no attempt to study them quantitatively.

In the course of diagnostic examination in the present study, it was observed that C. sclerosus and C. t. minutus were usually found at low infection levels on the gills of aquarium-kept fish. They were usually found on all of the different species of tilapia examined. It was also observed that there were certain sites on the gills preferred by the mature worms, although the juvenile worms did not seem to have such a marked preference. Therefore experiments, described below, were carried out, whereby the parasites were exposed to the host fish (cichlids) of two different species, and the respective levels of infection noted. Only two host species were used, because these were readily available, but it was observed casually that C. sclerosus was also found on other cichlid host species within the aquarium, such as S. nilotica and S. zilli. C. sclerosus has also been found on hybrid fish. Hybrids of S. homorum and S. mossambicus, examined from several fish ponds at the Universiti Pertanian Malaysia, Serdang, Malaysia, have all been observed to be infected with C. sclerosus (Mohd-Shaharom personal observations 1983).

4.2. MATERIALS AND METHODS

4.2.1. Host-specificity experiment

In this experiment three tanks were used. Each tank was divided into two equal areas by a netting divider. The volume of water was kept constant in each tank and the total volume in each half of the tank came to (26 x 43.5 x 60) cu. cm. The temperature of the water was kept constant at 28°C.

Fifty Sarotherodon mossambicus were kept in one-half of the tank and fifty S. spiluris were kept in the other. The fish were obtained at the fry stage, from the stock tanks kept in the tropical aquarium at the Institute of Aquaculture, University of Stirling. Twenty fry from this stock group were examined initially and they were all found to have no infection. Feeding was ad. lib. Ten S. niloticus were initially examined and they were all found to be infected. Hence five S. niloticus from this batch of fish were chosen as the source of infection. Five S. niloticus were then placed in square netlon baskets measuring (32 x 32 x 32) cu. cm. The baskets were kept suspended, one in each half of the tank.

After the first week of the experiment the two species S. mossambicus and S. spiluris became intermingled in two of the tanks. Since it is very difficult to separate these two species morphologically, this could have posed major problems. However, since the fish were from separate stocks, which had been closely monitored over several generations (MacAndrew and Majumbar, 1983), it was possible to make use of this by carrying out electrophoretic examination of the fish, in order to reassign them to the correct species at the completion of the experiment. The fact that they were completely intermingled during exposure added to the validity of the study. In order to perform electrophoresis the fish had to

be grown to a certain size. The fish, therefore, could not be sampled weekly as previously planned.

The method used for gel electrophoresis was as follows:-

- (1) 28 gms of starch and 220 mls of buffer solution (8.5 mls of buffer diluted to 220 mls with deionized water) were mixed in a buchner flask and shaken over a bunsen flame, until the mixture became clear and runny.
- (2) A vacuum pump was used to evacuate the air from the solution.
- (3) The mixture was poured over setting plates (perspex former between two glass plates). It was allowed to set.
- (4) Meanwhile, pieces of muscle tissue were cut from each fish and placed in holes in a perspex block. 1 ml of distilled water was put in each hole. Pieces of filter paper were placed in each hole.
- (5) The prepared gel was taken from the plates. A cut was made about one inch from the base, and parted slightly. The pieces of filter paper (from step 4) were wiped slightly on filter paper, then placed in the parting about 1 cm from each other. The partition was then closed by pushing the cut ends together. The gel plate was then ready and it was placed in a dish containing tricitric buffer. The plates were put in a fridge and set at 40 milliamps and 150 volts. It was allowed to run for four hours.
- (6) For staining the gel the following chemicals were weighed out.
6 ml. PGDH
30 mg 6 phosphogluconate
3 mg NADP

7 mg MITT
10 mg Mg Cl₂
PMS (a small amount)
30 ml Tris HCl pH8
Also weigh PGI
30 mg F6P
4 mg NADP
20 mg Mg Cl₂
7 mg MITT
50 ml G6 PDH

After four hours the gel plates were sliced in half and placed between flat rectangular plates. The stain was mixed, poured over the plates and kept in the oven at 38°C.

(7) The gel plates exhibited typical electrophoretic banding that distinguished the two species of S. mossambicus and S. spiluris.

This experiment was conducted in the knowledge that it had several potential shortcomings. Firstly, the only available fish were S. spiluris and S. mossambicus, so that only two species were in fact used. Another problem concerned the source of infection. It was not possible to ensure, with absolute certainty, that all of the sources of infection (the S. niloticus) were clearly infected at the start, since testing required sacrifice of the fish.

4.2.2. Site-specificity experiment

The gills were taken from 928 fish, which were kept in the tropical aquarium at the Institute of Aquaculture, University of Stirling.

Each gill arch was arbitrarily divided into the following sections: A, B, C, D, E and F. C and D were the regions

pointing towards the anterior extremity of the fish while A and F lie towards the side, away from the gill arch (Fig. 10).

The position of C. sclerosus, C. t. minutus and juvenile parasites were noted as follows:-

Right side	A	B	C	D	E	F
1st gill arch						
2nd gill arch						
3rd gill arch						
4th gill arch						
Left side						
1st gill arch						
2nd gill arch						
3rd gill arch						
4th gill arch						

The lengths of the fish were also noted. The number of parasites on position A, on 1st gill arch of right side, for each of the 928 fish was counted. Similarly, the number of parasites on position B, C, D, E and F for the first gill arch of each fish, was counted. This was repeated for the 2nd, 3rd and 4th gill arches. Similarly, the same procedure was repeated for the gill arches on the left side of the heads of the 928 fish.

The results were analyzed using the following statistical methods:-

Analysis of Variance

Duncan's Multiple Range Test

Regression Analysis.

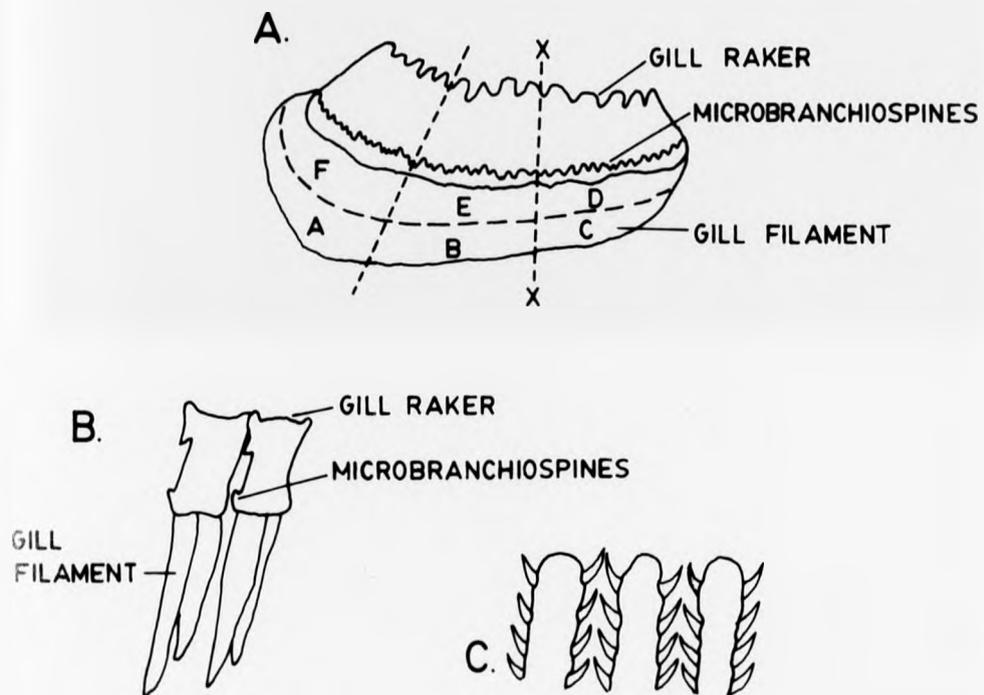


Fig. 10. Diagram of gill arch and microbranchiospines.

Legend for Fig. 10.

- A. Single gill arch (Not the outermost as seen from outer face)
- B. T.S. along XX (Through two adjacent gill arches)
- C. Three members of a row of microbranchiospines

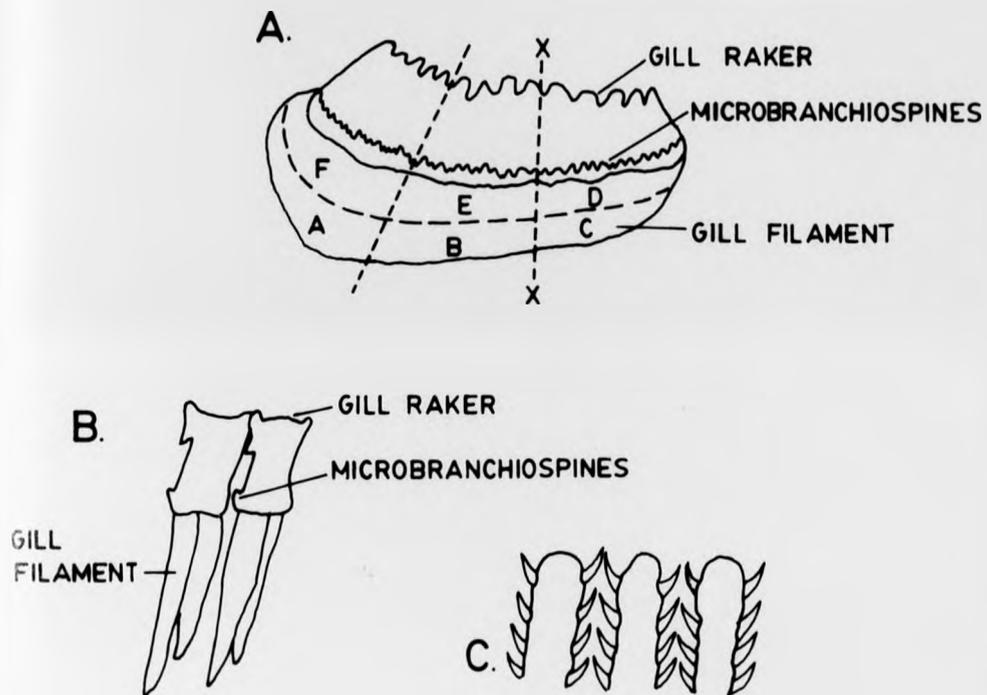


Fig. 10. Diagram of gill arch and microbranchiospines.

Legend for Fig. 10.

- A. Single gill arch (Not the outermost as seen from outer face)
- B. T.S. along XX (Through two adjacent gill arches)
- C. Three members of a row of microbranchiospines

Most of the statistical calculations were based on methods given by Sokal and Rohlf (1973). A Casio-fx 550 calculator was employed.

4.3. RESULTS

4.3.1. Host-specificity experiment

The overall results were shown as follows:-

TABLE IX. The infection level of *C. sclerosus* on two hosts, *S. spilurris* and *S. mossambicus*, in three tanks. (In Tank 9 there was no intermingling of fish as the barrier was intact till the end of the experiment).

Tank Number	9		7		6	
	<i>S. spilurris</i>	<i>S. mossambicus</i>	<i>S. spilurris</i>	<i>S. mossambicus</i>	<i>S. spilurris</i>	<i>S. mossambicus</i>
Numbers of fish at the start	50	50	50	50	50	50
Total numbers of fish at the end	38	37	33	25	38	19
Total numbers of fish infected	6	5	12	9	2	2
Total numbers of parasites	7	5	17	13	5	3
Prevalence	15.79	13.51	36.36	36.00	5.26	10.53
Mean worm numbers per infected fish (Mean intensity)	1.17	1.00	1.42	1.44	2.50	1.50
Standard deviation	0.41	0.00	0.30	0.73	2.12	0.71
Mean worm numbers per fish	0.18	0.14	0.52	0.52	0.13	0.16
Standard deviation	0.46	0.35	0.75	0.82	0.66	0.50

The numbers of fish in the various tanks had decreased at the end of the experiment because of natural mortality and cannibalism of smaller-sized fish by bigger fish during the course of the experiment.

The students t-test, to compare the mean intensity of C. sclerosus on S. spiluris and S. mossambicus in each of the tanks, was also carried out. In Tank 9, where no mixing of the fish had taken place, it was found that the value of t , $t_s = 1.02$, was not significant at the 5% level ($P > 0.05$). For Tank 6, the value of t , $t_s = 0.63$, and for Tank 7, the value of t , $t_s = 0.08$, were both found to be not significant at the 5% level ($P > 0.05$). Therefore, statistical results from all three tanks indicated that there was no significant difference between mean intensity of C. sclerosus in S. spiluris and S. mossambicus.

A t-test of the difference between means was carried out between prevalence in S. spiluris and prevalence in S. mossambicus. The results showed that $t = 0.07$ and was not significant at the 5% level ($P > 0.05$).

The results of pooled data from all tanks are shown in Table X. The definition of terms used in the following table were according to Margolis (1982).

Prevalence:-

Number of individuals of a host species infected with a particular parasite species divided by number of hosts examined.

Mean Intensity:-

Total number of individuals of a particular parasite species in a sample of a host species divided by number of infected

individuals of the host species in the sample.

TABLE X. Result of pooled data from all tanks.

Species of fish	<u>S. spiluris</u>	<u>S. mossambicus</u>
Numbers of fish started with	150	150
Total numbers of fish at the end of the experiment	109	81
Total numbers of fish infected	20	16
Total numbers of parasites	29	21
Prevalence (Percentage)	18.35	19.75
Mean parasite number per infected fish (Mean Intensity)	1.45	1.31
Mean parasite number per fish	0.266	0.259

A t-test of the difference between the two mean intensities, when carried out, gave the following results:-

$$t = 0.061$$

$$t_{.05} (188) = 1.98$$

t observed is smaller than t critical. Therefore, Null Hypothesis cannot be rejected. Therefore, there was no significant difference between the number of parasites on S. spiluris and the number of parasites on S. mossambicus.

The prevalence of C. sclerosus on S. mossambicus and S. spiluris, from individual tanks, is shown in Figure 11. The results seem to show that the prevalence alternated from one fish species to another. This is evident from Tank 9 where there was no mixing of fish. It should be noted that, here, the separation was

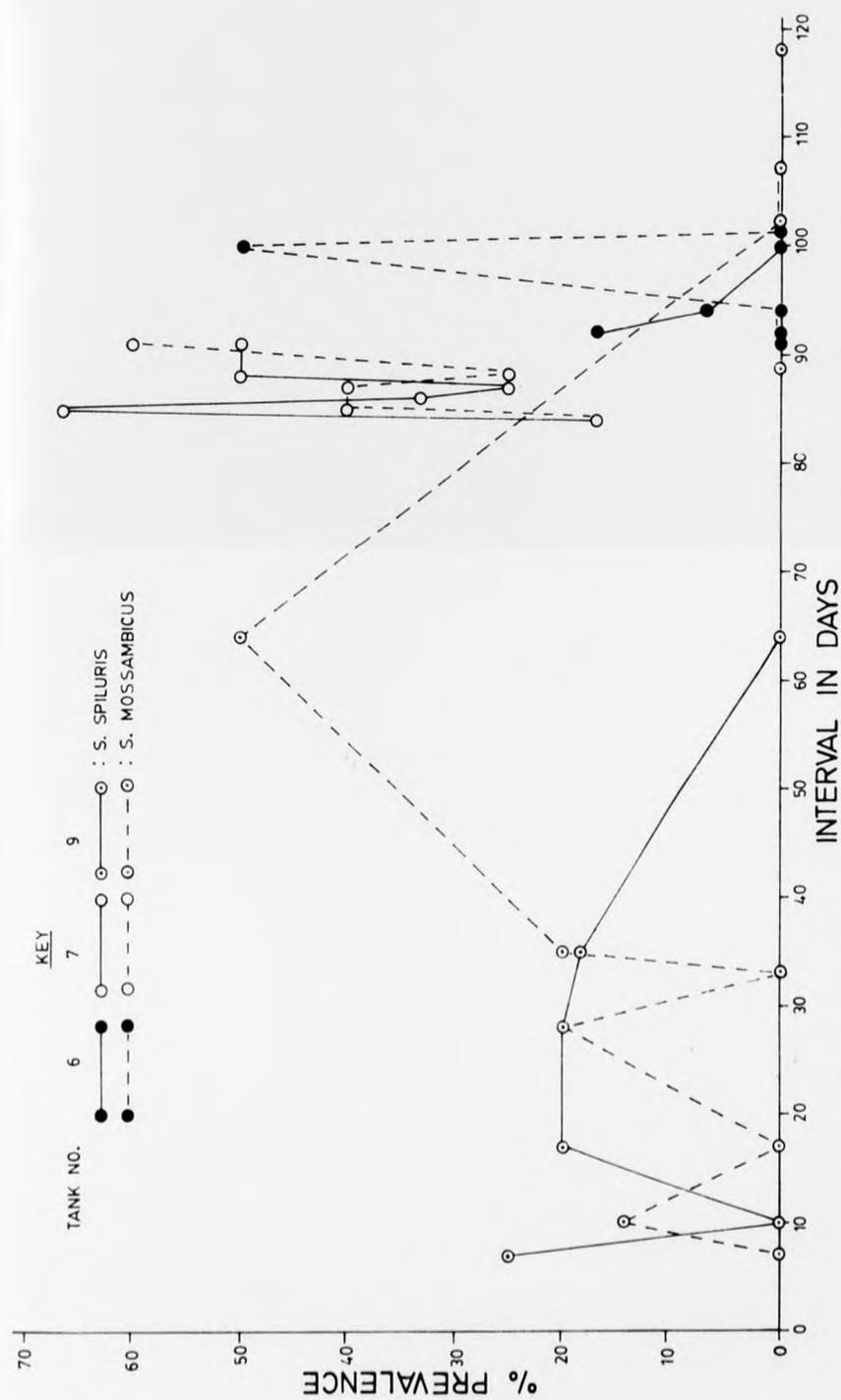


Fig. 11. Prevalence of C. sclerosus on S. spiluris and S. mossambicus, from individual tanks.

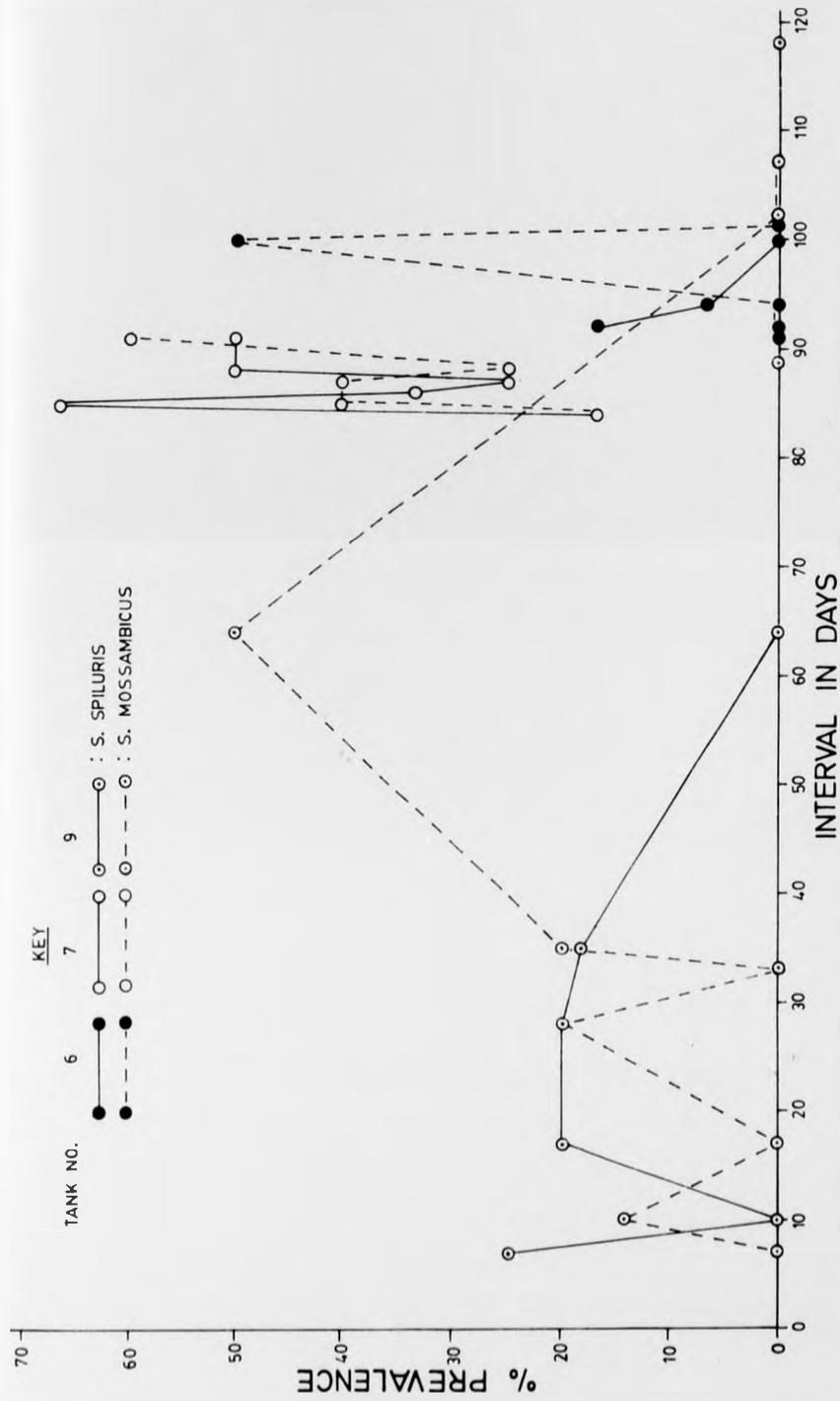


Fig. 11. Prevalence of *C. sclerosus* on *S. spiliuris* and *S. mossambicus*, from individual tanks.

a netting divider and there was still intermingling of the water. Hence eggs and free-swimming juveniles could still move from one side of the netting divider to another. Only from the twenty-eighth to the thirty-fifth day was the prevalence about the same in both species. After the sixty-fourth day, the infection in S. spiluris was zero percent while that in S. mossambicus was fifty percent. The prevalence in S. mossambicus decreased gradually, reaching zero percent after one hundred days.

In Tank 6 there was also alternation of prevalence, which was not apparent in Tank 7. Figure 12 shows results of pooled data of prevalence of C. sclerosus on S. spiluris and S. mossambicus from all tanks. It showed the same alternation in prevalence from the seventh day to the sixty-fourth day, from the time the experiment was started. On the eighty-fourth day the prevalence was equal (16.66%). From here on, there was only slight differences in prevalence of the two species, except on the one-hundredth day when S. spiluris dropped to zero percent while S. mossambicus peaked to 50%.

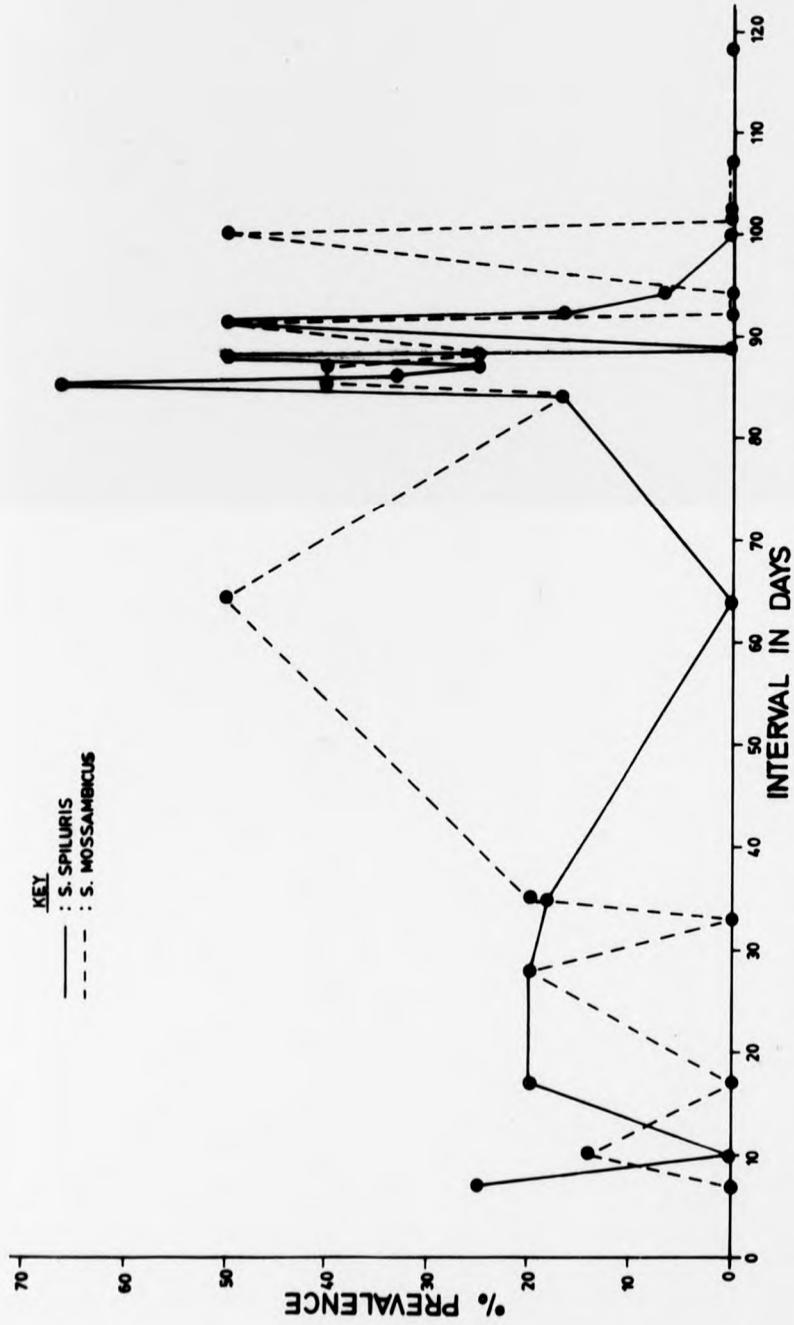


Fig. 12. Prevalence of C. sclerosus, on S. pilularis and S. mossambicus, from all tanks.

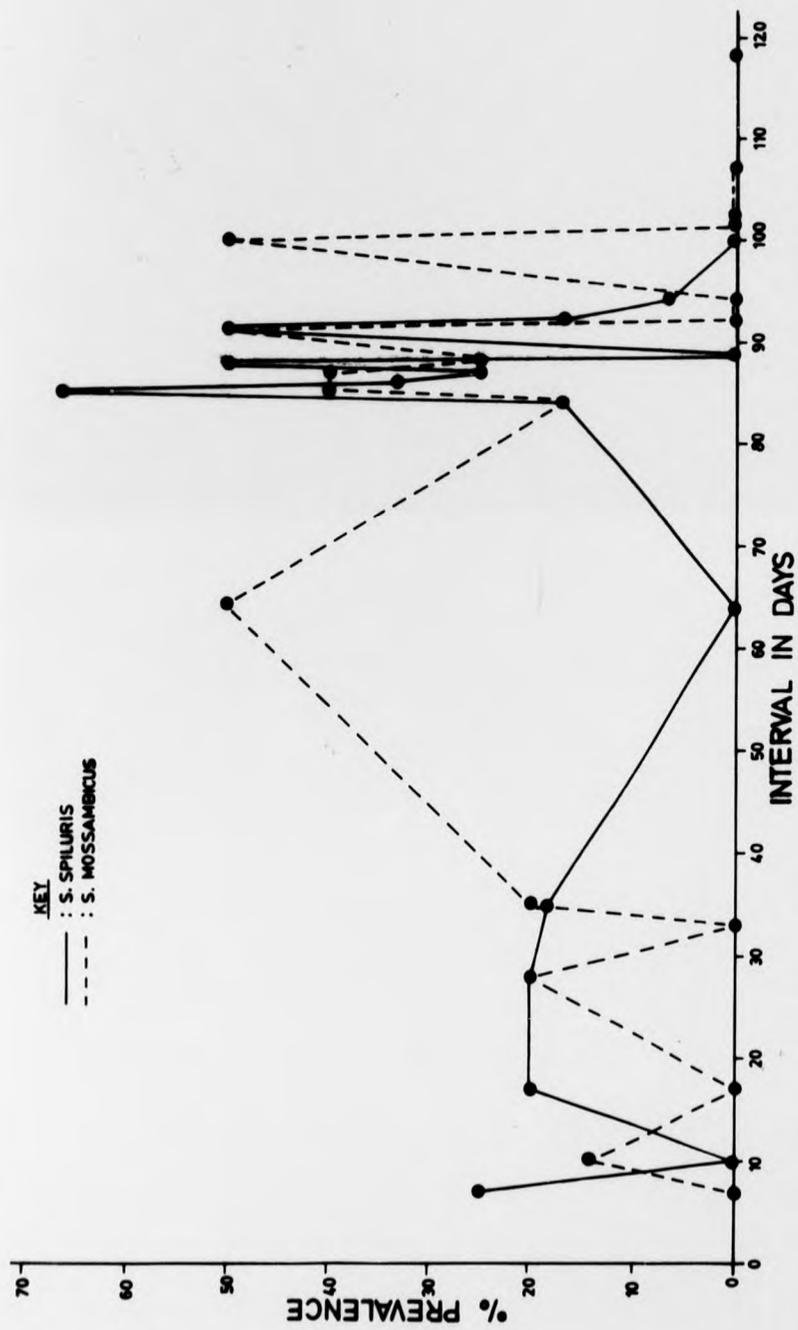


Fig. 12. Prevalence of *C. sclerosus*, on *S. spiluris* and *S. mossambicus*, from all tanks.

4.4. SITE-SPECIFICITY EXPERIMENT

4.4.1. Spatial distribution of C. sclerosus adults.

The overall results for all gill arches have been summed up as shown below:-

TABLE XI. The spatial distribution of C. sclerosus adults.

Right side	A	B	C	D	E	F	Total
1st gill arch	4	9	9	98	327	103	550
2nd gill arch	1	10	3	85	258	87	444
3rd gill arch	3	3	6	56	254	62	384
4th gill arch	2	4	4	31	190	63	294
Left side							
1st gill arch	5	13	10	87	291	104	510
2nd gill arch	4	11	6	63	278	100	462
3rd gill arch	4	5	2	60	207	80	358
4th gill arch	1	5	2	32	190	47	277

These results were well indicated in Figure 13. It was evident that sites E on the first gill arches were very much preferred.

It was also decided to analyze the results statistically, using an Analysis of Variance. In order to fit the assumptions for an Analysis of Variance, the above data had to be transformed. The transformed data was shown in the following Table (Table XII).

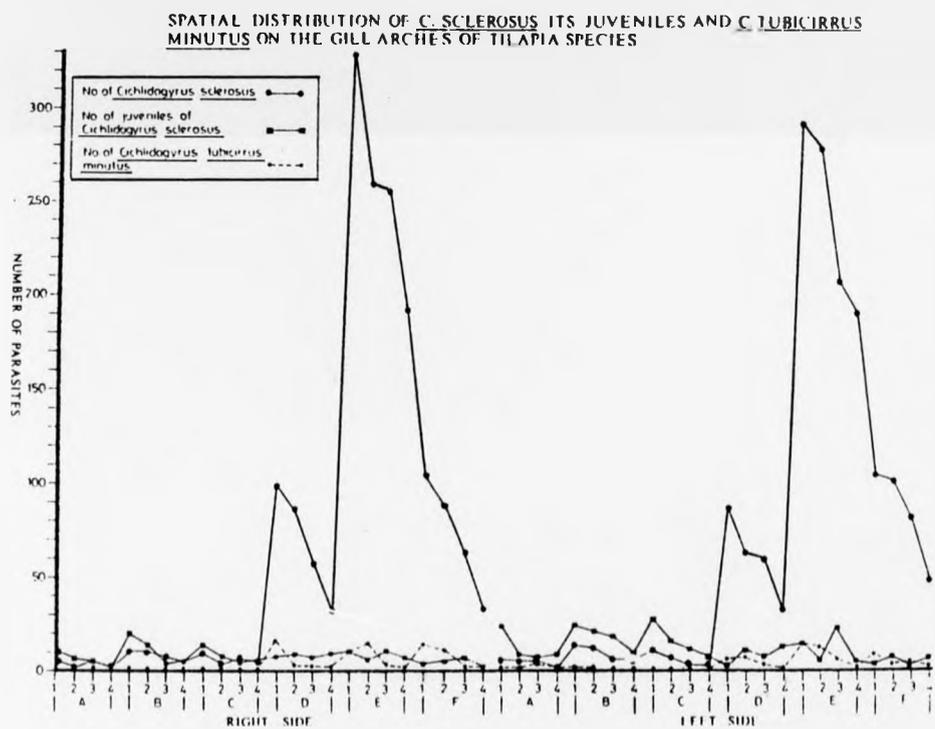


Fig. 13. Spatial distribution of *C. sclerosus*, its juveniles and *C. t. minutus* on the gill arches of *S. spiluris* and *S. mossambicus*.

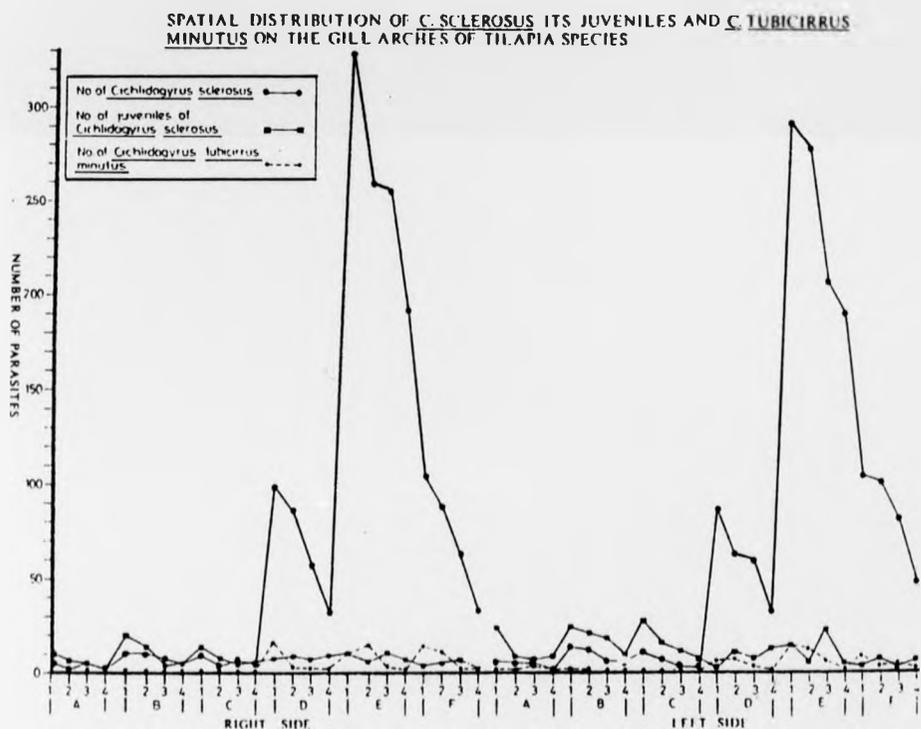


Fig. 13. Spatial distribution of C. sclerosus, its juveniles and C. t. minutus on the gill arches of S. spiluris and S. mossambicus.

TABLE XII. Square-root transformation of data for adult C. sclerosus distribution

Right side	A	B	C	D	E	F	Total
1st gill arch	2.00	3.00	3.00	9.90	18.08	10.15	46.13
2nd gill arch	1.00	3.16	1.73	9.22	16.06	9.33	40.50
3rd gill arch	1.73	1.73	2.45	7.48	15.94	7.87	37.20
4th gill arch	1.41	2.00	2.00	5.57	13.78	7.94	32.70
Total	6.14	9.89	9.18	32.17	63.86	35.29	156.53
Left side							
1st gill arch	2.24	3.61	3.16	9.33	17.06	10.20	45.60
2nd gill arch	2.00	3.32	2.45	7.94	16.67	10.00	42.38
3rd gill arch	2.00	2.24	1.41	7.75	14.39	8.94	36.73
4th gill arch	1.00	2.24	1.41	5.66	13.78	6.86	30.95
Total	7.24	11.41	8.43	30.68	61.90	36.00	155.66

4.4.2. Analysis of data for adult C. sclerosus distribution on the right gill arches.

A two-way Analysis of Variance was carried out to see if there was any significant difference between the numbers of adult C. sclerosus on sites A, B, C, D, E and F (refer to Appendix 3 for working). The results were summarized as follows:-

ANOVA TABLE

Source of Variation	df	ss	ms	F
A (columns)	5	623.64	124.728	166.30
B (rows)	3	15.99	5.33	7.11
Error	15	11.25	0.75	
Total	23	650.882		

$$F.05(5,15) = 2.90; \quad F.05(3,15) = 3.29$$

The results indicated that there was a highly significant difference between the numbers of C. sclerosus on sites A, B, C, D, E and F and also a significant difference between the numbers on the various gill arches.

Duncan's Multiple Range Test was then carried out to determine where the preferred sites lay. The results were summarized as follows:-

From TABLE XII.

	A	B	C	D	E	F
	6.14	9.89	9.18	32.17	63.86	35.29
\bar{y}	1.535	2.473	2.295	8.042	15.965	8.823
$R_p = q \alpha' \bar{s}_y$	$\alpha' = 1 - (1 - \alpha)^{P^{-1}} \quad p = 2, 3 \dots t$					
$p = 6$	$fe = 15$	$\bar{s}_y = \sqrt{\frac{0.75}{4}} = 0.433$				
p	2	3	4	5	6	
$q \alpha' (6,15)$	3.01	3.16	3.25	3.31	3.36	
R_p	1.303	1.368	1.407	1.433	1.455	

A summary of test results using underscores was as follows:-

A	C	B	D	F	E
<u>1.535</u>	<u>2.295</u>	<u>2.473</u>	<u>8.042</u>	<u>8.823</u>	15.965

The results indicated there was no significant difference between sites A, B and C. There was also no difference between D and F. E stood distinct from the others, therefore E was the most preferred site.

Duncan's Multiple Range Test was then employed again to determine which were the preferred gill arches. The results

were summarized as follows:-

From TABLE XII.			\bar{Y}
1st gill arch	46.13		7.69
2nd gill arch	40.50		6.75
3rd gill arch	37.20		6.20
4th gill arch	32.70		5.45
	$p = 4;$	$fe = 15$	$s\bar{y} = \sqrt{\frac{0.75}{6}} = 0.354$
p	2	3	4
$q \alpha' (4,15)$	3.01	3.16	3.25
R_p	1.066	1.119	1.151

A summary of test results using underscores was as follows:-

4th	3rd	2nd	1st
5.45	6.20	6.75	7.69

There was no significant difference between the 1st and 2nd gill arches but the 1st gill arch differed significantly from the 3rd and 4th gill arches.

4.4.3. Analysis of data comparing right and left gill arches for C. sclerosus distribution.

To see if there was a significant difference between the right and left gill arches for total adult C. sclerosus distribution, a one-way, two sample Analysis of Variance was carried out.

From TABLE XI

	Right side		Left side	
	Adult <u>C.</u> <u>sclerosus</u> (total numbers)	\bar{y}	Adult <u>C.</u> <u>sclerosus</u> (total numbers)	\bar{y}
1st gill arch	550	91.67	510	85.00
2nd gill arch	444	74.00	462	77.00
3rd gill arch	384	64.00	358	59.67
4th gill arch	294	49.00	277	46.17

In order to fit the assumptions for an Analysis of Variance, the above data had to be transformed. Transformation of variates by square-root transformation gave the following:-

	Right side	Left side
1st gill arch	9.57	9.22
2nd gill arch	8.60	8.77
3rd gill arch	8.00	7.72
4th gill arch	7.00	6.79

The Analysis of Variance was carried out on the above transformed data (refer to Appendix 4 for working).

The results were summarized as follows:-

ANOVA TABLE

Source of variation	df	ss	ms	F
Among groups	1	0.0514	0.0514	0.04
Within groups	6	7.0487	1.17478	
Total	7	7.1001		

$$F_{.05(1,6)} = 5.99$$

Therefore, there was no significant difference between the gill arches on the right and left sides for total C. sclerosus distribution.

4.4.4. The spatial distribution of juvenile C. sclerosus on the gills.

The overall results for the distribution of juvenile C. sclerosus were as shown below:-

TABLE XIII. Spatial distribution of juvenile C. sclerosus.

Right side	A	B	C	D	E	F	Total
1st gill arch	10	19	13	7	8	3	60
2nd gill arch	5	13	7	8	5	4	42
3rd gill arch	3	3	4	6	10	6	32
4th gill arch	1	5	4	8	6	1	25
Total	19	40	28	29	29	14	159
Left side							
1st gill arch	23	24	27	2	14	3	93
2nd gill arch	8	20	15	10	4	6	63
3rd gill arch	6	18	11	7	22	2	66
4th gill arch	8	9	7	12	4	5	45
Total	45	71	60	31	44	16	267

In order to fit the assumptions for an Analysis of Variance the above data was transformed using a square root transformation. The transformed data was as shown in the following table.

TABLE XIV. Square-root transformation of data for juvenile C. sclerosus distribution.

Right side	A	B	C	D	E	F	Total
1st gill arch	3.16	4.36	3.61	2.65	2.83	1.73	18.34
2nd gill arch	2.24	3.61	2.65	2.83	2.24	2.00	15.57
3rd gill arch	1.73	1.73	2.00	2.45	3.16	2.45	13.52
4th gill arch	1.00	2.24	2.00	2.83	2.45	1.00	11.52
Total	8.13	11.94	10.26	10.76	10.68	7.18	58.95
Left side							
1st gill arch	4.80	4.90	5.20	1.41	3.74	1.73	21.78
2nd gill arch	2.83	4.47	3.87	3.16	2.00	2.45	18.78
3rd gill arch	2.45	4.24	3.32	2.65	4.69	1.41	18.76
4th gill arch	2.83	3.00	2.65	3.46	2.00	2.24	16.17
Total	12.91	16.61	15.04	10.68	12.43	7.83	75.50

4.4.5. Analysis of data for juvenile C. sclerosus distribution on the right gill arches.

A two-way Analysis of Variance was carried out to see if there was any significant difference between the numbers of C. sclerosus juveniles on sites A, B, C, D, E and F (refer to Appendix 5). The results were summarized as follows:-

ANOVA TABLE

Source of variation	df	ss	ms	F
A (columns)	5	4.040	0.808	2.00
B (rows)	3	4.257	1.419	3.51
Error	15	6.068	0.4045	
Total	23	14.365		

$$F_{0.05}(5,15) = 2.90 \quad F_{0.05}(3,15) = 3.29$$

The results indicated that there were no significant differences between the number of juveniles on sites A, B, C, D, E and F. However, there was a significant difference between the numbers on the various gill arches.

Duncan's Multiple Range Test was then carried out to determine the individual differences amongst the various sites. The results were summarized as follows:-

From TABLE XIV

	A	B	C	D	E	F
	8.13	11.94	10.26	10.76	10.68	7.18
\bar{y}	2.03	2.99	2.57	2.69	2.67	1.80
$p = 6$	$fe = 15$	$s\bar{y} = \sqrt{\frac{0.4045}{4}} = 0.318$				
p	2	3	4	4	6	
$q \alpha (6,15)$	3.01	3.16	3.25	3.31	3.36	
	0.96	1.00	1.03	1.05	1.07	

A summary of test results using underscores was as follows:-

F	A	C	E	D	B
1.80	2.03	2.57	2.67	2.69	2.99

There was no significant difference between sites A, C, D, E and F. Sites A and F differed from B which did not differ from C, D and E.

Duncan's Multiple Range Test was then employed again to determine which were the preferred gill arches. The results were summarized as follows:-

From TABLE XIV

		\bar{Y}
1st gill arch	18.34	3.06
2nd gill arch	15.57	2.60
3rd gill arch	13.52	2.25
4th gill arch	11.52	1.92

$$p = 4 \quad fe = 15 \quad s\bar{y} = \sqrt{\frac{0.4045}{6}} = 0.2596$$

p	2	3	4	5	6
q (4,15)	3.01	3.16	3.25	3.31	3.36
Rp	0.7814	0.8203	0.8437	0.8592	0.8722

A summary of test results using underscores was as follows:-

4th	3rd	2nd	1st
1.92	2.25	2.60	3.06

There was no significant difference between 2nd, 3rd and 4th gill arches. The 1st gill arch differed significantly from the 3rd and 4th gill arches.

4.4.6. Analysis of data comparing right and left gill arches for C. sclerosus juvenile distribution.

To see if there was a significant difference between right and left gill arches for total C. sclerosus juvenile distribution, a one-way two sample Analysis of Variance was carried out.

From TABLE XIII

	Right side		Left side	
	Total number of juveniles	\bar{y}	Total number of juveniles	\bar{y}
1st gill arch	60	10.00	93	15.50
2nd gill arch	42	7.00	63	10.50
3rd gill arch	32	5.33	66	11.00
4th gill arch	25	4.16	45	7.50

In order to fit the assumptions for an Analysis of Variance the above data had to be transformed. Transformation of variates by square-root transformation gave the following:-

	Right side	Left side
1st gill arch	3.162	3.937
2nd gill arch	2.645	3.240
3rd gill arch	2.308	3.316
4th gill arch	2.039	2.738

The Analysis of Variance was carried out on the above transformed data (Refer to Appendix 6 for working). The results were summarized as follows:-

ANOVA TABLE

Source of Variation	df	ss	ms	F
Among groups	1	1.1837	1.1837	4.98
Within groups	6	1.4273	0.2378	
Total	7	2.611		

$$F_{.05(1,6)} = 5.99$$

Therefore, there was no significant difference between the gill arches on the right and left sides for total juvenile C. sclerosus distribution.

4.4.7. The spatial distribution of Cichlidoqvrus tubicirrus minutus.

C. t. minutus were found in very low numbers on the gills of tilapia. In figure number 13 their spatial distribution did not seem to differ very much from the juvenile worms of C. sclerosus, though most seemed to peak at site E on the first gill arches.

The overall result for the distribution of C. t. minutus were as shown below:-

TABLE XV. Spatial distribution of C. t. minutus.

Right side	A	B	C	D	E	F	Total
1st gill arch	0	0	0	15	9	14	38
2nd gill arch	0	0	0	2	14	10	26
3rd gill arch	0	0	0	1	2	1	4
4th gill arch	0	0	0	1	0	1	2
Total	0	0	0	19	25	26	70
Left side							
1st gill arch	1	1	0	5	13	8	28
2nd gill arch	0	0	0	6	11	2	19
3rd gill arch	3	0	1	2	5	3	14
4th gill arch	0	0	0	0	0	1	1
Total	4	1	1	13	29	14	62

In order to fit the assumptions for an Analysis of Variance the above data was transformed using a square-root transformation ($\sqrt{y + \frac{1}{2}}$). The transformed data was shown in the following table:-

TABLE XVI. Square-root transformation of data ($\sqrt{y + \frac{1}{2}}$) for C. t. minutus distribution.

Right side	A	B	C	D	E	F	Total
1st gill arch	0.707	0.707	0.707	3.937	3.082	3.808	12.948
2nd gill arch	0.707	0.707	0.707	1.581	3.808	3.240	10.750
3rd gill arch	0.707	0.707	0.707	1.225	1.581	1.225	6.152
4th gill arch	0.707	0.707	0.707	1.225	0.707	1.225	5.278
Total	2.828	2.828	2.828	7.968	9.178	9.498	35.128
Left side							
1st gill arch	1.225	1.225	0.707	2.345	3.674	2.915	12.091
2nd gill arch	0.707	0.707	0.707	2.549	3.391	1.581	9.642
3rd gill arch	1.871	0.707	1.225	1.581	2.345	1.871	9.600
4th gill arch	0.707	0.707	0.707	0.707	0.707	1.225	4.760
Total	4.510	3.346	3.346	7.182	10.117	7.592	36.093

4.4.8. Analysis of data for C. t. minutus on the right gill arches.

A two-way Analysis of Variance was carried out to see if there was any significant difference between the numbers of C. t. minutus on sites A, B, C, D, E and F. (Refer to Appendix 7 for working). The results were summarized as follows:-

ANOVA TABLE

Source of Variation	df	ss	ms	F
A (columns)	5	14.0664	2.813	4.312
B (rows)	3	6.73	2.2433	3.439
Error	15	9.785	0.6523	
Total	23			

$$F_{0.05}(5,15) = 2.90$$

$$F_{0.05}(3,15) = 3.29$$

The results indicated that there was a significant difference between the numbers of C. t. minutus on sites A, B, C, D and F, and there was a significant difference between the numbers on the various gill arches.

Duncan's Multiple Range Test was then carried out to determine where the preferred sites lay. The results were summarized as follows:-

From TABLE XVI

	A	B	C	D	E	F
	2.828	2.828	2.828	7.968	9.178	9.498
\bar{y}	0.707	0.707	0.707	1.992	2.295	2.375
p = 6	fe = 15	$s\bar{y} = \sqrt{\frac{0.6523}{4}} = 0.4038$				
p	2	3	4	5	6	
q α (6,5)	3.01	3.16	3.25	3.31	3.36	
Rp	1.21	1.27	1.31	1.33	1.35	

A summary of test results using underscores was as follows:-

A	B	C	D	E	F
0.707	0.707	0.707	1.992	2.295	2.375

The numbers of C. t. minutus on sites A, B and C did not differ significantly, but the numbers differed from those on sites D, E and F. Duncan's Multiple Range Test was then employed again to determine the individual differences amongst the various gill arches. The results were summarized as follows:-

The results indicated that there was a significant difference between the numbers of C. t. minutus on sites A, B, C, D and F, and there was a significant difference between the numbers on the various gill arches.

Duncan's Multiple Range Test was then carried out to determine where the preferred sites lay. The results were summarized as follows:-

From TABLE XVI

	A	B	C	D	E	F
	2.828	2.828	2.828	7.968	9.178	9.498
\bar{y}	0.707	0.707	0.707	1.992	2.295	2.375
$p = 6$	$fe = 15$	$s\bar{y} = \sqrt{\frac{0.6523}{4}} = 0.4038$				
p	2	3	4	5	6	
$q \alpha (6,5)$	3.01	3.16	3.25	3.31	3.36	
R_p	1.21	1.27	1.31	1.33	1.35	

A summary of test results using underscores was as follows:-

A	B	C	D	E	F
0.707	0.707	0.707	1.992	2.295	2.375

The numbers of C. t. minutus on sites A, B and C did not differ significantly, but the numbers differed from those on sites D, E and F. Duncan's Multiple Range Test was then employed again to determine the individual differences amongst the various gill arches. The results were summarized as follows:-

From TABLE XVI

		\bar{y}
1st gill arch	12.948	2.158
2nd gill arch	10.750	1.792
3rd gill arch	6.152	1.025
4th gill arch	5.278	0.879
p = 4	fe = 15	$s\bar{y} = \sqrt{\frac{0.6523}{4}} = 0.4038$

p	2	3	4
q α' (4.15)	3.01	3.16	3.25
Rp	1.215	1.276	1.312

A summary of test results using underscores was as follows:-

4th	3rd	2nd	1st
0.879	1.025	1.792	2.158

There was no significant difference between the numbers of C. t. minutus on the 2nd, 3rd and 4th gill arches. The 1st gill arch did not differ from the second and third gill arch but differed significantly from the 4th gill arches.

4.4.9. Analysis of data comparing right and left gill arches for C. t. minutus.

To see if there was a significant difference between right and left gill arches, for total C. t. minutus distribution, a one-way, two sample Analysis of Variance was carried out.

From TABLE XV

	Right side		Left side	
	<u>C. t. minutus</u> (total numbers)	\bar{y}	<u>C. t. minutus</u> (total numbers)	\bar{y}
1st gill arch	38	6.333	28	4.666
2nd gill arch	26	4.333	19	3.166
3rd gill arch	4	0.666	14	2.333
4th gill arch	2	0.333	1	0.166

In order to fit the assumptions for an Analysis of Variance, the above data had to be transformed. Transformation of variates by square-root transformation gave the following:-

	Right side	Left side
1st gill arch	2.517	2.160
2nd gill arch	2.082	1.779
3rd gill arch	0.816	1.527
4th gill arch	0.577	0.407

The Analysis of Variance was carried out on the above transformed data (Refer to Appendix 8 for working). The results were summarized as follows:-

ANOVA TABLE

Source of Variation	df	ss	ms	F
Among groups	1	0.0019	0.0019	0.003
Within groups	6	4.3939	0.7323	
	7	4.3958		

$$F_{.05(1,6)} = 5.99$$

∴ there was no significant difference between right side gill arches and left side gill arches for spatial distribution of C. t. minutus.

4.4.10. Summation of result for site-specificity experiment.

The preferred sites and preferred gill arches established above for C. sclerosus, its juveniles and C. t. minutus can be expressed as follows:-

	Gills on right side		Gills on left side	
	preferred site	preferred arch	preferred site	preferred arch
<u>C. sclerosus</u>	E	1 (2)	E	1, 2
<u>C. sclerosus</u> juvenile	B	1 (2)	B (C)	1, 2, 3, 4
<u>C. t. minutus</u>	F, E, D	1 (2)	E (F)	1, 2, 3

There was no overall preference for either right or left sides by any of the above parasites.

4.5. THE RELATIONSHIP BETWEEN LENGTHS OF FISH AND NUMBER OF PARASITES

4.5.1. Linear regression analysis.

It was observed that the infection in fish smaller than 1 cm in length was rather infrequent. Infection levels usually occurred in fish greater than 2 cm in length. In fish of lengths between 5 cm and 14 cm, the infection level was quite high. Hence it was decided to carry out a regression analysis of lengths of fish against the number of parasites, to see if there was a relationship.

The results of the regression analysis were as follows:-

Lengths of fish (x)	Number of parasites (y)
$\sum x^2 = 42326.38$	$\sum y^2 = 81126$
$\sum x = 5555.71$	$\sum y = 3690$
$n = 914$	$n = 914$
$\bar{x} = 6.078$	$\bar{y} = 4.037$
$\sum xy = 34086.2$	
Regression coefficient by .x	= 0.8053
Y intercept a	= -0.8576
Regression equation is \hat{y}	= 0.8053x - 0.8576
Explained sums of squares $\sum y^2$	= 27450.23
Unexplained sums of squares $\sum d^2 y.x$	= 53675.77

Results of the Analysis of Variance were as follows:-

Analysis of Variance Table

Source of Variation	df	ss	ms	F
Explained	1	27450.23	27450.23	466.40
Unexplained	912	53675.77	58.855	
	913	81126		

Therefore, a large and significant portion of the variance of y had been explained by regression on x.

The regression line was as shown in Figure 14.

Significant tests of regression statistics were as shown below:-

Standard error of regression coefficient

$$sb = 0.037289$$

Testing significance of the regression coefficient

$$ts = \frac{b - 0}{sb}$$

$$= 21.596$$

Therefore t was significant at $P < 0.001$.



Fig. 14. Linear regression of numbers of C. sclerosus on lengths of tilapia.



Fig. 14. Linear regression of numbers of C. sclerosus on lengths of tilapia.

4.6. DISCUSSION

In the host-specificity experiment, in Tank 9, the S. spiluris and S. mossambicus were separated from each other by a netting divider, but there was still intermingling of the water. In Tanks 6 and 7 there was intermingling of the species, and they had to be identified using gel electrophoresis.

It was noted that the prevalence appeared to alternate from one fish species to another and was very evident in Tank 9, where there was a high level of prevalence in S. spiluris and zero prevalence in S. mossambicus. The reason for this could be the territorial and aggressive behaviour of each species. This will lead to aggregation of one species as compared to another. The infection dynamics could also be related to the spatial distribution of the oncomiracidia. Hence, if the oncomiracidia are grouped in one area then there is a greater possibility for fish around that area to become heavily infected. The spatial distribution of infective stages could influence the acquisition rate by the prospective hosts, as has been proved true in the works of Crofton (1971), Anderson (1978) and Keymer and Anderson (1979).

It will be seen in the next chapter that the parasite distribution is not random but over-dispersed. Hence there is the probability that when one fish gets infected there are more chances for the next fish to be infected. Possibly all the available oncomiracidia would have infected one lot of fish and hence none are left to infect the other group of fish. When the host defence of the infected fish develop, the parasites are rejected, becomes zero prevalence and any available oncomiracidia will infect a new lot of fish which has not yet developed a resistance.

Hybridization of certain fish could also result in the host-specificity of its parasites, as shown by the works of Bychowsky (1933) and Hargis (1953c). The former showed that certain European carp which show susceptibility to the same species of Dactylogyrus are those forming hybrids in their natural habitats. Hargis (1953c) found that Urocleidus ferox occurred on Lepomis microchirus macrochirus and Lepomis gibbosus whereas other centrachids taken from the same pond were not infected. As these two lepomids form hybrids in their natural environment, this could be an explanation for the host-specificity of U. ferox.

Intraspecificity is defined as the occurrence of a single monogeneid species on members of a single fish taxon (Hargis, 1957). The subsidiary term, species-specificity, applies to the occurrence of the monogenean on one host and genus-specificity if the occurrence is on several hosts of the same genus. Hargis (1957) also stated that intraspecificity could be either physiological or ecological in nature, or a combination of both.

It can be said that Cichlidogyrus is intraspecific, as it is strictly restricted to cichlid fish. In the host-specificity experiment reported here C. sclerosus is genus-specific as it infects both S. mossambicus and S. spiluris, which both belong to the genus Sarotherodon. It is not species-specific because it is present on all of the different species of Sarotherodon which were examined, namely S. mossambicus, S. spiluris, and S. zilli.

S. mossambicus and S. spiluris form hybrids in nature. Hybrids of S. hornorum and S. mossambicus are also infected with C. sclerosus. Therefore, the intraspecificity could be due to close

genetic similarity of the hosts. It was observed that rapid embryonic development of C. sclerosus eggs occurred in the presence of host mucus. According to Shepherd (1962), nematodes of the genus Heterodera, which respond to specific hatching factors from the host plant, show a high degree of specificity. Llewellyn (1957) suggested that such strict host-specificity could possibly be due to physiological adaptation to the blood-borne antibodies of the host, a relationship which would promote a high degree of host-specificity at all taxonomic levels.

From the results of the site-specificity experiment it was clearly evident that the most preferred site on the gill arches was site E, which was the anterior medial section of the hemibranch. This corresponds with the findings of Hanek and Fernando (1978a) who found that there was a significant preference for the anterior medial section of the hemibranchs by the monogeneans of Lepomis gibbosus and Ambloplites rupestris. Duncan's Multiple Range also showed that E was the most preferred site for C. sclerosus. Similar results were also obtained for the sites E on the gill arches on the left side of the fish's head. When both the gill arches on either side of the head are considered, Analysis of Variance indicates that there is no significant difference between the two groups. Duncan's Multiple Range indicated that the first gill arch on the right side of the head does differ significantly from all other gill arches. This differed from the findings of Hanek and Fernando (1978b) who found that there was a well-defined preference by Monogenea for gill arches in descending order two, three, one and four. In contrast, with regard to Monogenea on marine fish, Llewellyn and Macdonald (1980) found that there was a

distinct preference for the 2nd and 3rd gill arches by diclidophoran (Monogenea), gill parasites of trisopteran (gadoid) fishes at Plymouth. Most parasites attach themselves to the middle third of the gill arches and to proximal regions of the primary lamella. Another worker, Rohde (1978b), showed that the most frequent location of parasites, on the gills of marine fish caught off the Great Barrier Reef of Australia, is gill arch I with moderate levels in II and III and least frequently on gill arch IV.

So far none of these workers have reported on the spatial distribution of juvenile parasites. The author has also looked at the spatial distribution of the young of C. sclerosus which were the transition stage of the oncomiracidium attaching to the gills, developing to adult size but not yet reaching the egg-laying stage or maturity; that is, the reproductive structures had not yet developed.

Spatial distribution could be due to centrifugal migration of the parasites on the gill arches, as stressed by Oliver (1976) and Lambert and Maillard (1975). The writer has also observed that, when an oncomiracidium lands on the gills, it does not attach immediately to a certain fixed position. There is usually a migration process. The juvenile appears to execute a series of looping movements along the gill filaments. This is almost equivalent to the searching patterns of the juveniles of other vertebrates and invertebrates (Ulmer, 1970). But, once the juveniles develop into adults, they appear to concentrate mainly around the medial sections of hemibranchs of the first gill arch and therefore exhibit site-specificity. The pattern is the same in all the different tilapias examined. Of interest is the

acanthocephalan Acanthocephalus clavula in eels, Anquilla anquilla. This was reported by Kennedy and Lord (1982) as exhibiting a preference for a particular region in the alimentary canal, but there was considerable variation between hosts.

There are many empty niches on the gills of tilapia, the C. sclerosus preferring to aggregate in one place. It has been seen that the population of Cichlidoqyrus is very low on its wild hosts (Paperna, 1960a). This is also true of tilapia kept under experimental conditions. Rohde (1977) says that low density populations increase their chances of mating and cross-fertilizing by restricting niches, thus bringing conspecific individuals into contact with each other. This is also agreed upon by Lebedev (1978) and Ktari (1971). But C. sclerosus does not exhibit mating behaviour and, being hermaphrodite, it normally fertilises its own eggs. The one explanation is perhaps the favourableness of the site which they have chosen. The first gill arch is usually nearest the operculum. This serves as a protective cover for the gills. Hence, the parasites are sheltered from the strong water currents. Another advantage is that movements of the operculum could help to dissipate eggs, laid by the parasite, which will fall and settle to the bottom.

Another interesting factor is that newly hatched oncomiracidium, when placed with a small fish in a dish, appear to be engulfed by water currents flowing into the fish's mouth. There is the possibility that the oncomiracidium could be sheltering amongst the mucus in the buccal cavity of the fish, until it develops slightly bigger before attaching to the gills.

As in most fresh-water Monogenea, the infection is

direct; that is, the oncomiracidia attach themselves directly to the gills and there are no intermediate hosts involved, as is the case with some marine fish (Bychowsky and Nagibina, 1967).

It should be noted that tilapia are mouth brooders and hence this mechanism could be an excellent way to infect juvenile tilapia. Thurston (1970) reported that very few specimens of Cichlidogyrus were found on fry or fingerlings of Haplochromis tilapia at Kajansi experimental ponds in Uganda, even though there were numerous young of S. niloticus or S. mossambicus in the ponds where all mature fish were infected.

Differential distribution could be due to factors operating at the time of infestation of the larvae, as assumed by Frankland (1955) and Llewellyn (1956). The infection of host fish is by the free-swimming larvae being passively taken into the mouth by the host and subsequently effecting attachment when being passed over the gills. Kearns (1968b) also thinks that the larvae of Merizocotyle species swim directly into the nasal fossae of Raia undulata, or they are carried through the nostrils by the olfactory water current.

Site-selection in Dactylogyrus could be due to host age, rather than age of parasite (Molnar, 1971b). There was also a relationship between host age, and thus size, with the number of D. paradoxum on any fish (Anderson, 1974). He found that, in small fish, the parasites tend to be underdispersed and have a more regular distribution. Here the author has found that the juvenile parasite distribution is regular and there is no site-specificity on the gills, irrespective of the lengths of the fish. The mature parasites tend to be overdispersed and show site-specificity. It has also been observed in some cases

where fish are very small (1-2 cm) and infected by 1 or 2 worms. These are usually located at site E on the 1st gill arch. This medial section of the hemibranch has a large area of gill filament or "spatial heterogeneity" and also probably a greater supply of mucus and blood supply or "productivity" - terms coined by Rohde (1976).

The spatial distribution of Dactylogyrus amphibothrium on the gills of ruffe, Gymnocephalus cernua, was worked out by Wooten (1974). He found that site of attachment of D. amphibothrium is influenced by direction and force of respiratory current over the gills. Most of the D. amphibothrium selected sites which are not subjected to the full force of the current. This also seems to be the case with C. sclerosus. The first gill arch, which is nearest the operculum, could be the area where there is least current flow. Sydam (1971) also suggested that site-specificity may be the result of force and direction of ventilating current. He divided, arbitrarily, the branchial baskets of each of three species of fish, Urophycis regius, Stenotonus chrysops and Orthopristis chrysopterus which were parasitized by Diclidophora maccallumi, Microcotyle stenotomi and Pseudotaqia cupida respectively. D. maccallumi, the only parasite to occur in sufficient numbers, showed site-specificity.

The final suggested explanation for site-specificity of C. sclerosus on gills of tilapia is that it could be due to the presence of microbranchiospines, present on arches 2, 3 and 4 and not present on the first gill arch (refer Figure 10). These lateral spines form a "fence" which sieves the water passing through the gap. Fryer and Iles (1972) stated that the final

closure of the operculum was delayed until after the mouth began to open. This, they said, would reverse the current and blow the filtered material into the mucous-containing buccal cavity.

If this is the case, then it is possible that any oncomiracidium passing with the water current could be trapped by the microbranchiospines on gill arches 2, 3 and 4. But, on expulsion of water from the buccal cavity due to the operating of the opercular flap, the filtered material is blown out from its trapped site to the mucus of the buccal cavity. In the case of the first gill arch there are no microbranchiospines to trap the oncomiracidia and, therefore, they can subsequently attach themselves to the gill filaments during the passage of water current over the first gill arch.

There is a significant regression between lengths of fish and number of parasites. It is shown that most C. sclerosus are found on fish of lengths greater than 1 cm and smaller than 17 cm. Therefore, it can be assumed that, as lengths of fish increase, that is as the fish grows older (length proportional to age) the number of parasites will increase. This is similar to the findings of Dogiel (1961) who studied extensively the changes in parasite fauna of host with age. The way of life of the host can influence its parasitic fauna. For eg. Paling (1965) found that lake trout only become infected with the monogenean Discocotyle sagittata when they are two years old, that is when the trout move out from the nursery streams into the deeper waters of the lake, where they are infected. It has also been observed that tilapia fingerlings are normally found at the sides of ponds whereas the adults are found in the centre of the lakes.

Hence, probably, as the tilapia gets older, it migrates to the centre where it is liable to become more infected.

It could also be possible that small fry are not easily infected, due to the undifferentiation of the gills (Prost, 1963; Iziunova, 1956). Prost (1963) found that most carp fry get infected with Dactylogyrus only at thirteen days old because the gills have become differentiated. In 4, 6 and 8-day old carp, the gill bars are only single tissue axons of very scanty structure. The author had also found that very young fry of tilapia, less than 1 cm, were very rarely infected in the aquarium. Hence, this could be due to the undifferentiation of the gills at this stage.

As the fish larvae became more active and as their gill filaments enlarged, the prevalence of *Monogenea* also increased (Komarova, 1976). *Monogenea* could not fix themselves to the gills of larval fish only a few days old, hence infection in fish hatchery is usually not possible, as confirmed by Molnar (1971b). He presumed infection only commenced at 5-7 days of age of the fry.

The curve of change of anchor length of *Monogenea* and their points coincided with the growth of thickness of secondary gill filaments as the host grows older, as elucidated by Gussev and Kulemina (1971).

Larger fish probably lose their infection or do not get infected, due to age resistance. Radha (1971) showed that the highest incidence of Gastrocotyle indica occurred on hosts Caranx kalla of lengths 2.0 to 3.9 cm (47.4%) and the lowest incidence was 11.1% in fish 16.0 to 17.9 cm. The host required 8 months to acquire immunity against infection.

In the case of tilapia, fish of lengths greater than 17 cm do not seem to get infected and this could probably be due to age resistance too. It is also possible for the fish to have lost its infection because fish of length 20 cm have been found to be lightly infected. The linear regression analysis shows that, as the fish get older, the number of parasites will increase. But the increase will level off and become steady when the fish reach a certain age when, possibly, their host-defence system have fully developed.

The change in age of host could also mean changes in structure, behaviour or diet or a change in the probability of infection. Older tilapia are bottom dwellers and hence are less likely to encounter larval stages of the parasite, which usually remain in the surface waters.

CHAPTER FIVE

The effect of density of tilapia, Sarotherodon mossambicus, and the effect of hydrocortisone on the build-up of Cichlidogyrus sclerosus infections under controlled conditions.

5.1. INTRODUCTION

Little published information is available on the population build-up of dactylogyrids on fish kept under crowded conditions in fish ponds and nurseries except for Paperna (1960b; 1963b). Esch et al. (1975), while referring to general ecological principles, stated that there is a tendency for a change in both the density of the infra-populations and supra-populations of parasites on the hosts if there is a shift in the density of the host population, due to stress or other related factors. The only significant work done in this field was by Molnar (1971b) who studied the monogenean parasites, Dactylogyrus lamellatus, infection on grass carp (Ctenopharyngodon idella) which were kept under crowded conditions. He reported that under medium stocking density (90 fish/m²) infestation remained low (30-100 parasites/host) but at a higher stocking density (200 fish/m²) some of the fish developed clinical dactylogyrosis during six weeks exposure.

In earlier parts of the present study it was reported that C. sclerosus infection on S. mossambicus was low (Chapter Four). In the host-specificity experiment (Chapter Four) no infection was observed even after 100 days exposure. The failure to induce the build-up of parasite population, even when exposed to very crowded conditions by the host fish, and under poor quality conditions, prompted the initiation of the present

experiment to investigate:

- (1) whether the parasites could increase in population under a very high density population of host tilapias and whether density of the host could regulate the numbers of these parasites and
- (2) to determine the effect of hydrocortisone administration on the host with regards to the levels of C. sclerosus on the gills of tilapia. The result from the hydrocortisone experiment could indicate the possibility of a control mechanism moderated via the endocrine system, since hydrocortisone is a hormone which inter alia depresses the immune response of the host.

Hydrocortisone has been shown to promote the build-up of parasitic infection levels in higher vertebrates (Robinson, 1961; Olivier, 1962; Campbell, 1963; Esch, 1967; Macinnes and Voge, 1970). So far it has been little used in lower vertebrate work, especially in parasitic infections in fish. Robertson (personal communication) has used this drug to successfully build up the infections with the protozoan parasite Ichthyobodo necator in rainbow trout. This success was only achieved, however, when the drug was applied by the use of slow release of pellets of hydrocortisone inserted into the peritoneal cavity of the fish, allowing a continuous low level of cortisone to circulate in the fish. Intramuscular injection of hydrocortisone did not have any effect. Paperna (1960a) has described an acquired resistance or "self-cure" to Cichlidogyrus sp. by tilapias. He does not call it an immune response and has not published any experiments with regard to this aspect which would confirm his casual observations.

5.2. MATERIALS AND METHODS

5.2.1. Experiments to determine the effect of density of tilapia on the build-up of C. sclerosus infections.

Three glass tanks of dimensions (43.5 x 60 x 43.5) cu. cm. were used. The water in the tanks was recirculated and all were maintained at a temperature of 30°C. Each tank was divided into two compartments, one having a density of 100 S. mossambicus and the other 30 S. mossambicus and their densities were henceforth known as density 1 and density 2 respectively. The range of lengths of fish at the start of the experiment were 1 cm. to 2 cm.

The source of infection was S. niloticus. Five S. niloticus were placed in netlon baskets measuring (32 x 32 x 32) cu. cm. and kept floating in the tanks. The small size of the netlon baskets led to increased aggression among the S. niloticus, and contributed to some mortality amongst the source of infection, which had to be replaced continually. The S. niloticus from stock tanks were examined frequently and were found to have a level of infection much greater than S. mossambicus. There was an average of twelve parasites in fish greater than 10 cm. in length.

Some fish also died in the experimental tanks. On examination of these dead fish they were found to have a large number of parasites in proportion to their size (5 parasites in fish less than 2 cm. in length). There was also some cannibalism of small fish by bigger fish. As the fish initially obtained were fry of two days old, these fish had to be acclimatized to the experimental tanks and allowed to grow before killing off for examination. Hence a period of 28 days was allowed to elapse before batches of fish were killed and examined.

A further experiment was conducted using three tanks, each of which was divided into two separate halves by a glass screen. One-half had a density of 150 S. mossambicus and the other a density of 37 S. mossambicus initially and the densities of these halves were henceforth known as density A and density B respectively. 150 fish was used because, according to Macintosh, (personal communication) it was a density which would not cause undue stress to the fish host when the fish had grown bigger. The results of the experiments were analyzed by hand computation using a Casio-Fx Scientific calculator.

The frequency distributions of the parasites were found for all the tanks. The data were analyzed to see if they fitted a Poisson Distribution (using a Texas Personal Computer) or a Negative Binomial Distribution by the Maximum Likelihood Method (Bliss and Fisher, 1953).

If parasites are distributed at random amongst their hosts the variance and mean should be equal and the distribution Poissonian (Sokal and Rolf, 1973). When the variance is greater than the mean the distribution is overdispersed and a Negative Binomial Distribution can be fitted, which is appropriate for the description of host-parasite distributions (Crofton, 1971). The mathematics of the Negative Binomial Distribution have been derived from Fisher (1941), Anscombe (1950) and Bliss and Fisher (1953). Negative Binomial Distribution (according to Pennycuick, 1971).

General Introduction

This is given by the expansion of the expression
 $(q - p)^{-k}$ where $q = 1 + p$.

The mean is $\mu = pk$ and the variance s^2 is $s^2 = pkq$.

The Negative Binomial Distribution can arise

- (1) Due to the host being exposed to several waves of infection, each being a random attack and this gives rise to a series of Poissons.
- (2) The infective stages of the parasite are not randomly distributed.
- (3) The infection in a host increases or decreases the chances of a further infection.
- (4) As a result of the variation in host individuals which makes the chances of infection unequal.

5.2.2. Hydrocortisone experiment

Two experiments were carried out. In the first experiment, five S. aureus were used as controls and five were used as treated fish. These fish were taken from stock tanks where all fish were known to be lightly infected. The control fish and treated fish were kept in separate tanks. Water was aerated and recycled, as the tanks were part of a recirculating water system. The temperature of the water was 30°C.

In the second experiment, four fish were used for controls and four fish for the treatments. The species here was S. mossambicus. The treated fish were injected with 0.2 ml of hydrocortisone, intramuscularly. The control fish were injected with glucose in cholesterol.

The hydrocortisone powder was prepared as follows. Cortisole and cholesterol were weighed out in the ratio of 1:2. The compounds were dissolved in ethyl ether, then blown off in a stream of nitrogen in a fume cupboard, leaving a well-mixed powder. The injection was done by first anaesthetizing the fish

slightly, using benzocaine and alcohol, and the needle containing hydrocortisone was injected into the muscle of the lateral line system. There was a tendency for the fluid to come out of the needle sinus once the needle was pulled out, so a piece of tissue was applied to the infected spot for two minutes before the fish was released into fresh copper-free water. One or two fish died after the injection and had to be replaced with new fish. At the end of eleven days, one fish from each of the control and treated groups were killed by pithing and the gills examined for C. sclerosus. Subsequently, one fish from each set of tanks was killed at one day intervals.

5.3. RESULTS

5.3.1. Results of the experiments on the effect of density of tilapia on the build-up of C. sclerosus.

The results of the preliminary experiment on the effect of density of tilapia on the build-up of C. sclerosus are shown in Tables XVII and XVIII and Figures 15 and 16. These results indicated that the number of parasites per fish was variable in the different tanks (0-2.25 parasites/fish). There was an increase in the mean numbers of parasites per fish in all tanks on day 42, which was 14 days after the commencement of the experiment.

The variation in results in mean number of parasites for infected fish in this experiment prompted the author to carry out a second experiment where the density of the host fish was increased to 150 and 37 from 100 and 30 respectively. The infection of C. sclerosus on S. mossambicus, at weekly intervals, is given in Tables XIX and XX and Figures 17-20.

The results obtained from the second experiment was similar to the preliminary experiment, with the parasite numbers building up and declining at intervals of 30 days. This is well illustrated in Figure 21. The fluctuation of the parasite population at an interval of 15 days could be related to the life span of C. sclerosus. From observations it is noted that C. sclerosus takes 4 days to hatch at 25°C. Thus it takes about 15 days or several generations of parasites for the parasites to build up their numbers. The building up of the parasite population induces the host to produce a defence mechanism which result in the decline of the parasites at the end of the next 15 days.

TABLE XVII. The level of infection of C. sclerosus on S. mossambicus at weekly intervals, for two different densities of fish.

Interval in days	Tank No.	Numbers of fish	Numbers of fish infected	Numbers of parasites	Mean parasite per fish	Standard deviation
28	32	12	2	3	1.50	0.62
	30	9	2	4	2.00	0.88
	29	4	0	0	0.00	0.00
	31	5	2	3	1.50	0.89
35	32	12	0	0	0.00	0.00
	30	9	2	4	2.00	1.01
	29	4	1	1	1.00	0.50
	31	5	0	0	0.00	0.00
42	32	12	3	5	1.67	0.90
	30	9	1	2	2.00	0.67
	29	4	2	3	1.50	0.96
	31	5	1	1	1.00	0.45
49	32	12	4	5	1.25	0.65
	30	9	3	3	1.00	0.50
	29	4	0	0	0.00	0.00
	31	5	2	2	1.00	0.55
56	32	12	1	1	1.00	0.29
	30	9	2	2	1.00	0.44
	29	4	0	0	0.00	0.00
	31	5	4	9	2.25	1.10
63	32	5	2	2	1.00	0.55
	29	3	0	0	0.00	0.00

TABLE XVIII. Results of preliminary experiment on the effect of density of tilapia on the build-up of C. sclerosus.

	Density 1		Density 2	
Tank number	32	30	31	29
Total numbers of fish at the end of the experiment	65	45	25	23
Total numbers of <u>C. sclerosus</u>	16	15	15	4
Total numbers of fish infected	12	10	9	3
Mean numbers of parasites per fish (Mean Intensity)	1.33	1.50	1.67	1.33
Standard deviation	0.64	0.76	0.24	0.59
Prevalence	18.46	22.22	36.00	13.04
Pooled data				
Total numbers of fish	110		48	
Total numbers of fish infected	22		12	
Total numbers of parasites	31		19	
Total percentage infection	20		25	
Mean numbers of parasites per infected fish	1.41		1.58	
Standard deviation	1.63		1.82	

GRAPH OF NUMBERS OF C. SCLEROSUS PER SAMPLE AGAINST INTERVAL IN WEEKS FOR TWO DENSITIES OF S. MOSSAMBICUS.

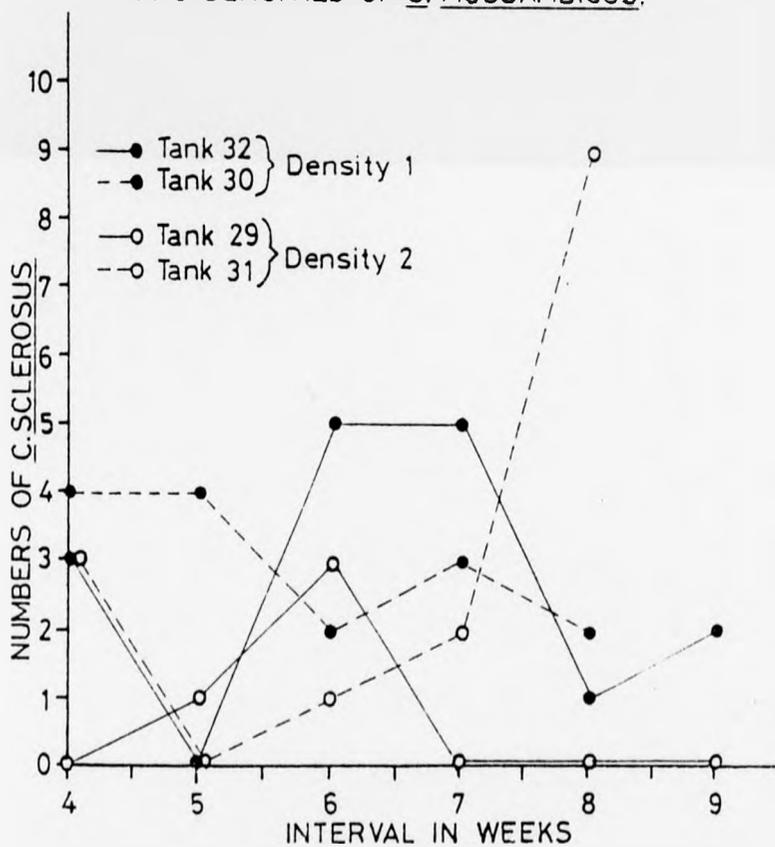


Fig. 15. Graph of numbers of *C. sclerosus* per fish against interval in weeks, for two densities of *S. mossambicus*.

GRAPH OF NUMBERS OF C. SCLEROSUS PER SAMPLE AGAINST INTERVAL IN WEEKS FOR TWO DENSITIES OF S. MOSSAMBICUS.

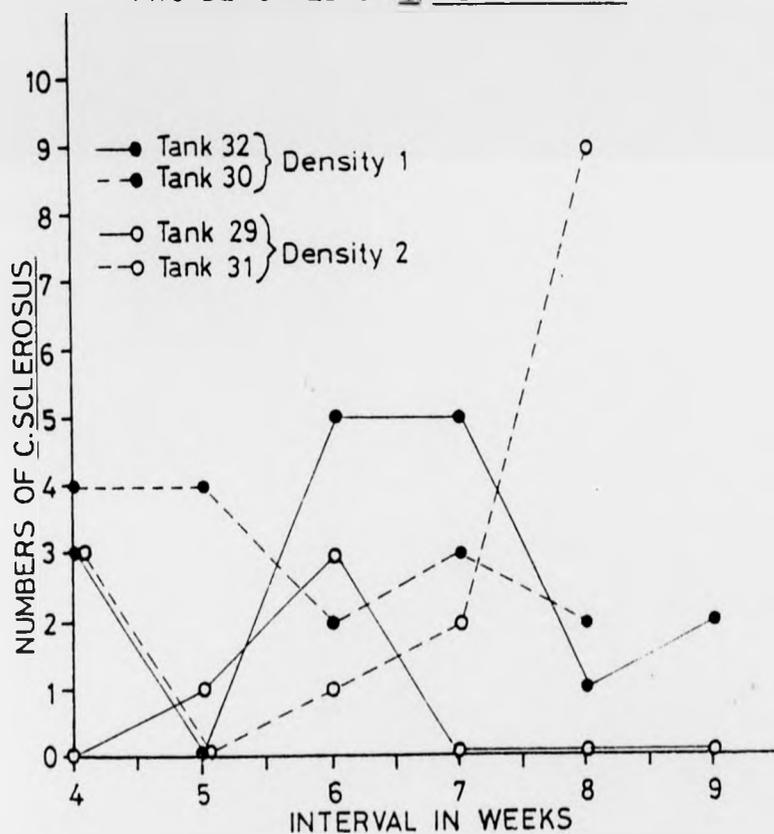


Fig. 15. Graph of numbers of C. sclerosus per fish against interval in weeks, for two densities of S. mossambicus.

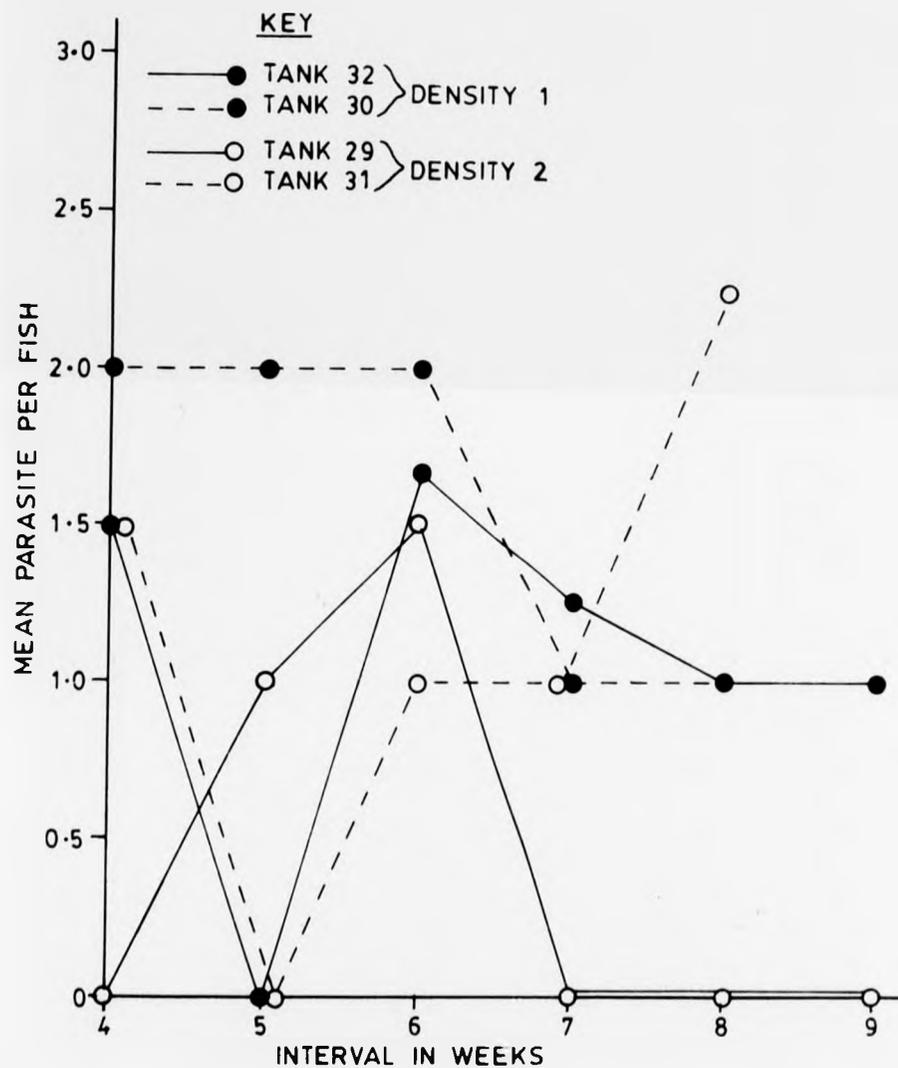


Fig. 16. Graph of mean C. sclerosus per fish against interval in weeks, for two densities of S. mossambicus.

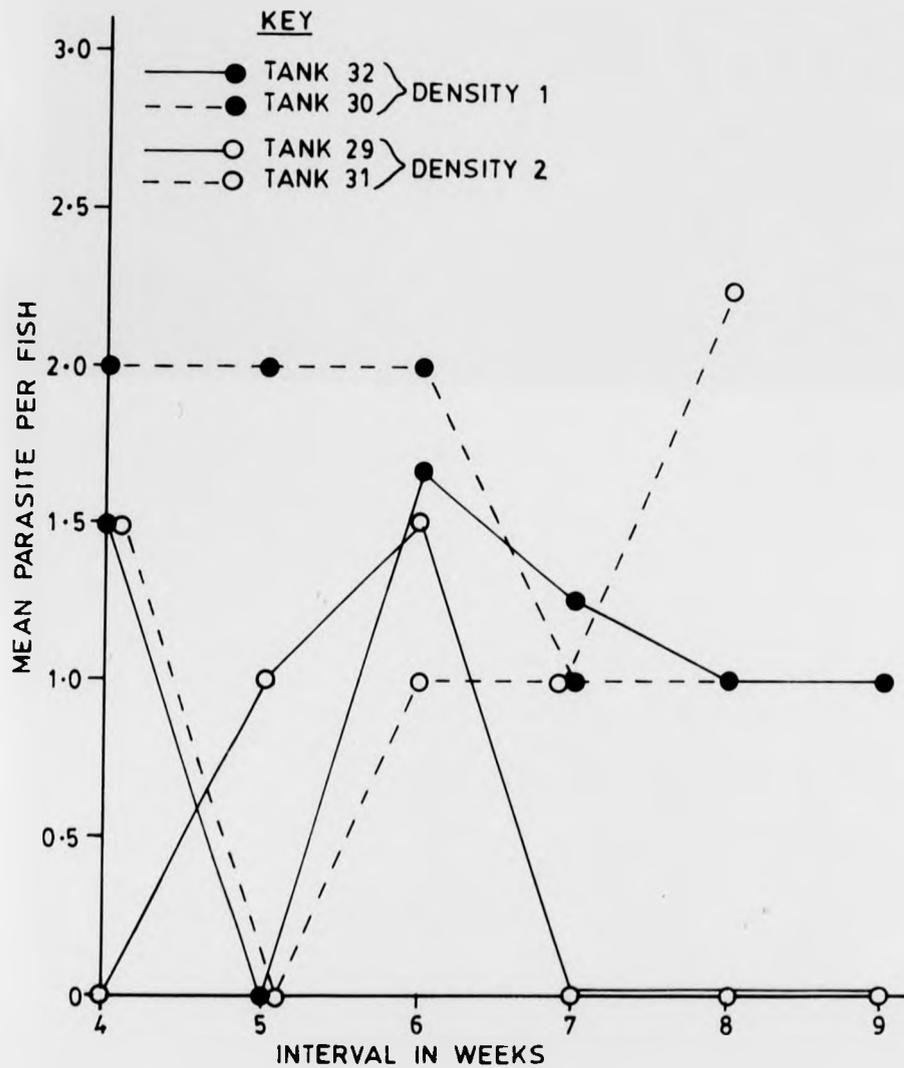


Fig. 16. Graph of mean C. sclerosus per fish against interval in weeks, for two densities of S. mossambicus.

TABLE XIX. The mean number of C. sclerosus on S. mossambicus at weekly intervals.

The results are from the Density A group

Tank Number	Interval in days	Numbers of fish examined	Numbers of fish infected	Prevalence %	Numbers of <u>C. sclerosus</u>	Mean intensity	Standard deviation
5A	37	20	9	45	18	2.00	1.12
	44	20	11	55	21	1.91	1.28
	51	20	8	40	18	2.25	1.29
	58	20	12	60	38	3.17	2.15
	65	20	15	75	48	3.20	2.39
	72	20	14	70	30	2.14	1.50
	79	17	9	53	27	3.00	1.94
Total		137	78		200	Mean 2.52	Mean 1.67
15A	39	20	15	75	76	5.07	4.18
	46	20	16	80	64	4.00	3.56
	53	20	14	70	42	3.00	2.19
	60	20	16	80	51	3.19	2.91
	67	20	18	90	87	4.83	3.17
	74	20	16	80	76	4.75	3.30
	81	16	6	38	18	3.00	1.93
Total		136	101		414	Mean 3.98	Mean 3.03
16A	41	20	13	65	28	2.15	1.70
	48	20	9	45	24	2.67	1.85
	55	20	12	60	20	1.67	1.08
	62	20	6	30	10	1.67	0.89
	69	20	9	45	15	1.67	1.02
	76	20	12	52	23	1.92	1.39
	83	15	2	13	3	1.50	0.56
Total		135	63		123	Mean 1.89	Mean 1.21

The results for the density B group are shown as follows:-

TABLE XX. The mean number of C. sclerosus on S. mossambicus at weekly intervals.

Tank Number	Interval in days	Numbers of fish examined	Numbers of fish infected	Prevalence %	Numbers of <u>C. sclerosus</u>	Mean Intensity	Standard deviation
5B	37	8	4	50	10	2.50	1.58
	44	8	4	50	15	3.75	2.75
	51	8	7	88	26	3.71	2.49
	58	8	5	63	7	1.40	0.83
	65	8	6	75	15	2.50	2.30
	72	8	6	75	11	1.83	1.30
	79	7	3	43	5	1.67	0.95
Total		55	35		89	Mean 2.48	Mean 1.74
15B	39	4	4	100	23	5.75	2.63
	46	4	4	100	17	4.25	2.87
	53	4	3	75	5	1.67	0.96
	60	4	4	100	25	6.25	5.12
	67	4	4	100	16	4.00	2.58
	74	4	3	75	16	5.33	4.24
	81	2	2	100	7	3.50	2.12
Total		26	24		109	Mean 4.39	Mean 2.93
16B	41	4	2	50	9	4.50	2.63
	48	4	2	50	3	1.50	0.96
	55	4	1	25	4	4.00	2.00
	62	4	3	75	7	2.33	1.26
	69	4	3	75	7	2.33	1.71
	76	4	3	75	8	2.67	1.63
	83	4	2	50	6	3.00	2.06
	90	3	2	67	18	9.00	7.94
Total		31	18		72	Mean 3.67	Mean 2.52

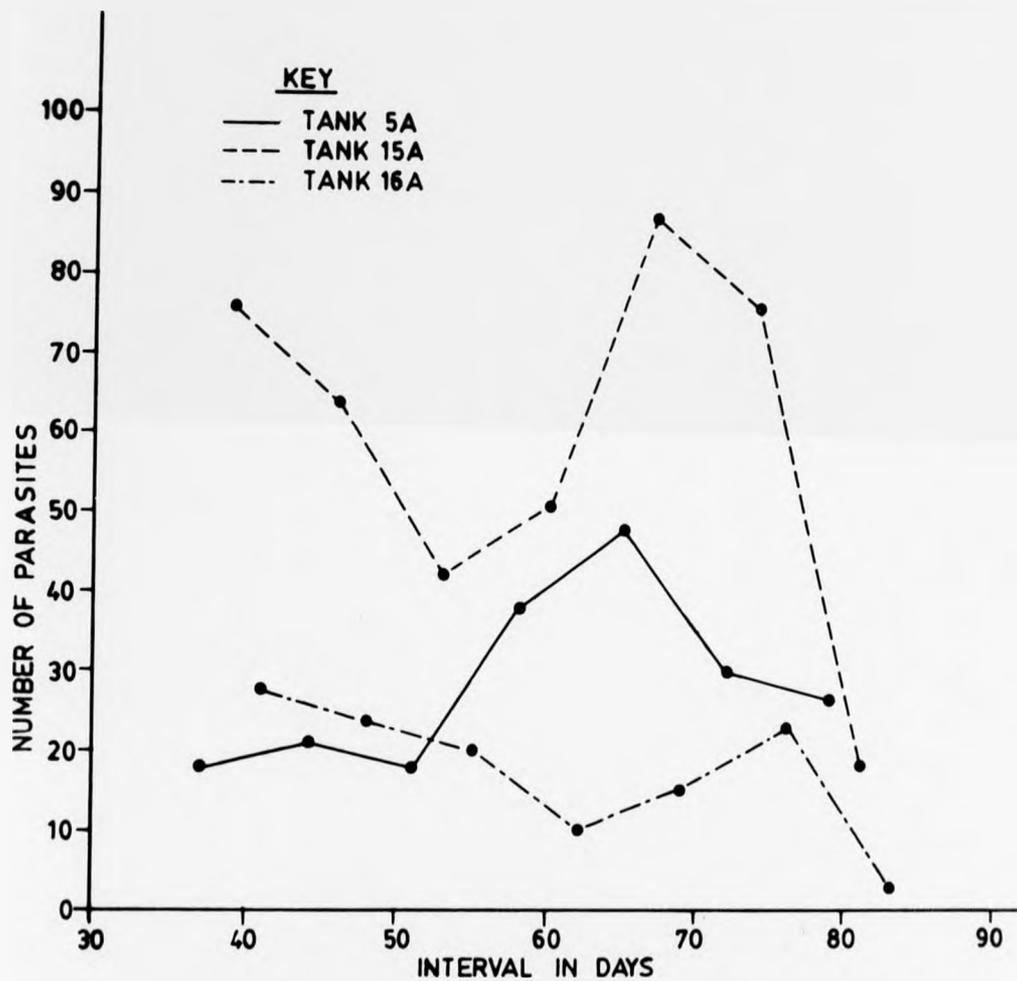


Fig. 17. Graph of numbers of *C. sclerosus* per sample, against interval in days, for Density A tanks.

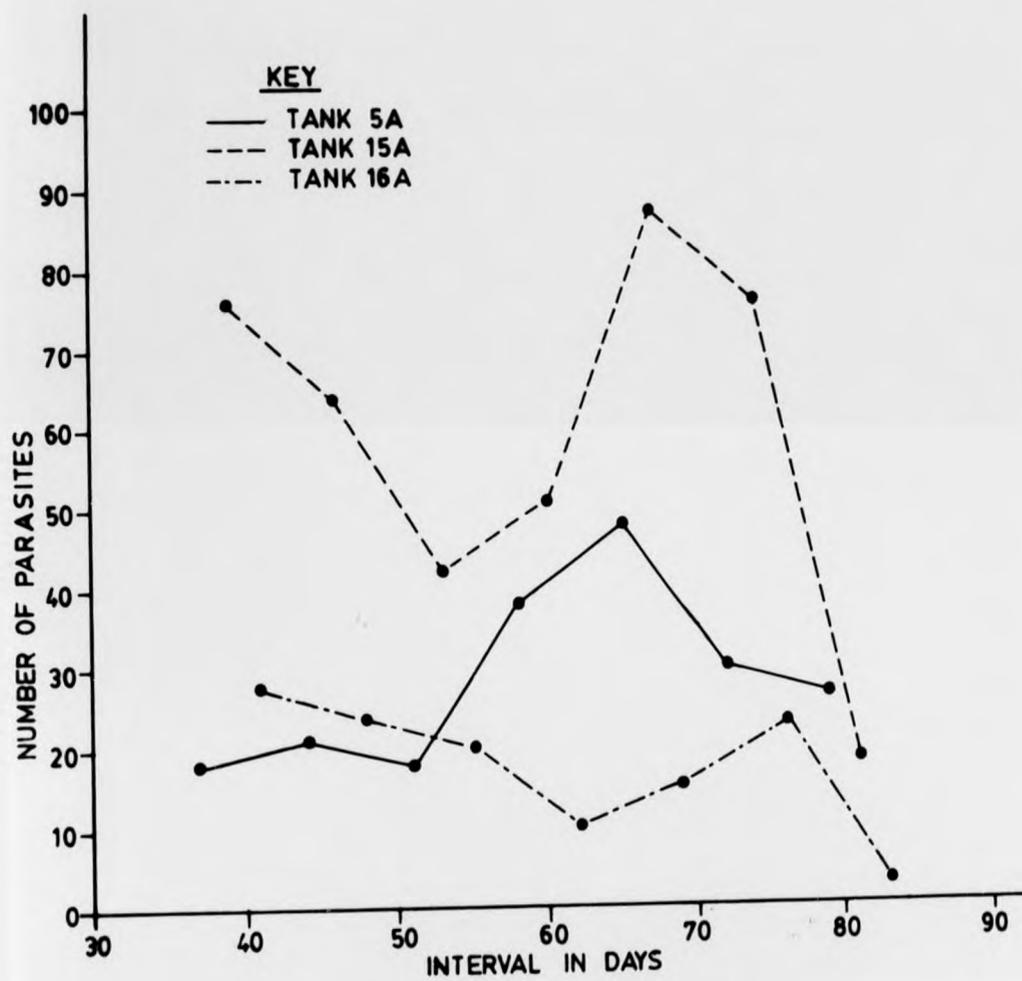


Fig. 17. Graph of numbers of *C. sclerosus* per sample, against interval in days, for Density A tanks.

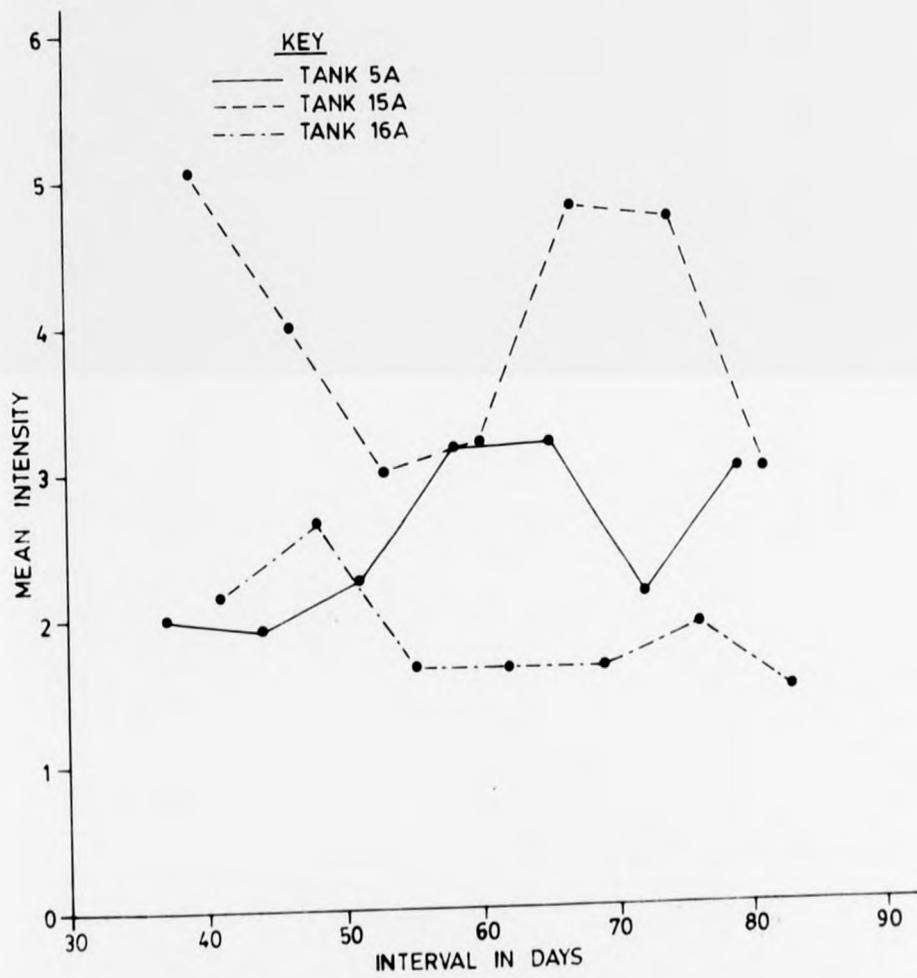


Fig. 18. Graph of mean intensity of *C. sclerosus* per fish, against interval in days, for Density A tanks.

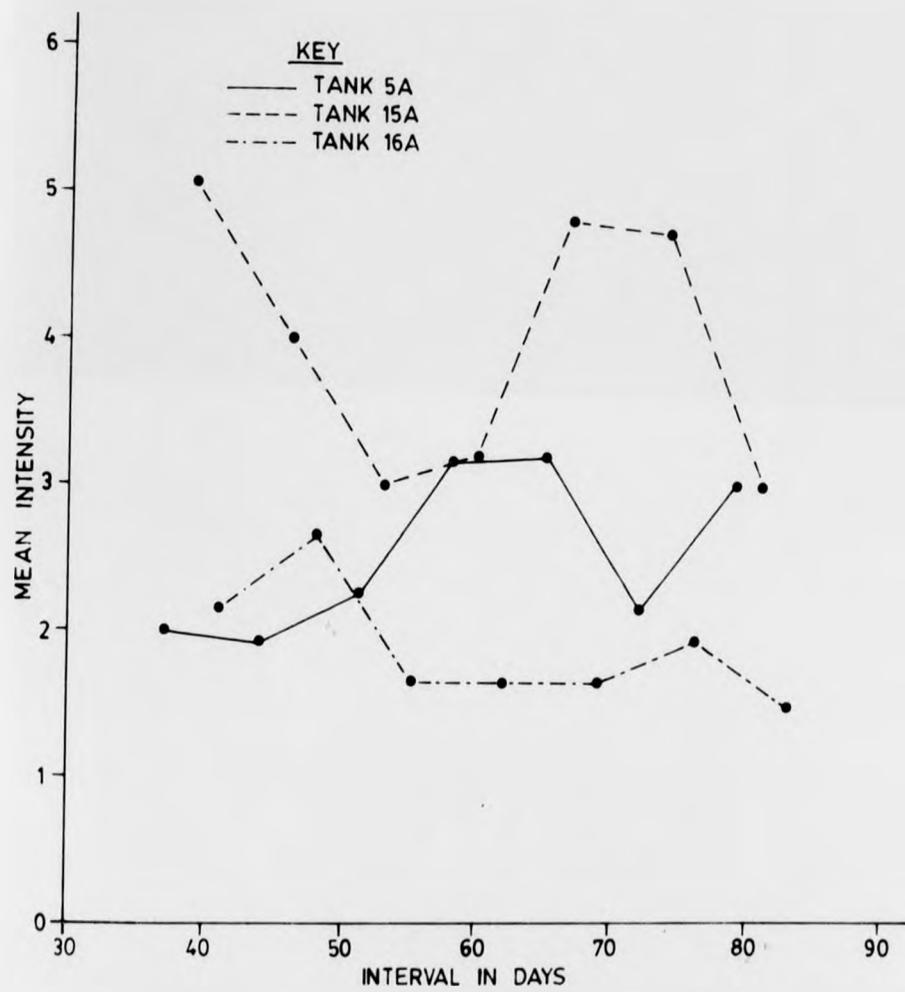


Fig. 18. Graph of mean intensity of *C. sclerosus* per fish, against interval in days, for Density A tanks.

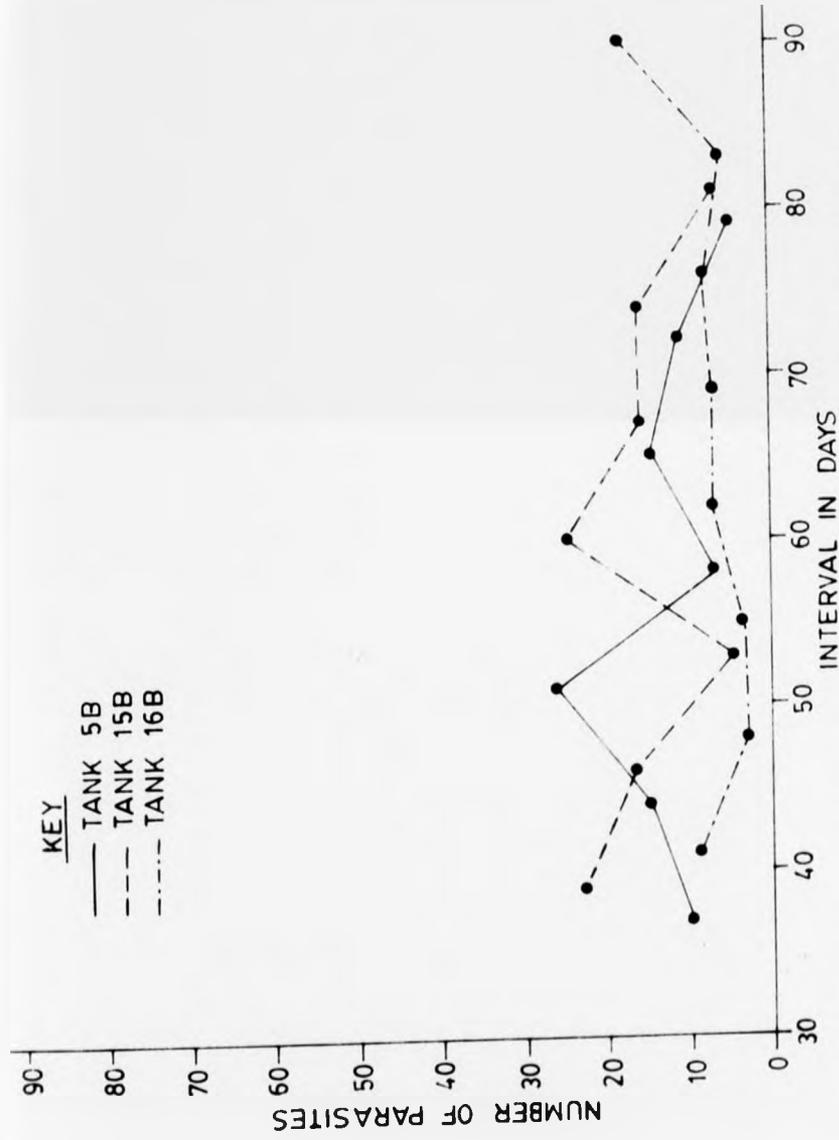


Fig. 19. Graph of numbers of *C. sclerosus* per sample, against interval in days, for Density B tanks.

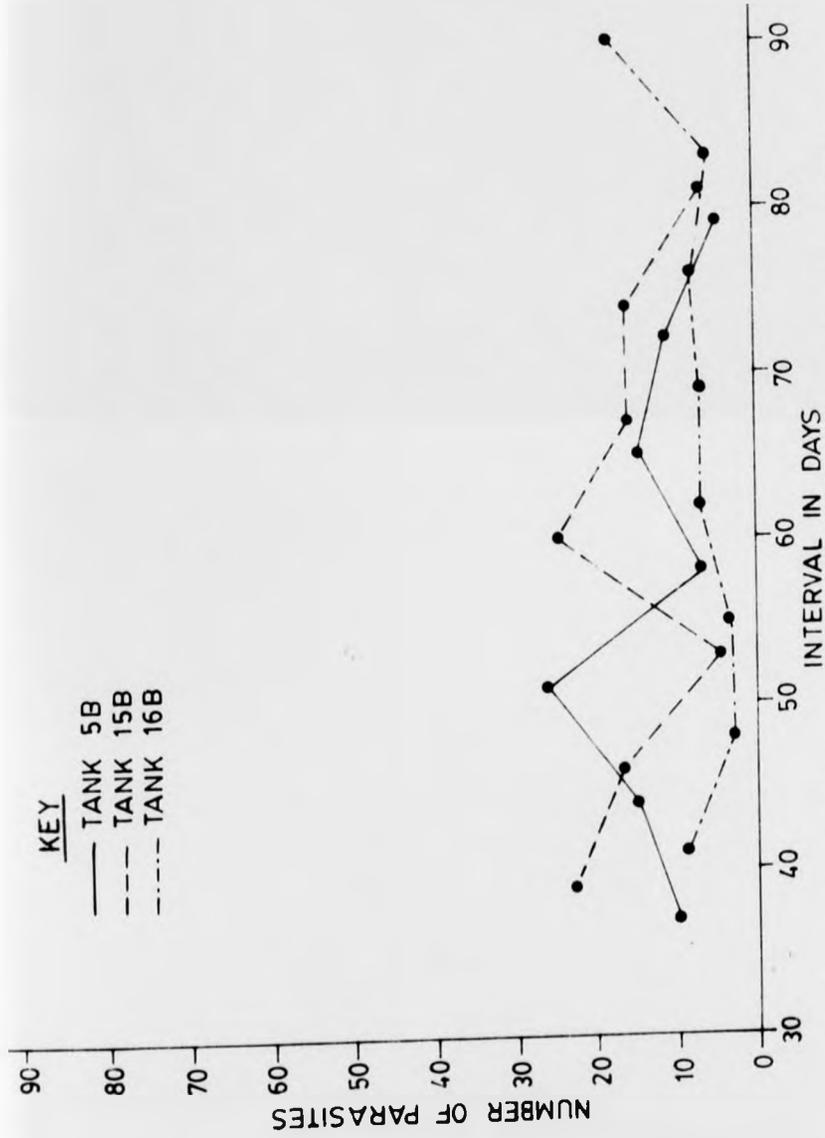


Fig. 19. Graph of numbers of *C. sclerosus* per sample, against interval in days, for Density B tanks.

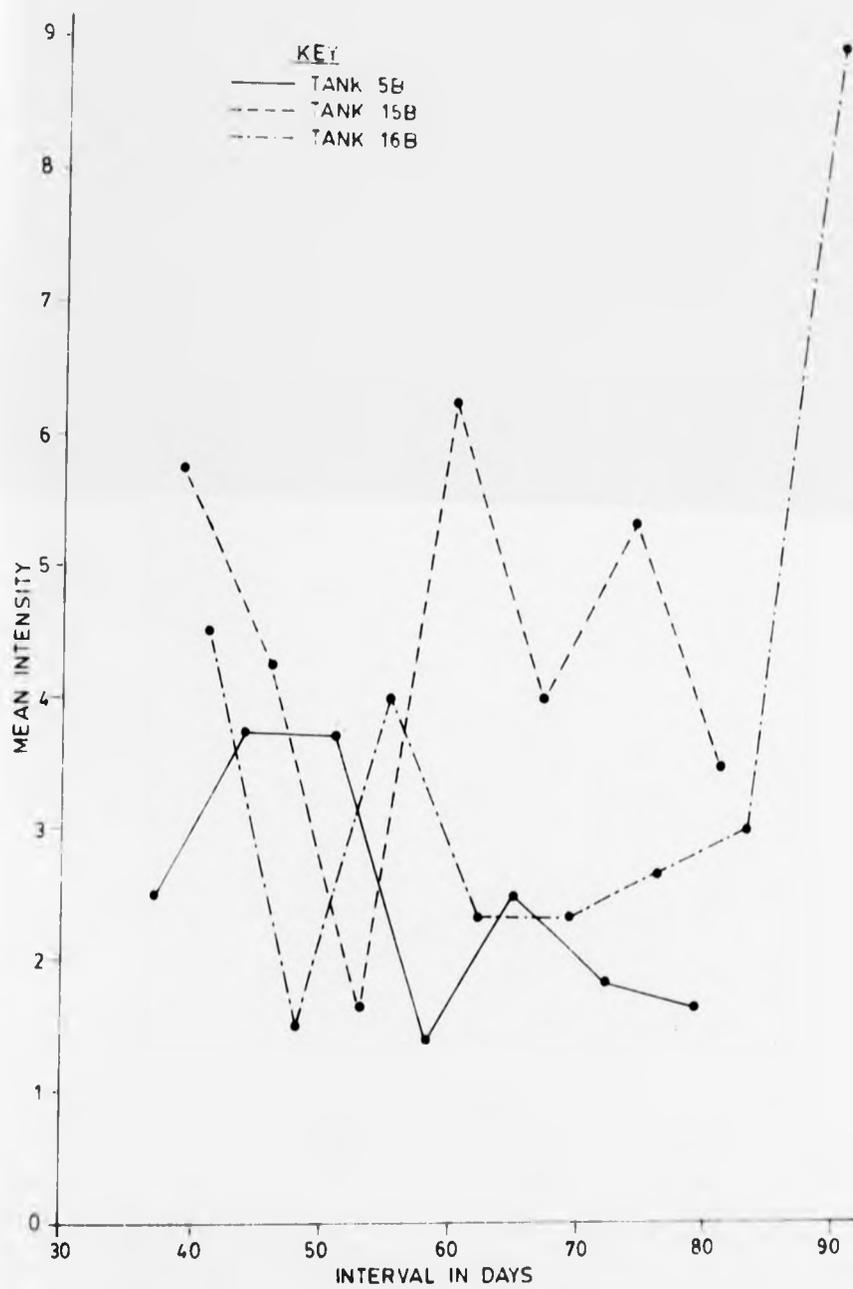


Fig. 20. Graph of mean intensity of *C. sclerosus* per fish, against interval in days, for Density B tanks.

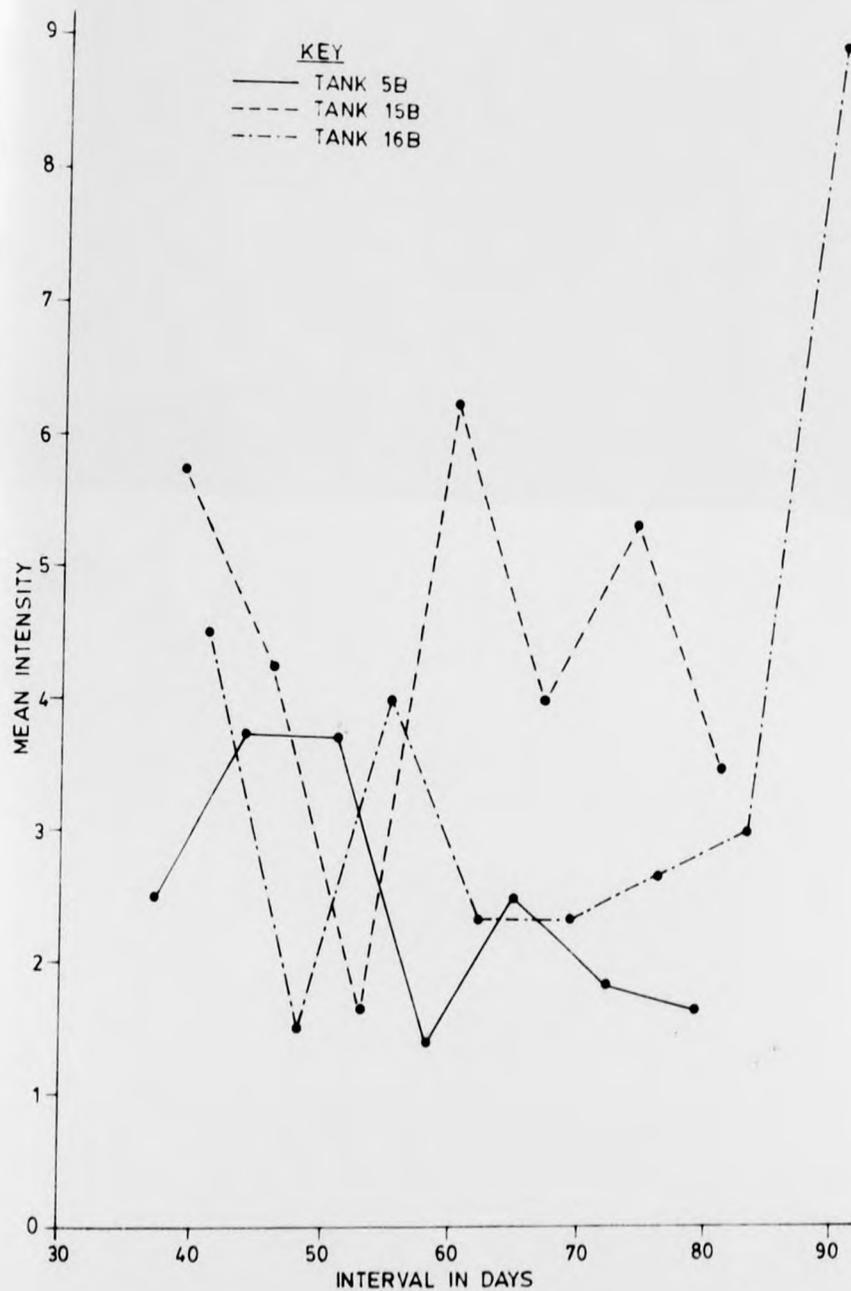


Fig. 20. Graph of mean intensity of *C. sclerosus* per fish, against interval in days, for Density B tanks.

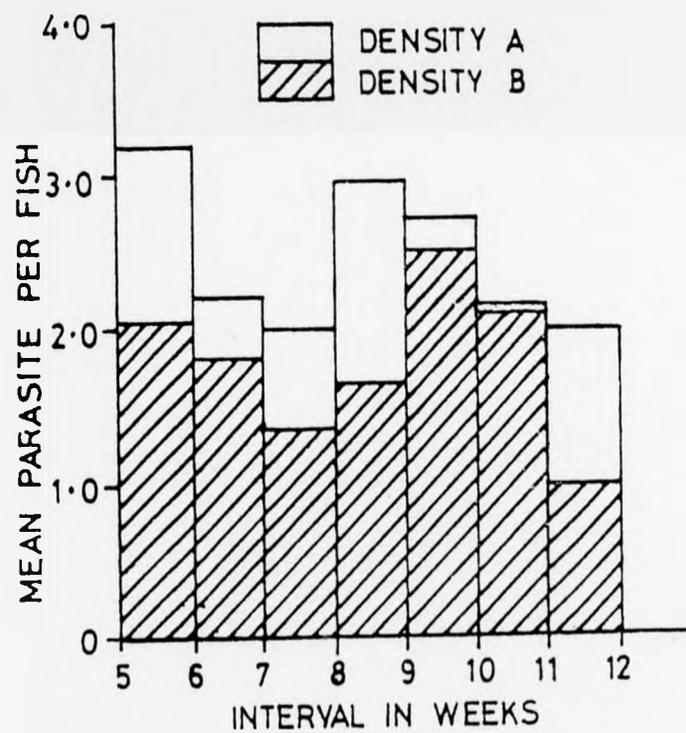
AVERAGE WEEKLY LEVEL OF INFECTION
PER FISH

Fig. 21. Average weekly infection of *C. sclerosus* on tilapia.

AVERAGE WEEKLY LEVEL OF INFECTION
PER FISH

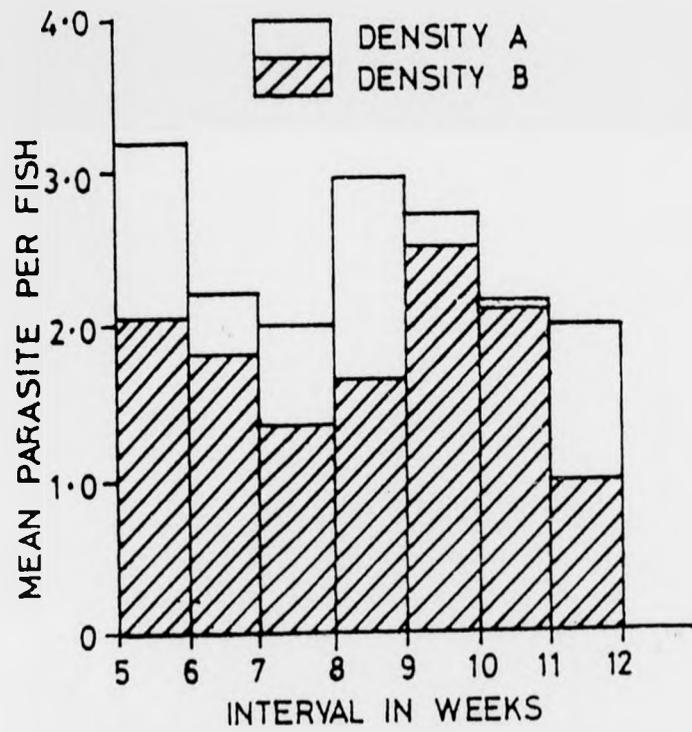


Fig. 21. Average weekly infection of *C. sclerosus* on tilapia.

In order to establish if there was a significant difference between the levels of infection in the density A and density B tanks, a Mann-Whitney statistical test was employed. Firstly, the results were arranged in ascending order of value with the density A values being underlined to distinguish them.

0.991.351.651.816

2.00 2.00

2.0332.12

2.13

2.21

2.50

2.71

2.96

3.17

Each density B value was taken in turn and the number of density A values numerically lower than it was recorded. The following figures were obtained:-

Sum of counts

$$4 + 4 + 6 + 6 + 7 + 7 + 7 = 41$$

The Mann-Whitney statistics is the greater of the two quantities C and $n_1 n_2 - C$, in this case 41 and $(7 \times 7) - 41 = 8$

$$\text{Since } U \leq 0.025 (7, 7) = 41$$

The Null Hypothesis is rejected. The two samples are significantly different at $P < 0.05$.

5.3.2. Results of Poisson Distribution and Negative Binomial Distribution fitted to data from all tanks at weekly intervals.

The observed frequency distributions for each tank, at different time intervals, and the weekly frequency distributions, obtained by combining these results, together with fitted Poisson and Negative Binomial Distributions are shown in Figures 22 to 28 for both A and B densities. The total frequency distribution for each density, together with fitted Poisson and Negative Binomial Distributions, are shown in Figures 29 and 30.

Detailed results for the Poisson Distribution and calculations for tests of fit are shown in Appendix 9. The calculation procedures for the Negative Binomial values are shown in Appendix 10. The statistics of the original distribution are given in Table XXI. The detailed calculations for the Negative Binomial Distributions are shown in Appendix 11, 12 and 13.

For density A (Table XXI) the overdispersion ranged from 2.05 - 4.23 with an average of 3.08. For density B the overdispersion ranged from 2.24 - 6.43 with an average of 3.50. The zero class was the largest in each weekly distribution for density A by at least 10%, with the exception of week 10. This was not the case with the B density weekly distributions where the zero class was equalled by classes 1 and 2 for weeks 8 and 10 and was less than class 1 for week 9 (Fig. 22-28).

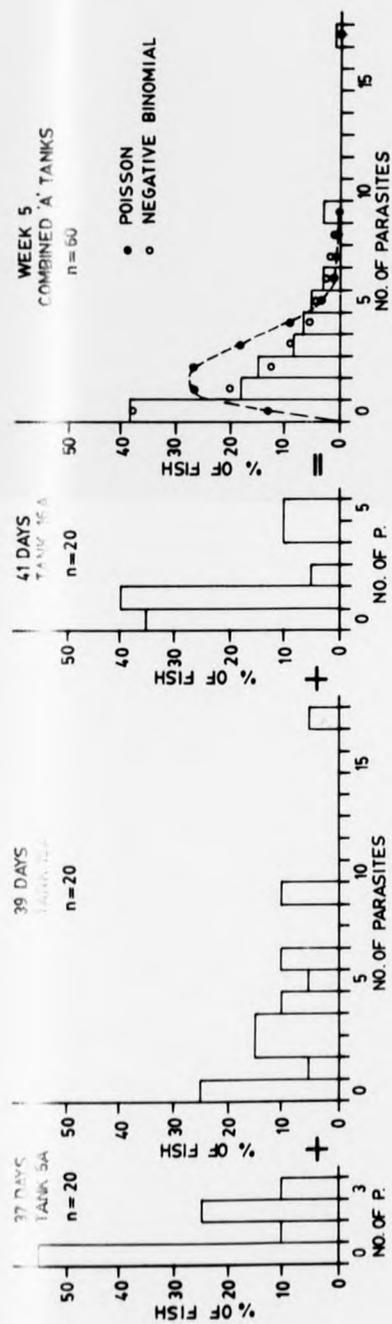
For the overall results, for density A the overdispersion was 3.27 and for density B, 3.33 (Table XXI). The zero

TABLE XXI. Statistics of original distribution and fitted Negative Binomial Distribution - Time Interval Results.

A - High Density Results							
Original Distribution				Negative Binomial			
Week No.	% infection	\bar{x}	s^2	$\frac{s^2}{\bar{x}}$	p	k	pqk
5	61.67	2.03	8.58	4.23	2.85	0.71	7.79
6	60.00	1.82	6.69	3.68	2.55	0.71	6.43
7	56.67	1.33	2.73	2.05	1.23	1.08	2.96
8	56.67	1.65	5.21	3.16	2.34	0.70	5.47
9	70.00	2.50	7.61	3.04	2.48	1.01	8.72
10	70.00	2.15	6.27	2.92	1.97	1.09	6.38
11	35.42	1.00	2.47	2.47	3.09	0.32	4.04

B - Low Density Results							
Original Distribution				Negative Binomial			
Week No.	% infection	\bar{x}	s^2	$\frac{s^2}{\bar{x}}$	p	k	pqk
5	62.50	2.63	7.58	2.88	3.11	0.84	10.74
6	62.50	2.19	7.10	3.24	2.62	0.84	7.97
7	68.75	2.19	5.10	2.33	1.56	1.41	5.63
8	75.00	2.44	11.20	4.59	2.54	0.96	8.63
9	81.25	2.38	5.32	2.24	1.21	1.96	5.24
10	75.00	2.19	6.16	2.81	1.58	1.38	5.63
11	56.25	2.25	14.47	6.43	4.74	0.47	12.79

Total results							
Tank 5A	59.31	1.81	5.92	3.27	2.45	0.74	6.25
Tank 5B	68.75	2.32	7.72	3.33	2.37	0.98	7.83



OBSERVED FREQUENCY DISTRIBUTIONS

WEEK 5

AND COMBINED RESULTS WITH FITTED POISSON AND NB DISTRIBUTIONS.

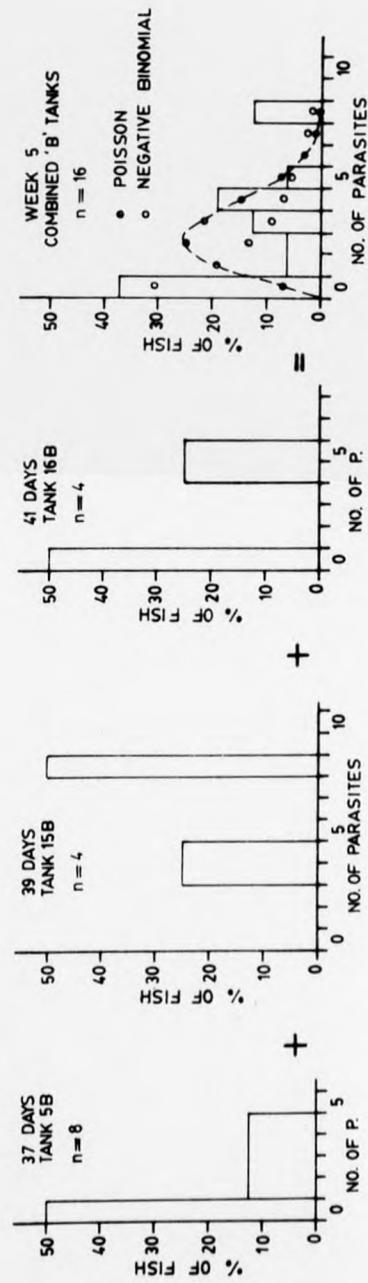


Fig. 22. Observed frequency distributions for week 5 and fitted Poisson and Negative Binomials for combined results.

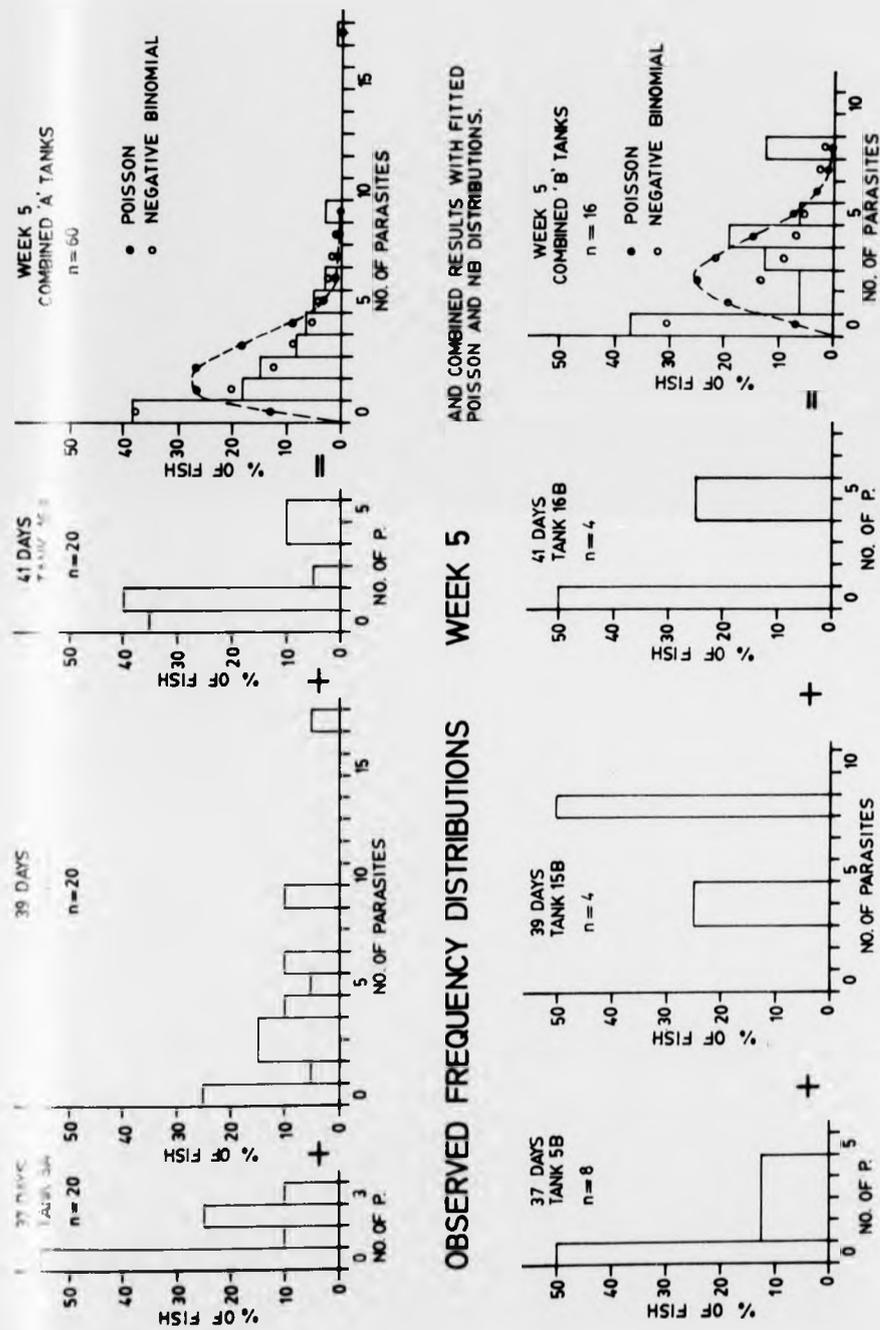
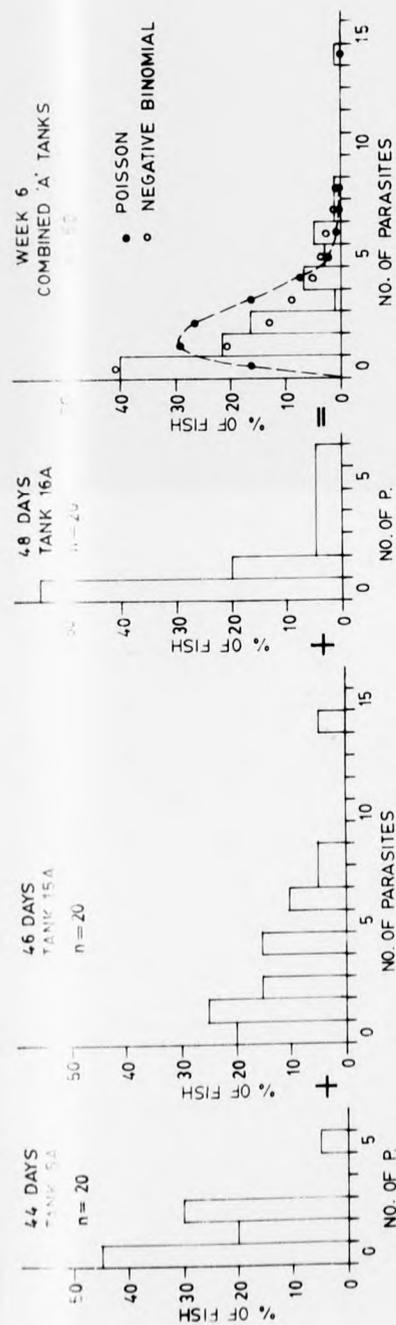


Fig. 22. Observed frequency distributions for week 5 and fitted Poisson and Negative Binomials for combined results.



AND COMBINED RESULTS WITH FITTED
POISSON AND NB DISTRIBUTIONS.

WEEK 6

OBSERVED FREQUENCY DISTRIBUTIONS

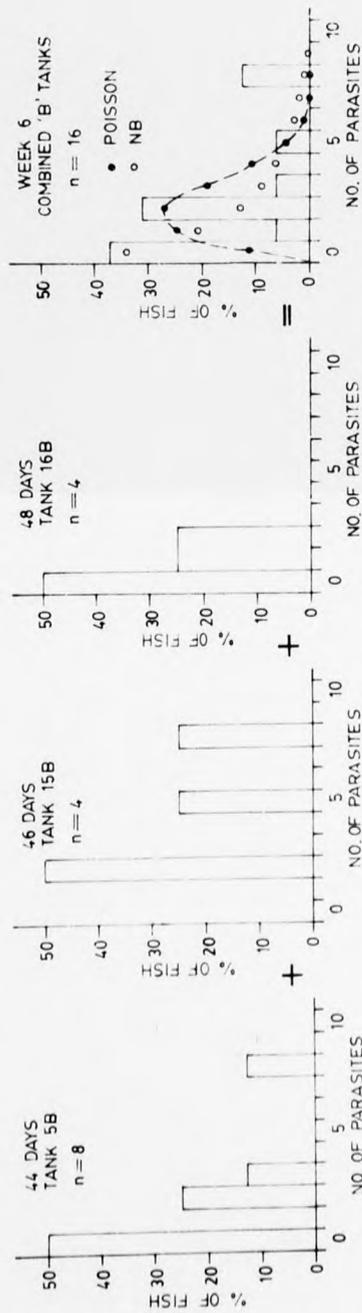
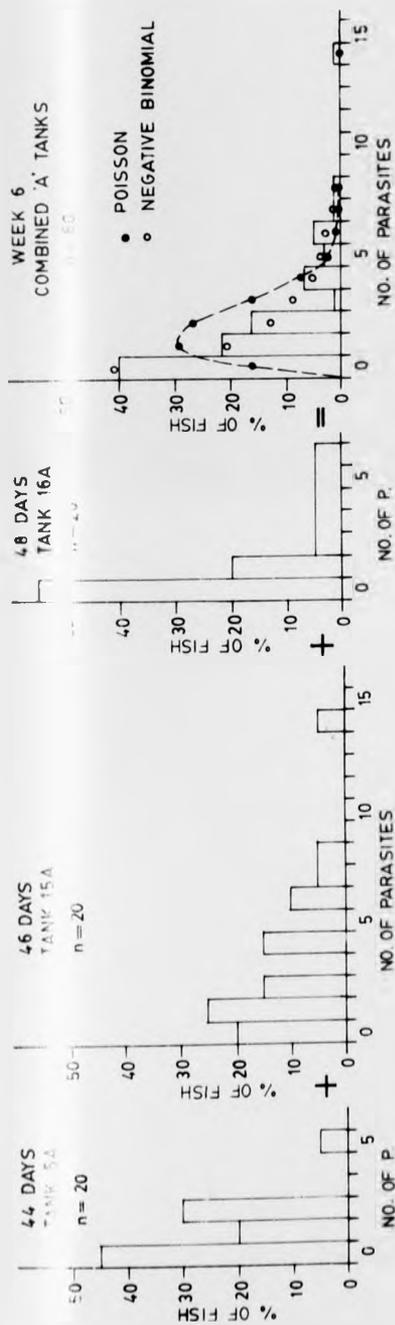


Fig. 23. Observed frequency distributions for week 6 and fitted Poisson and Negative Binomials for combined results.



OBSERVED FREQUENCY DISTRIBUTIONS

WEEK 6

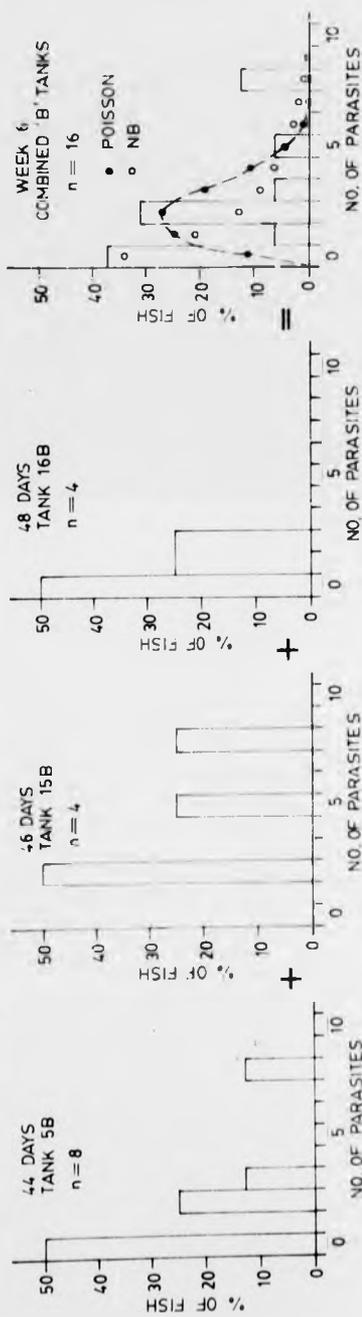
AND COMBINED RESULTS WITH FITTED
POISSON AND NB DISTRIBUTIONS.

Fig. 23. Observed frequency distributions for week 6 and fitted Poisson and Negative Binomials for combined results.

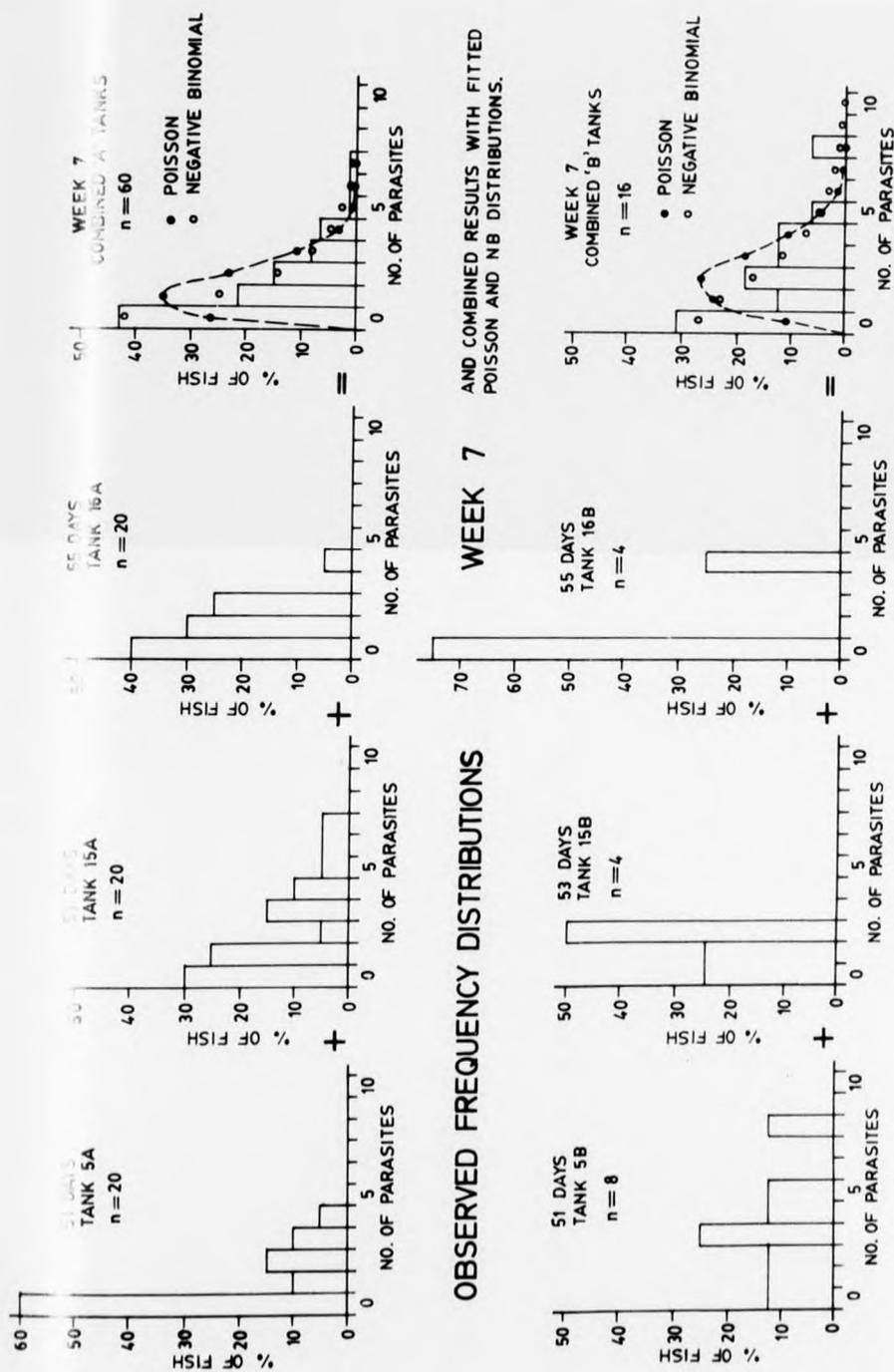
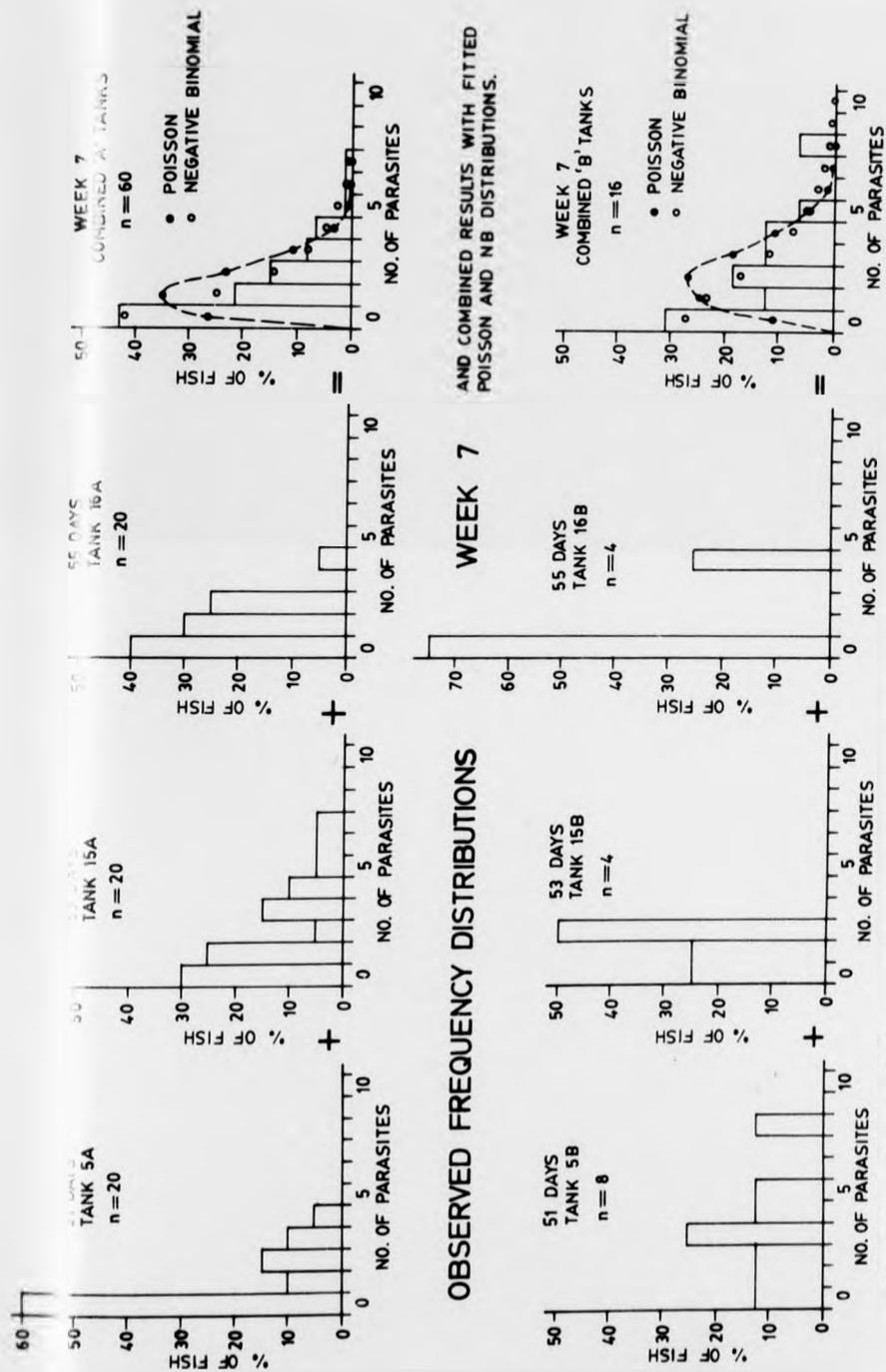
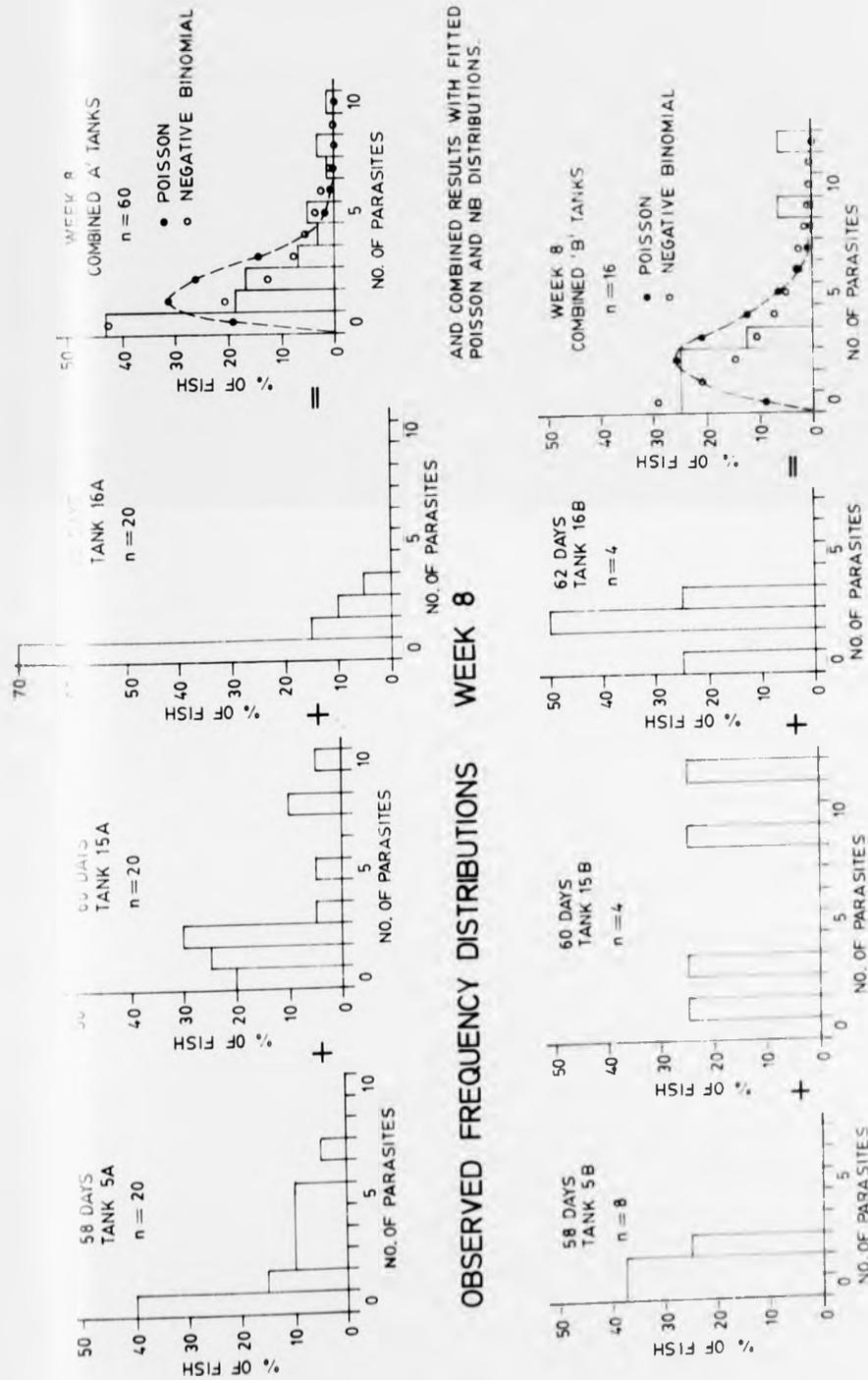


Fig. 24. Observed frequency distributions for week 7 and combined results with fitted Poisson and Negative Binomial Distributions.





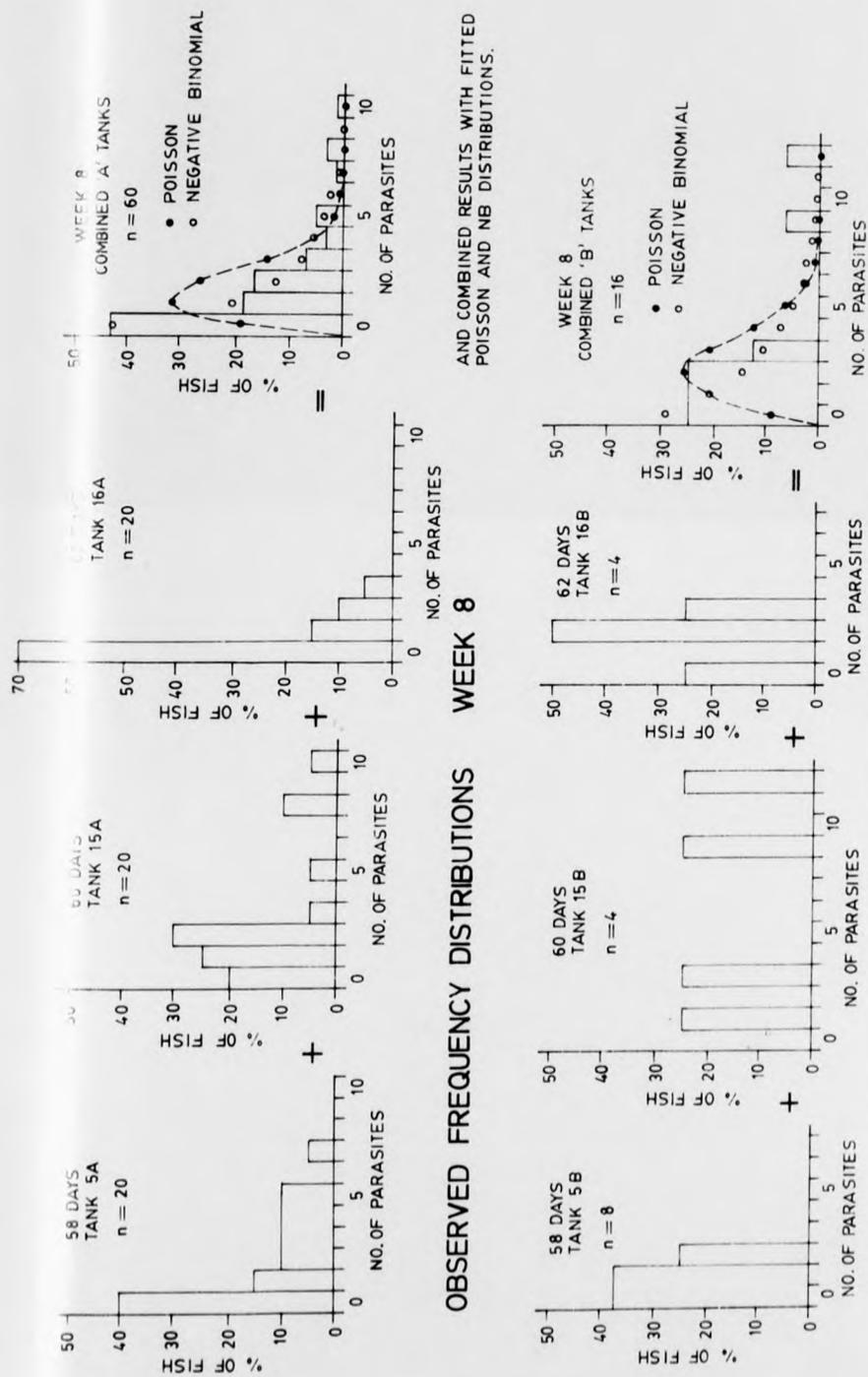
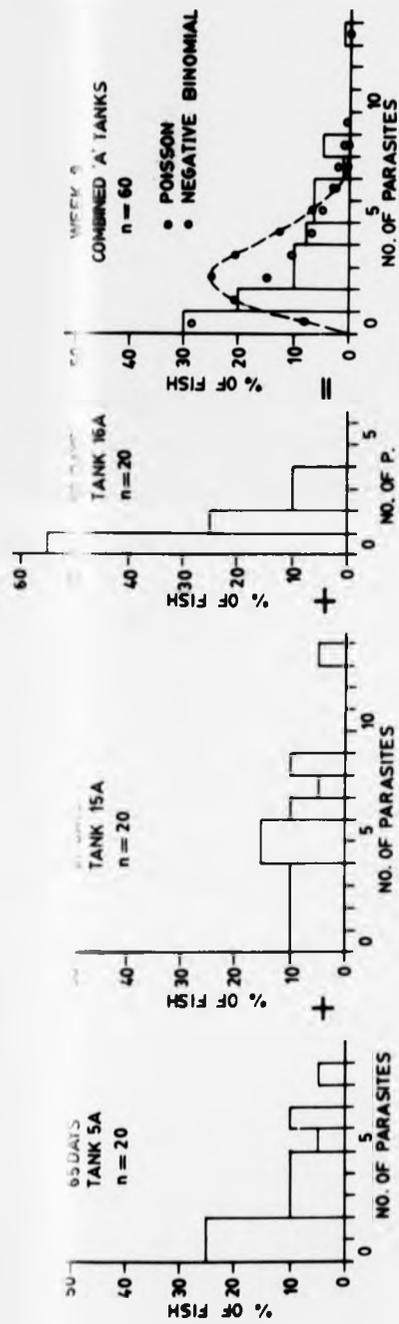


Fig. 25. Observed frequency distributions for week 8 and combined results with fitted Poisson and Negative Binomial Distributions.



OBSERVED FREQUENCY DISTRIBUTIONS

WEEK 9

AND COMBINED RESULTS WITH FITTED POISSON AND NB DISTRIBUTIONS.

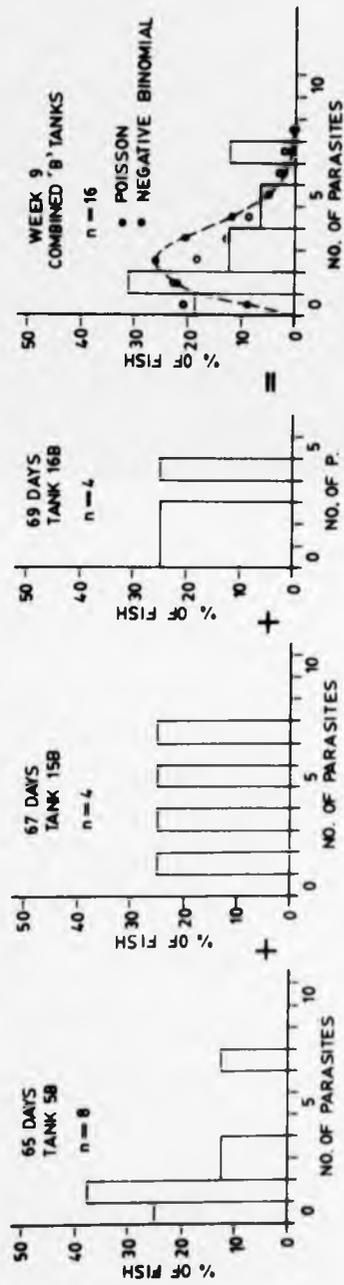
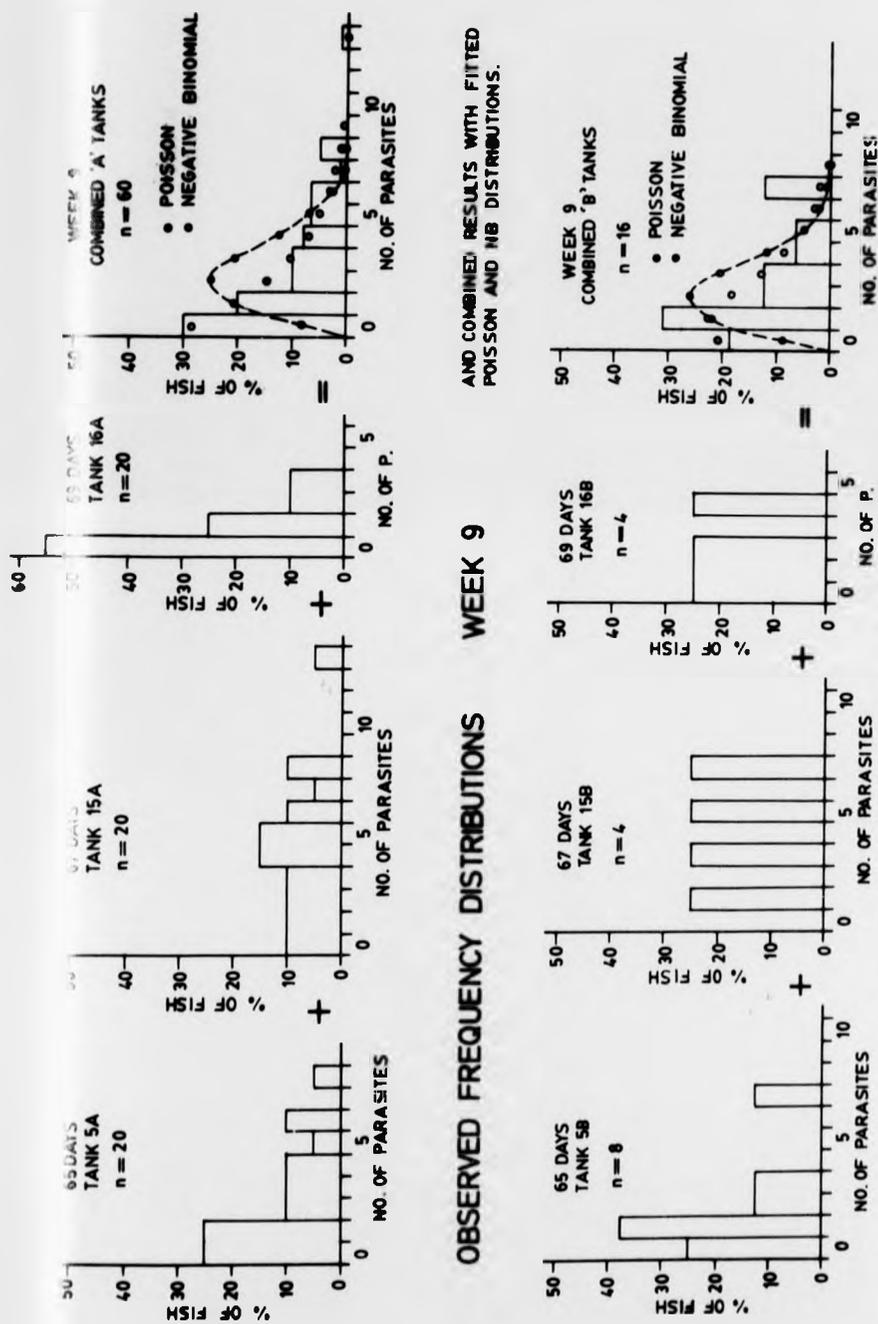
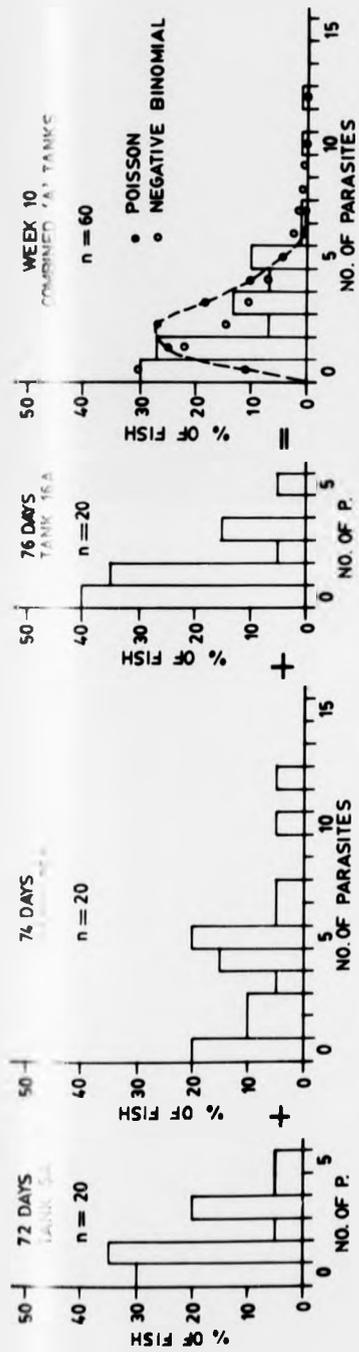


Fig. 26. Observed frequency distributions for week 9 and combined results with fitted Poisson and Negative Binomial Distributions.





OBSERVED FREQUENCY DISTRIBUTIONS

WEEK 10

AND COMBINED RESULTS WITH FITTED POISSON AND NB DISTRIBUTIONS.

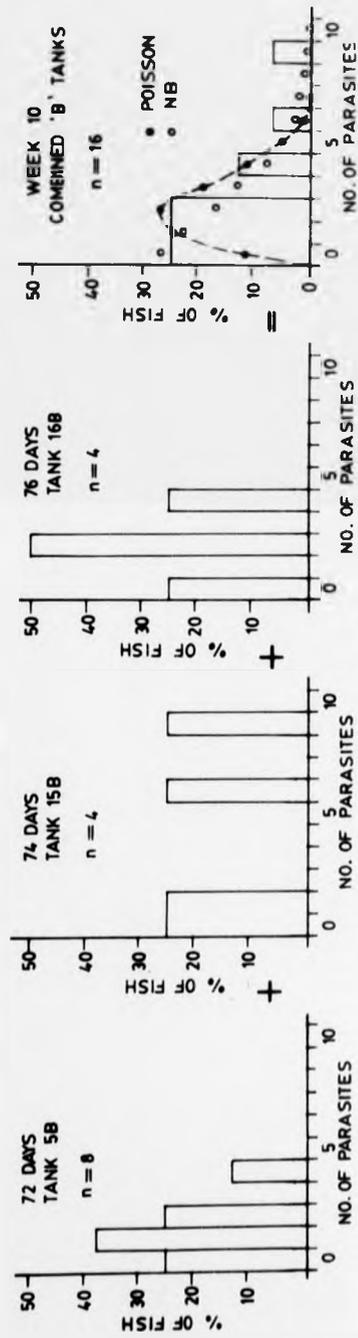
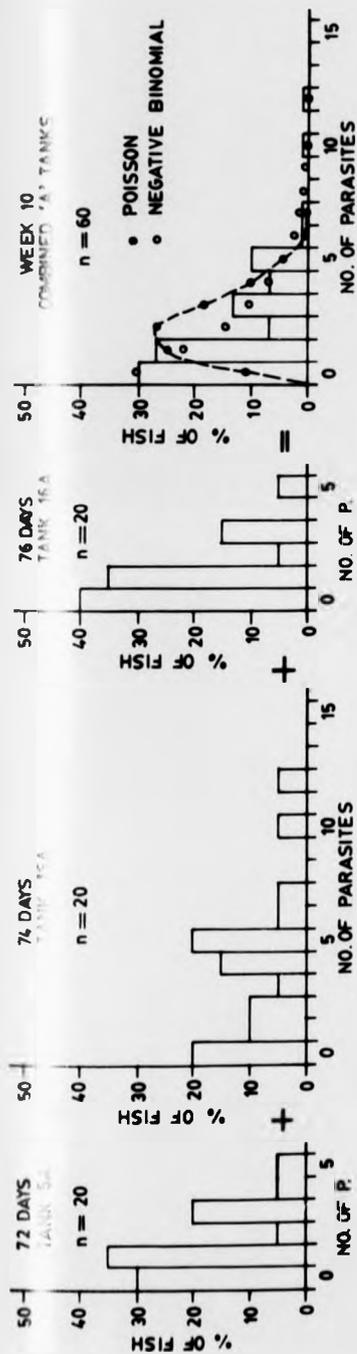


Fig. 27. Observed frequency distributions for week 10 and combined results with fitted Poisson and Negative Binomial Distributions.



OBSERVED FREQUENCY DISTRIBUTIONS

WEEK 10

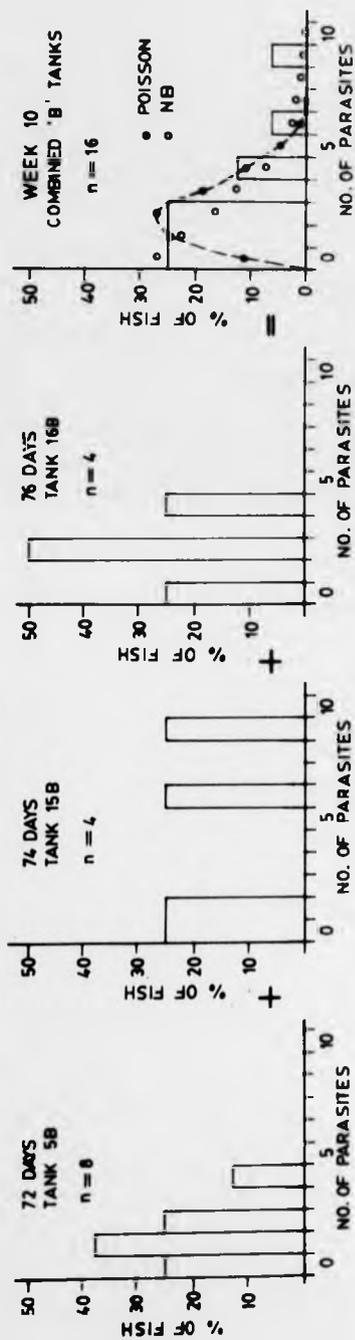
AND COMBINED RESULTS WITH FITTED
POISSON AND NB DISTRIBUTIONS.

Fig. 27. Observed frequency distributions for week 10 and combined results with fitted Poisson and Negative Binomial Distributions.

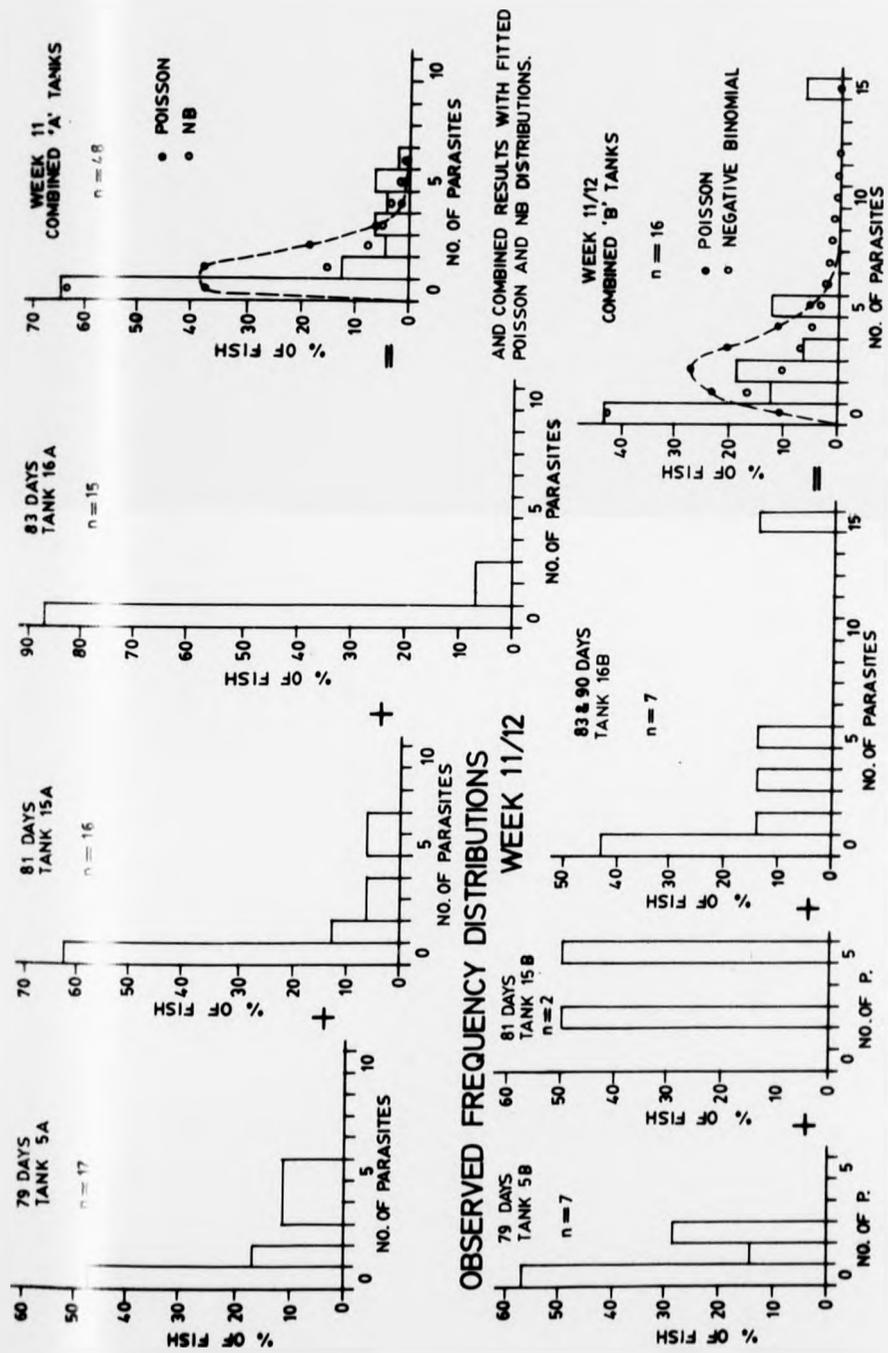


Fig. 28. Observed frequency distributions for week 11/12 and fitted Poisson and Negative Binomial Distributions for combined results.

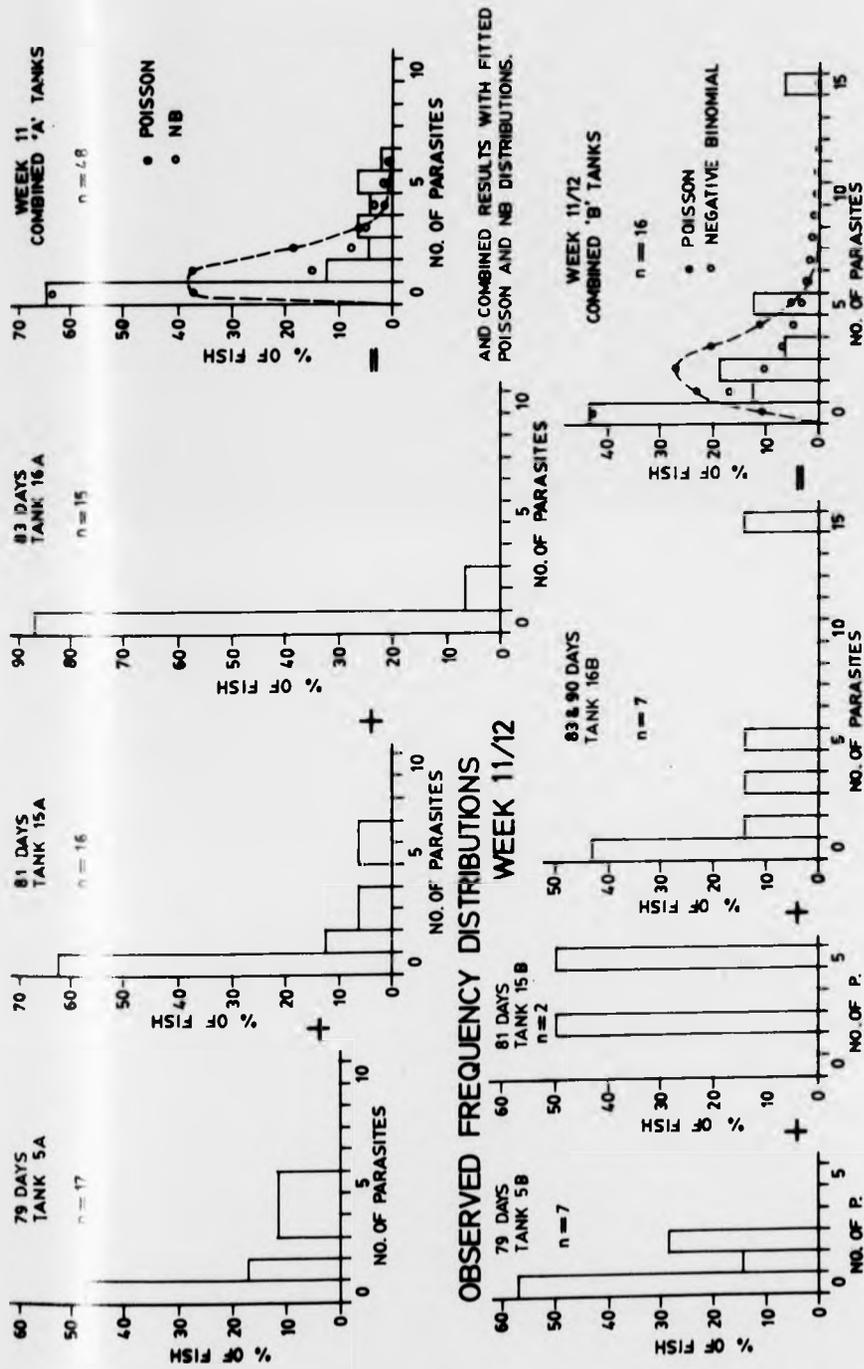


Fig. 28. Observed frequency distributions for week 11/12 and fitted Poisson and Negative Binomial Distributions for combined results.

class was the largest for both densities by about 20% for density A and 13% for density B (Fig. 29 and 30).

It was generally found that the Poisson Distribution did not fit the observed frequency distribution at a significance level of 0.005. The greater the overdispersion of the original distribution, the worse the fit of the Poisson.

The closeness of the Negative Binomial fit can be seen in Figures 22-30. The values of the parameters, p and k , plotted against the percentage infection, mean, variance and overdispersion of the distributions for both density A and density B results are given in Figures 31 and 32 respectively.

It was found that P tended to vary inversely with the percentage infection, particularly for the B results. p tended to vary as the mean for the A results, but appeared to be independent of the mean for the B results. For both density A and density B, p tended to vary as the variance, s^2 , but was correlated most closely with the overdispersion $\frac{s^2}{\bar{x}}$. The line $\frac{s^2}{\bar{x}} = 1 + p$ is shown (Fig. 31) and it can be seen to fit most of the points quite well (as can be expected from the general equations of the Negative Binomial), particularly for the A results.

However, there were certain weeks where the points were noticeably off this line. For density A, one week in particular, week 11, had a value of p which was too high for the value of the overdispersion (Table XXI). This was due to a low percentage infection as many fish were not infected. For density B results, 3 points were off this line noticeably. The value of p for week 5 was again too high for the overdispersion value, due to a high mean. For the other two points the value of the over-

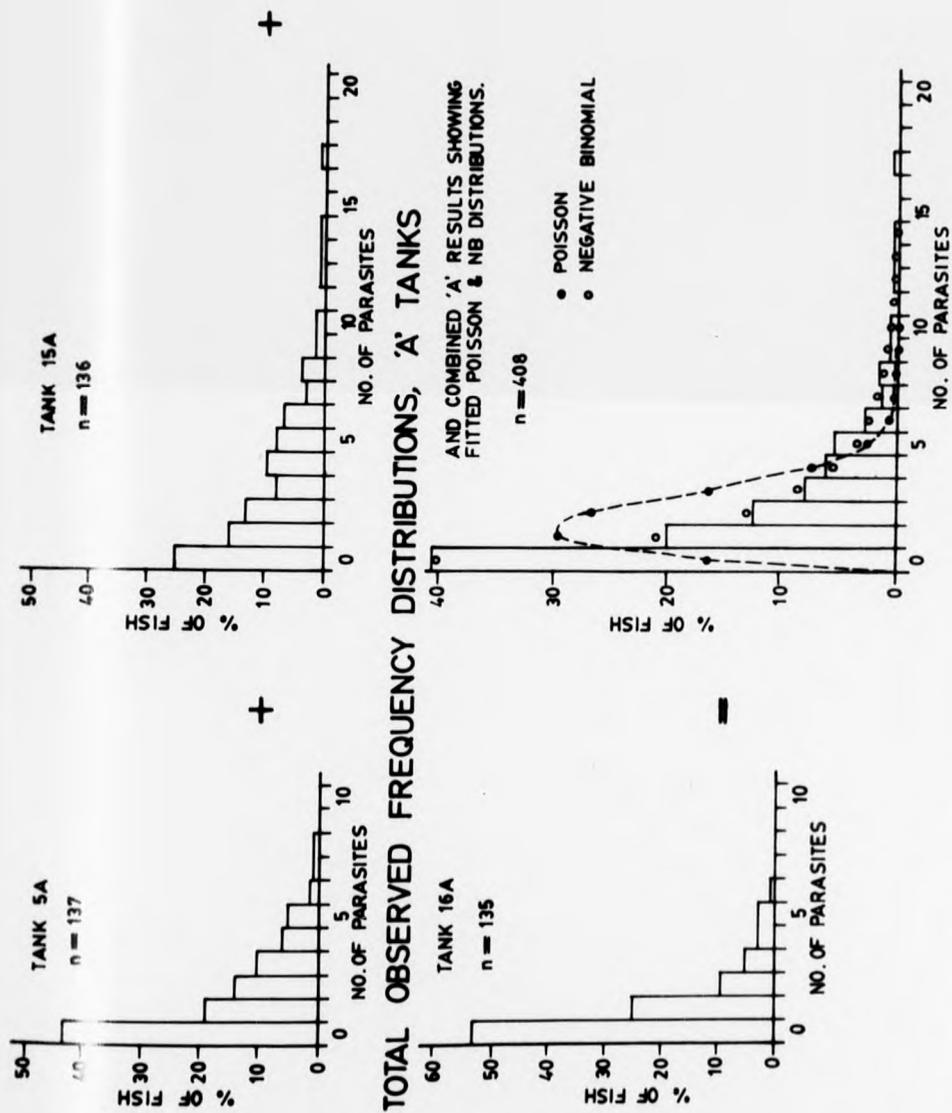


Fig. 29. Total observed frequency distributions and combined 'A' results showing fitted Poisson and Negative Binomial Distributions.

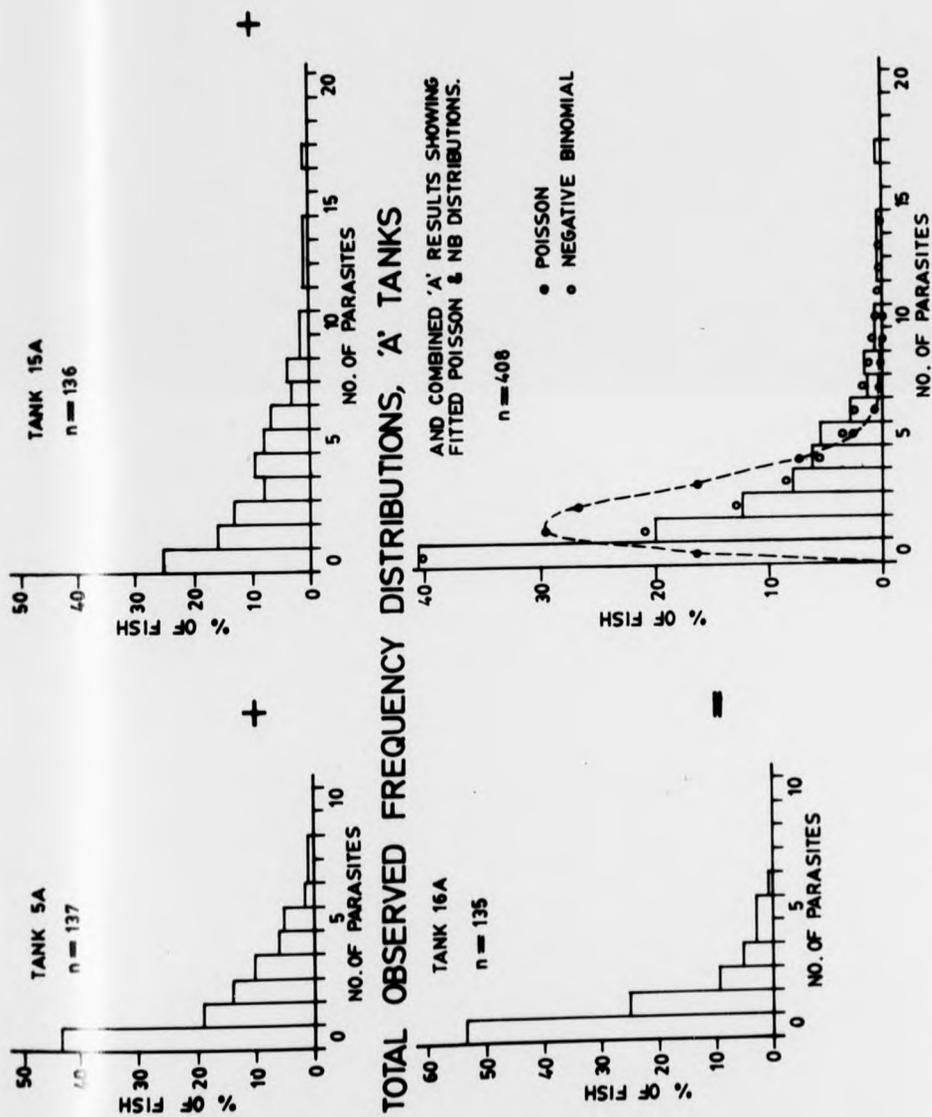


Fig. 29. Total observed frequency distributions and combined 'A' results showing fitted Poisson and Negative Binomial Distributions.

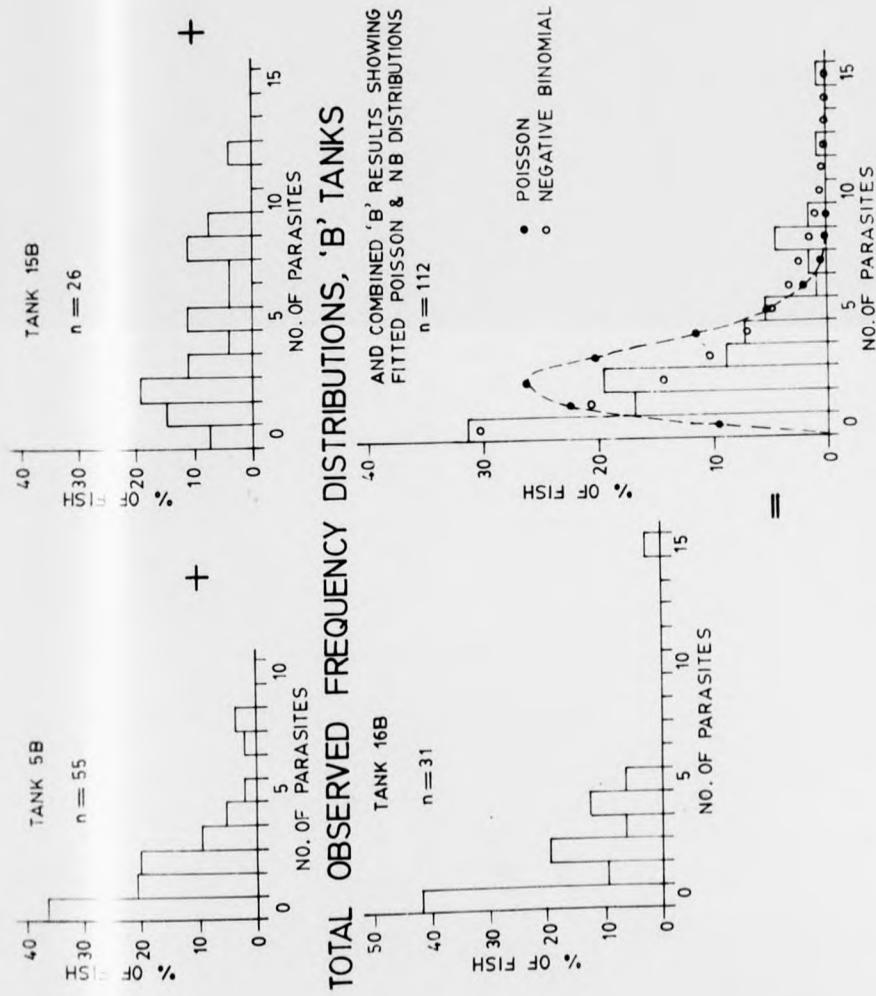


Fig. 30. Total observed frequency distributions and combined 'B' results showing fitted Poisson and Negative Binomial Distributions.

dispersion was too high for the value of p . This was for weeks 8 and 11 and was due to high variances in both cases (Table XXI).

The value of k tended to vary with the percentage infection. It also varied inversely with the overdispersion. The values of p and k tended to vary inversely (See Table XXI).

Figure 33 shows the variance of the observed distribution, plotted against pkq , the Negative Binomial variance. The variance of the Negative Binomial was close to the sample variance in all but the few examples described above. The three which belonged to density B all had high variances. This was due generally to the presence of one or two heavily infected fish and was more evident for density B results, where these fish formed a higher percentage of the total sample size.

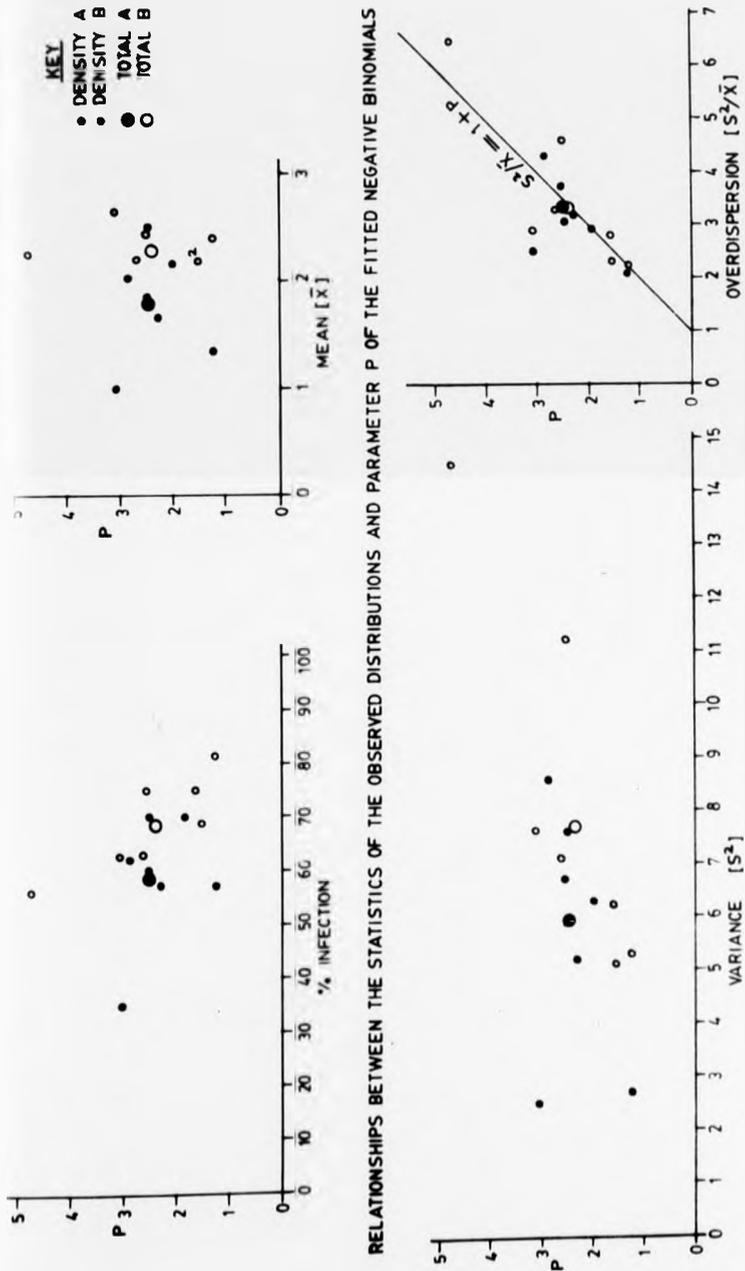


Fig. 31. Relationships between the statistics of the observed distributions and parameter P of the fitted Negative Binomials.

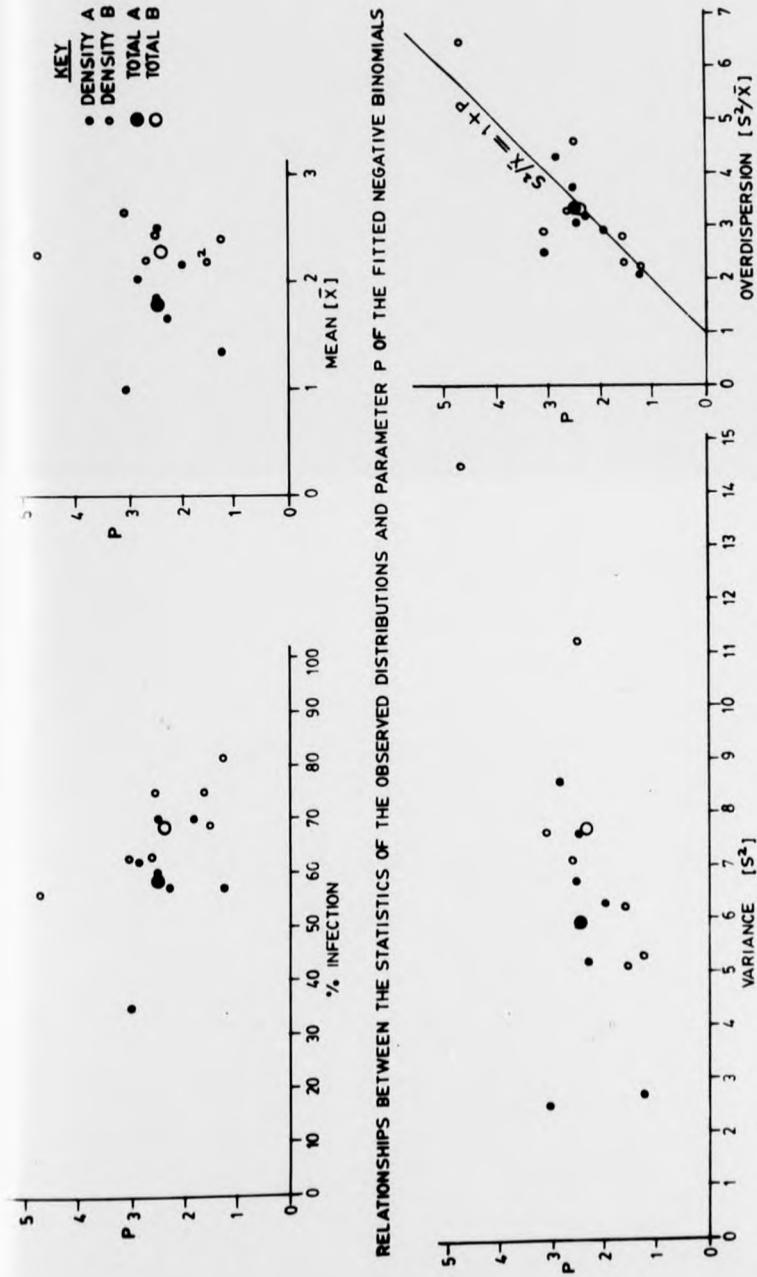
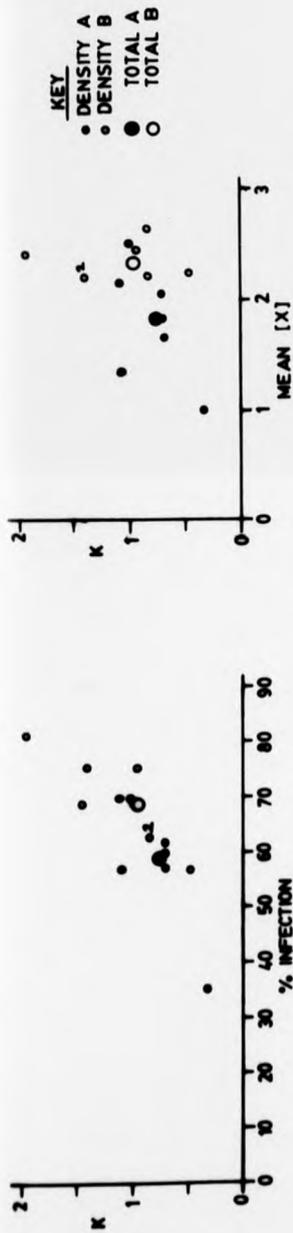


Fig. 31. Relationships between the statistics of the observed distributions and parameter P of the fitted Negative Binomials.



RELATIONSHIPS BETWEEN THE STATISTICS OF THE OBSERVED DISTRIBUTIONS AND PARAMETER K OF THE FITTED NEGATIVE BINOMIALS

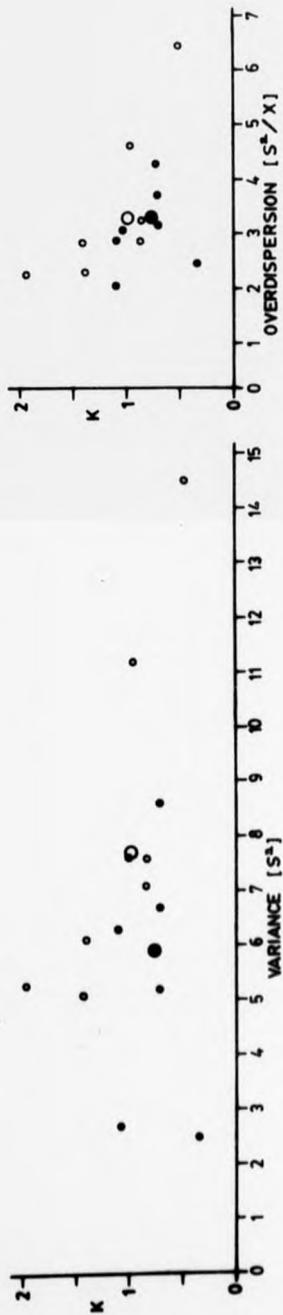
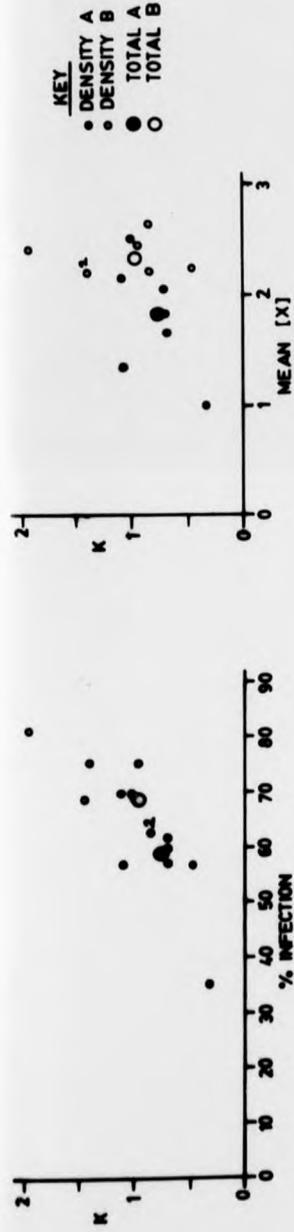


Fig. 32. Relationships between the statistics of the observed distributions and parameter k of the fitted Negative Binomials.



RELATIONSHIPS BETWEEN THE STATISTICS OF THE OBSERVED DISTRIBUTIONS AND PARAMETER K OF THE FITTED NEGATIVE BINOMIALS

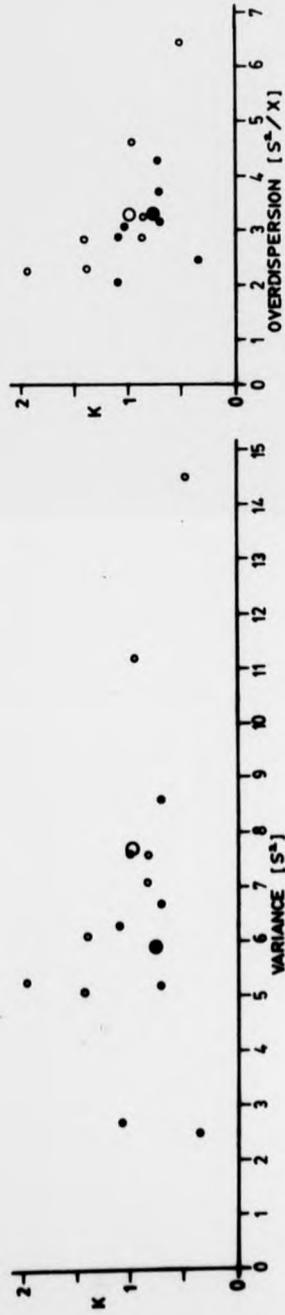
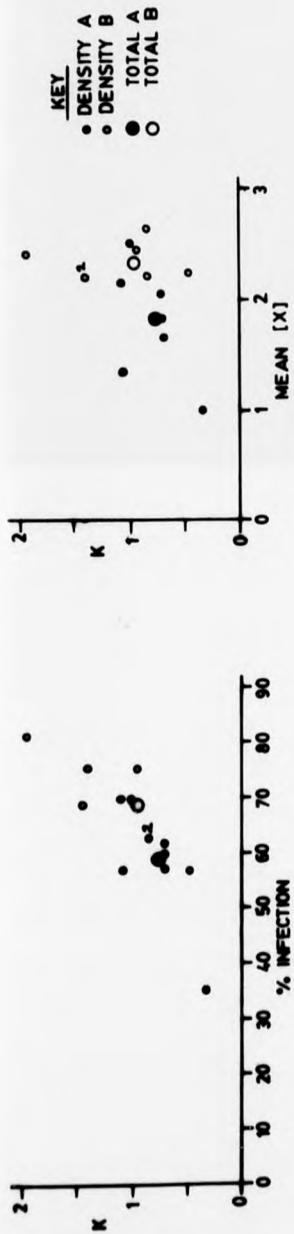


Fig. 32. Relationships between the statistics of the observed distributions and parameter k of the fitted Negative Binomials.



RELATIONSHIPS BETWEEN THE STATISTICS OF THE OBSERVED DISTRIBUTIONS AND PARAMETER K OF THE FITTED NEGATIVE BINOMIALS

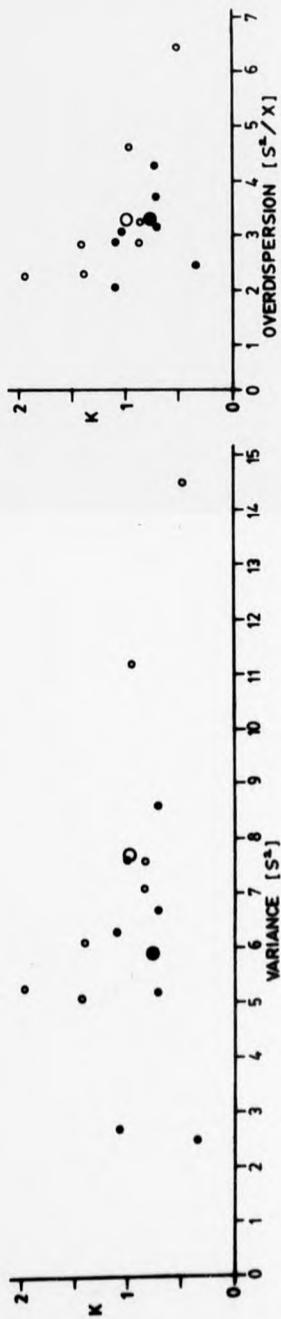
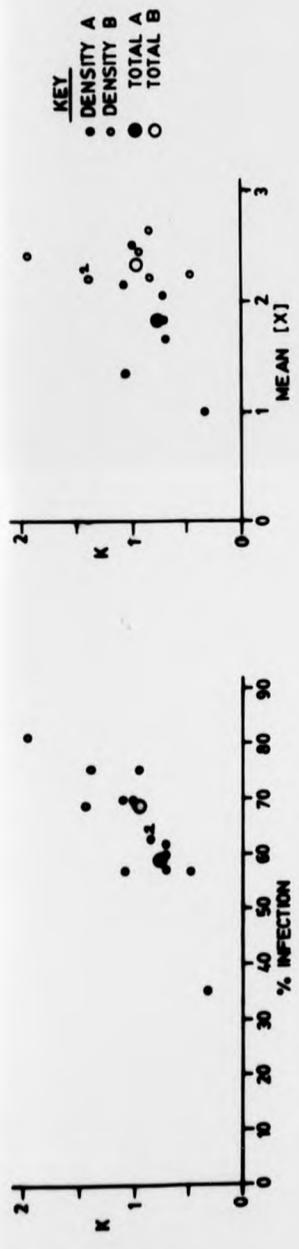


Fig. 32. Relationships between the statistics of the observed distributions and parameter k of the fitted Negative Binomials.



RELATIONSHIPS BETWEEN THE STATISTICS OF THE OBSERVED DISTRIBUTIONS AND PARAMETER K OF THE FITTED NEGATIVE BINOMIALS

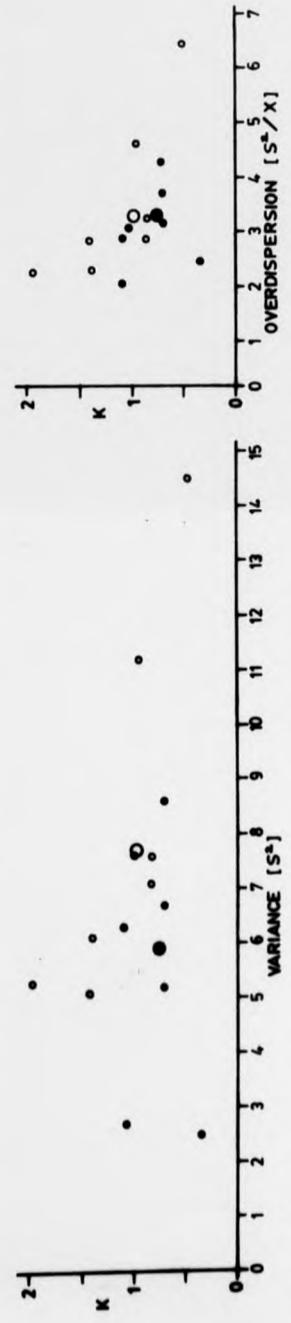


Fig. 32. Relationships between the statistics of the observed distributions and parameter k of the fitted Negative Binomials.

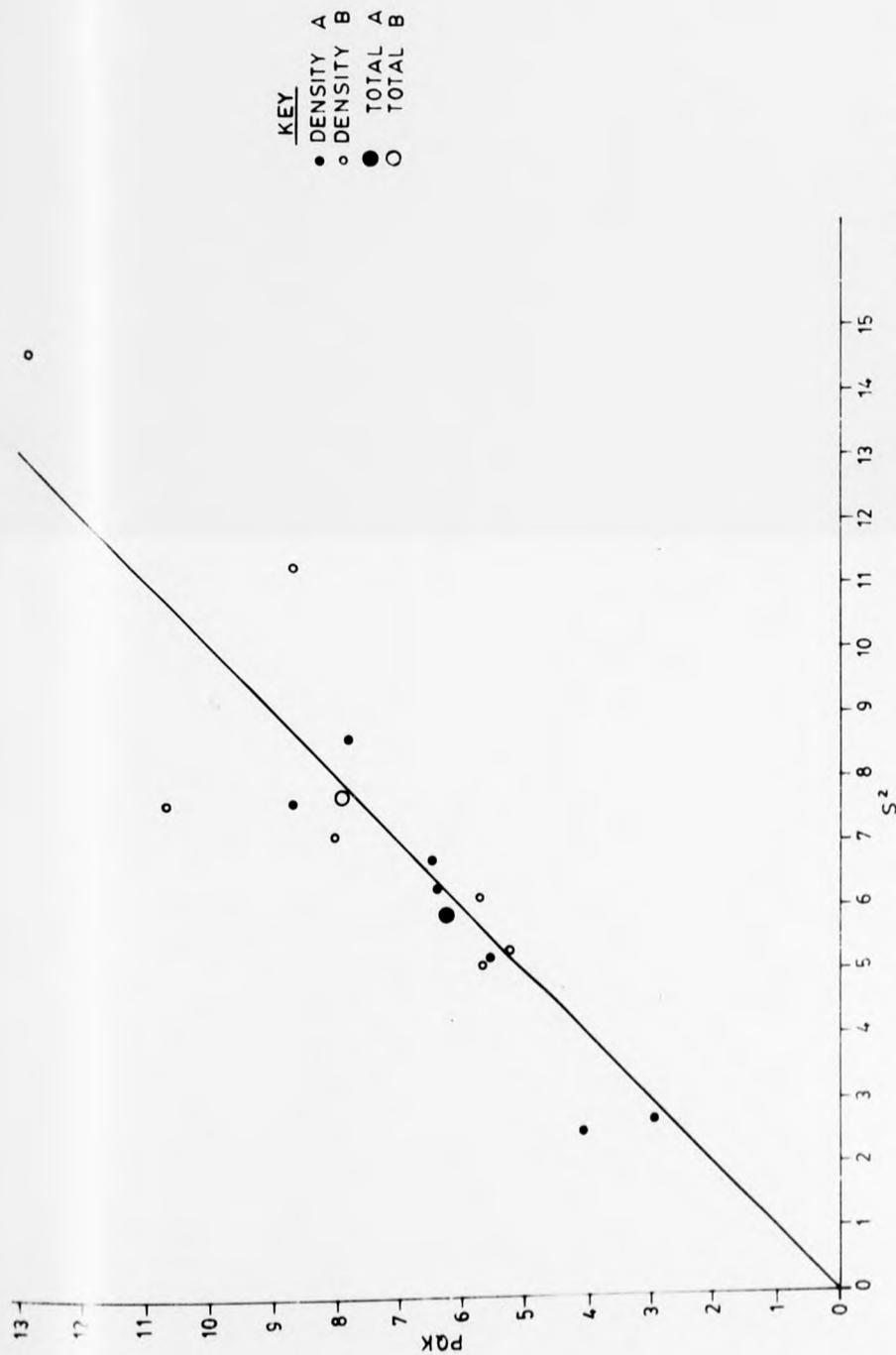


Fig. 33. Relationship between the variance of the Negative Binomial Distribution (pqk) and the variance of the observed distribution (s^2).

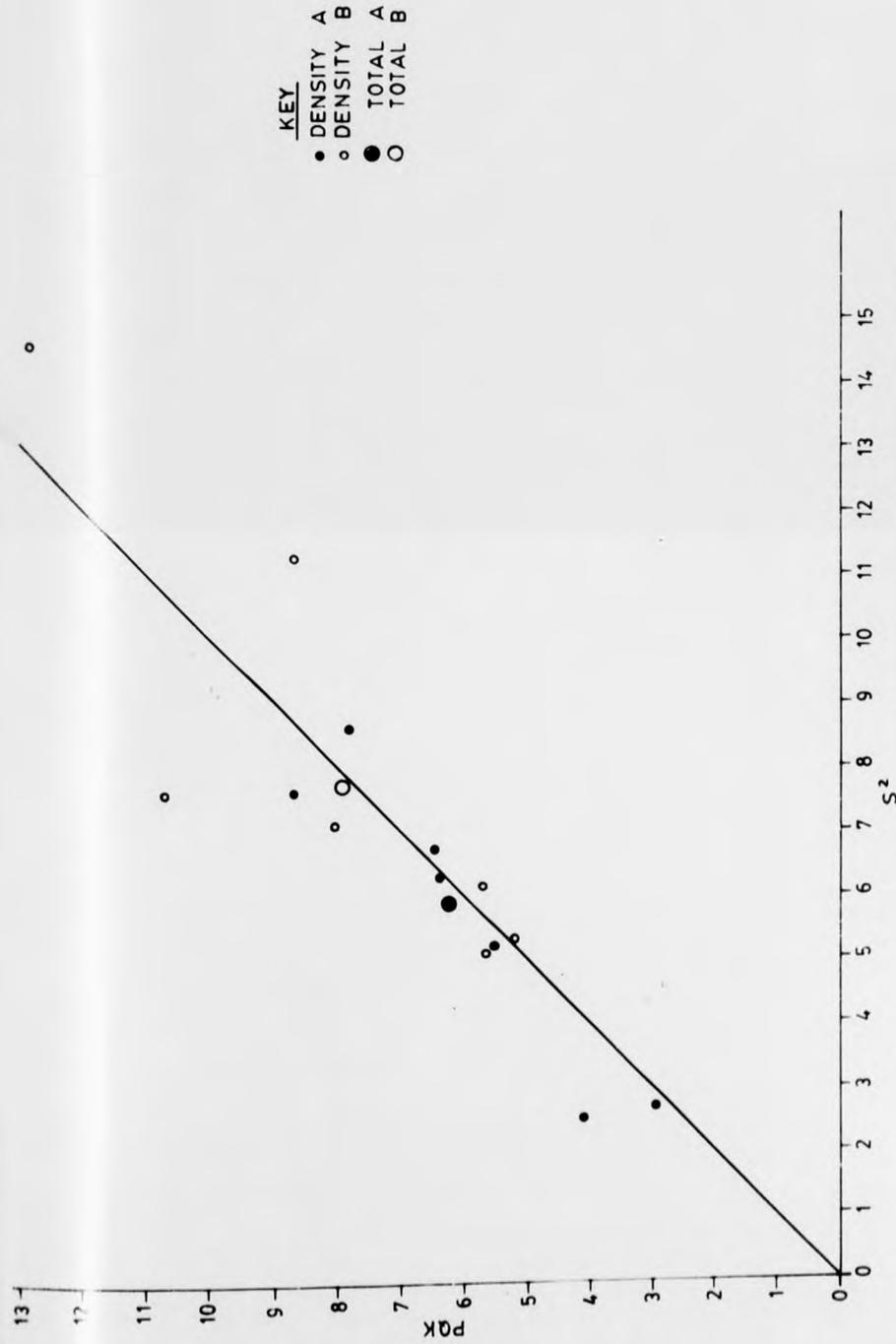


Fig. 33. Relationship between the variance of the Negative Binomial Distribution (pqk) and the variance of the observed distribution (s^2).

5.3.3. Results of the hydrocortisone experiment.

The results of the experiment on the effect of hydrocortisone on the build-up of C. sclerosus on S. aureus are given in Table XXII and Figure 34a. The results showed that there is a significant difference between the control and treated fish ($F = 11.94$ at $P < 0.05$) (refer Appendix 14) and the mean parasite per fish is 3.4 ± 2.88 as compared to 27.2 ± 12.62 parasite per fish.

A second experiment was carried out using S. mossambicus as experimental fish. The results of the experiment are given in Table XXIII and Figure 34b. The results indicated that there is a significant difference between the control fish and the treated fish ($F = 13.67$; $P < 0.05$) (refer Appendix 15) and the mean parasite per fish is 14.8 ± 10.90 as against 159 ± 68.13 parasites per fish.

In both experiments it is evident that there is a significant build-up of the parasites on the treated fish (Tables XXII and XXIII and Figure 34).

The Analysis of Variance for both control and treated fish are shown in Appendices 14 and 15)

TABLE XXII. The level of C. sclerosus infections on S. aureus in the experiment with hydrocortisone.

Control Fish		
Length of fish	Interval in days	Numbers of <u>C. sclerosus</u>
7	3	7
5.5	6	1
5.5	9	1
9	12	6
5	15	2
Mean		3.4 ± 2.88

Treated Fish		
Length of fish	Interval in days	Numbers of <u>C. sclerosus</u>
9	3	9
9	6	35
9	9	35
7.5	12	19
7.6	15	38
Mean		27.2 ± 12.62

TABLE XXIII. The level of C. sclerosus infections on S. mossambicus in the experiment with hydrocortisone.

Control Fish		
Length of fish	Interval in days	Numbers of <u>C. sclerosus</u>
14.8	11	29
15	12	11
14.5	13	3
18	14	16
Mean		14.8 \pm 10.90

Treated Fish		
Length of fish	Interval in days	Numbers of <u>C. sclerosus</u>
15	11	57
15	12	196
16	13	196
17	14	187 (fish died)
Mean		159 \pm 68.13

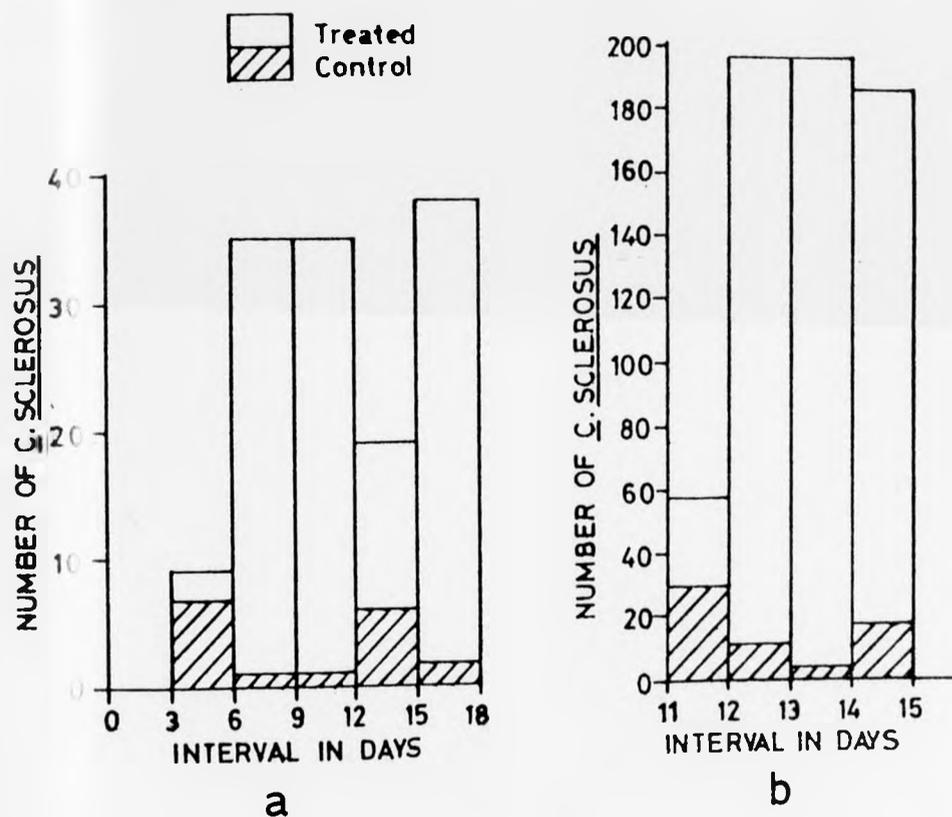


Fig. 34 a. The level of *C. sclerosus* infection for hydrocortisone - treated and control, *S. aureus*.

Fig. 34 b. The level of *C. sclerosus* infection for hydrocortisone - treated and control, *S. mossaebicus*.

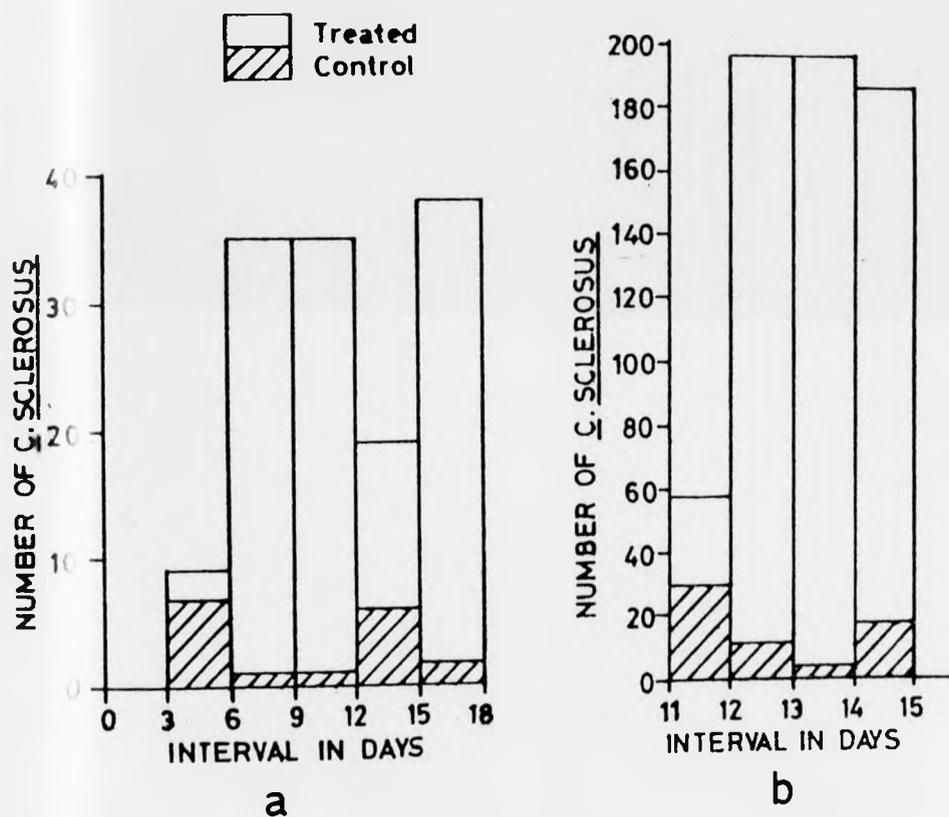


Fig. 34 a. The level of *C. sclerosus* infection for hydrocortisone - treated and control, *S. aureus*.

Fig. 34 b. The level of *C. sclerosus* infection for hydrocortisone - treated and control, *S. mossambicus*.

5.4. DISCUSSION

The overdispersed distributions of C. sclerosus on tilapia from the density experiments (Table XXI, Fig. 22-30) could possibly be the result of a non-random distribution of the infective larval stages, which in this case is the oncomiracidia. Jarrol (1979) also made similar observations in his study on the distribution and abundance of the cestode, Bothriocephalus rarus, infecting red-spotted newts, Notophthalmus viridescens. It could also be due to variability in the behaviour of the host, the distribution sample being clumped (Pennycuik, 1971).

The results of the density experiments also showed that the parasite numbers appeared to build up after two weeks and decline after two weeks (Tables XVII, XIX, XX and figure numbers 17-20). Hence a period of approximately 30 days elapsed between periods of build-up or periods of decline of C. sclerosus. This could be related to the life span of C. sclerosus. It has been seen that eggs of C. sclerosus hatch out after 4 days. These possibly develop and mature within 6 days, as is typical of dactylogyrids (Bychowsky, 1957; Prost, 1963). By the 15th day the parasite numbers will build up. As the numbers build up the defense mechanism of the host starts to operate and hence this leads to a decline in numbers in the next 15 days. Immune responses in dactylogyrids have been known to operate over a minimum period of 30 days (Vladimirov, 1971). During routine examination of stock fish, there were certain periods when there were many moribund parasites on the gills of the fish. The presence of dying juveniles did not favour the suggestion that death could be due to senility of fish parasites. Therefore, there would appear to be

some host-induced toxic or antibody component, possibly in the mucus.

Other workers have also reported on the ability of monogeneans to inhibit or eliminate their parasites. Wunder (1929) and Paperna (1964c) reported that proliferation of gills contributed to the decrease of parasite numbers due to the unfavourableness of the gills. Nigrelli and Breder (1934) found that Epibdella survived longer in mucus from susceptible fish, than in mucus from "immune fish". O'Rourke (1961) found antigens in the skin mucus of fish. Immunoglobulins were found to be present in the serum and mucus of the plaice, Pleuronectes platessa (Fletcher and Grant, 1969). Fletcher and White (1973a, 1973b) also indicated lysozyme activity and antibody production in the plaice, P. platessa. Harris (1973) and Hines and Spira (1974) also showed that an epidermal immune response is evident in fish. Lester (1972) has reported that, in sticklebacks, sloughing of the mucus takes place every two weeks and the parasites are shed together with the mucus. Fish immune responses are still in an evolutionary state of development (Kennedy, 1977) and this is reflected in the number of claims that immune responses play no part in the regulation of all stages of parasite populations (infrapopulations) in fish (Rees, 1967, Kennedy, 1977). Nevertheless, host-defense mechanisms and host responses play a significant part in regulation of parasite numbers and it would be of future interest to elucidate the true nature of the host defense mechanism of tilapia towards C. sclerosus.

It was observed in the host-specificity experiment and the density experiments that one or two individuals in a population had died. On examination of the fish, which had not been dead long, they had a higher number of parasites in relation to their

size. Could this be one way they regulated their parasite population? Crofton (1971) postulated that there was a level of parasitization which could kill the host, and this was termed the lethal effect. These parasites had an aggregated distribution on the host and their numbers as well as the host population densities were regulated by death of a few heavily infected hosts.

Parasite transmission from one host to another is through a free-living infective stage, which in the case of C. sclerosus is the oncomiracidium. Crofton (1971) and Anderson (1978) stated that the rate of encounter between an infective stage and its hosts depends on the density of the organism and how it is spatially distributed. This also agrees with the findings of Keymer and Anderson (1979). Keymer (1982) showed that, under specified experimental conditions, increased host density of Tribolium confusum resulted in an increase to a plateau in the total number of infections of Hymenolepis diminuta eggs and there was a simultaneous exponential decline in the mean number of infections per host. But here, infection is achieved by the ingestion of infective stages by the host. This pattern will be different where the infective agent attaches, as in C. sclerosus oncomiracidia, or penetrates through the body wall of the host, as with miracidia of most digenetic trematodes namely the work of Anderson (1978).

C. sclerosus oncomiracidium has a very short life span and will die readily if it does not attach to a host. In other monogeneans the period of free swimming is longer, about 20-30 hours at 7°C and 9-14 hours at 17°C in Entobdella; up to 10 hours in Diplozoon and 4-6 hours in Discocotyle (Llewellyn,

1972). The chance of attachment will therefore depend on the spatial distribution of the oncomiracidia and also on the density of fish hosts (Crofton, 1971; Anderson, 1978; Keymer and Anderson, 1979). When the fish were widely dispersed there was less chance for the free-swimming juvenile stages to find the host readily. Chubb (1979) commented that the invasion of the fish Brachydario reriae was proportional to density of cercariae present in the habitat and was a matter of chance contact between cercariae and fish.

The behaviour of the tilapiae could also influence the recruitment of the oncomiracidia. According to Schwanck (1977) there was a relationship between territorial behaviour, food abundance and population density in juvenile Tilapia mariae. He found that, at high densities, dominant fish tend to establish territories and be more spread out. But if fish were in lesser numbers they tend to aggregate around each other more frequently, hence ensuring easier access of contact by the oncomiracidia.

Of interest is an example from the insect world. Manfred (1982) found that the mean daily oviposition rate is higher in the high density aphid host but the overall proportion of aphids parasitised is lower. On the other hand, Pandey *et al.* (1982) found that at low density (≤ 10) Trioxys indicus, an aphidiid parasitoid of Aphis craccivora, could not locate the host in 50% of the cases. At high host densities (≥ 20) the parasitoid approaches the host quicker and stays on the leaf of the host plant longer than at low host densities.

It has also been stated (Kennedy, 1977) that a fish parasite population will grow exponentially until constraints act

upon it. These constraints could operate over a whole range of densities (density independent) or the constraints become more severe as parasite population increased (density dependent). Density-dependent regulation of fish parasite populations are rare and the first evidence of this was reported by Mills (1979) who showed that the survival and reproduction of Transversotrema patialense decreased at high population density. Another example of density-dependent regulation was that of Deretrema on Anomalops as reported by Burn (1980).

The regulation of parasite numbers is also by inhibition of parasite reproduction or through density-dependent immune responses (Bradley, 1974). This type III regulation of Bradley's could be applicable to C. sclerosus. Paperna (1960a) found that, in natural conditions, Cichlidoqyrus sp. did not reach high levels of infection and there is a self-cure process which periodically clears the fish of their parasites. The author has also shown that, when the immune depressor hydrocortisone was injected into the tilapia, there was a huge build-up of C. sclerosus. This indicates the possibility of a host defense mechanism in tilapia towards C. sclerosus, which could be in the form of an immune response.

Li and Hsu (1951) have stressed the importance of the knowledge of frequency distributions of parasites in their hosts, while Williams (1964) discussed the use of frequency distributions to analyze parasitological and biological data.

Frequency distributions have been calculated from the data from the density experiment. The frequency distributions were overdispersed, the variance being greater than the mean. In

an overdispersed distribution a larger number of parasites is accommodated in a smaller number of hosts. The results are similar to other authors (Cassie, 1962; James and Llewellyn, 1967; Srivastava and James, 1967; James and Srivastava, 1967; Pennycuik, 1971; Hirsch, 1980).

Injection with hydrocortisone led to increased levels of infection of C. sclerosus (Table XXII and XXIII). There was also a huge build up of Gyrodactylus infections. Under normal circumstances Gyrodactylus is practically nil on tilapia, except when fish are highly stressed. Hence their massive build-up after injection of host with hydrocortisone suggest the presence of a host defense mechanism which is suppressed by the cortisone.

There is also some evidence that ACTH may influence factors which control the rate at which corticosteroids and their metabolites are catabolized (Dougherty, 1959). According to Batchelor (1971) the administration of ACTH or adrenal steroids inhibits the formation of antibody. These adrenocorticoids produce destructive effects upon lymphocytes (Corbell, 1975). Cortisole is the most potent steroid which can result in loss of cytoplasm and karyorhexis of lymphocytes. The effect of cortisone is more indirect as it has to be converted to cortisole.

The blood characteristics of Tilapia zilli has been elucidated by Ezzart (1974). He showed that lymphocytes were present in greater numbers in blood cells of tilapia compared to other species of fish. The large lymphocyte counts fluctuated at monthly intervals. It was also observed by the author that there were fluctuations of 30 days in mean numbers of parasites per fish. From this it can be deduced that the host defense

mechanism of tilapia could be in the form of antibodies but more work will be needed to elucidate this.

CHAPTER SIX

GENERAL CONCLUSIONS

The statement "Classification of Monogenea is in a state of flux" Hargis (1959) is still a valid comment about modern monogenean taxonomy, especially with regard to Ancyrocephalid infections.

Mueller (1934) considered the genus, Ancyrocephalus, as a catch-all "genus" and divided it into 3 subgenera: (Ancyrocephalus, Cleidodiscus and Urocleidus) on the basis of the presence and location of the vaginal duct. However, these features are not always distinct and the duct may open on the right or the left, depending on the individual specimens. Based on the above mentioned features, different authors assigned the genus from one to the other, thus resulting in further confusion.

Because of these confusions there is a considerable amount of synonymy involved in the genera of this subfamily; for example, Ancyrocephalus aculeatus is synonymous with Cleidodiscus aculeatus (Van Cleave and Mueller, 1932) cited in Bykhovskaya - Pavlovskaya et al. (1962). This is principally because of the close similarity of the opisthaptoral armature of the different genera in the family. There is also no standard or near standard terminology for anatomical structure, nor consistency in the methods of preparation of fixed specimens.

Hoff (1943) recommended that the taxonomic description of a species must be detailed and specific so that it can withstand the passage of time. Taking this recommendation into consideration, Price and McMahon (1967) proposed a standardised system of anatomical terminology. In the present study, the author made every

effort to follow this system in describing Cichlidoqyrus from the gills of tilapia. It has been found that there are considerable differences in measurements of the Cichlidoqyrus species from the archetypes dealt with by Paperna and Thurston (1969). The main differences are in the measurements of the reproductive structures and the marginal hooks (Fig. 3, 4 and 6). The different range of values for hooks of opisthaptor, by different authors, could be due to different rates of growth of hooks on the hard parts of the opisthaptor at different temperatures. Ergens (1975) found that the hard parts of the opisthaptor of Gyrodactylus aphae and G. pannonicus decreased in accordance with an increase in water temperature and vice-versa.

The vaginal prop was not visible in the specimens studied by Paperna and Thurston (1969) though Kritsky and Thatcher (1974b) described it as a highly sclerotised tube which flared at one end. Paperna and Thurston (1969) further emphasized that the different methods of fixation could result in distortion of the chitinous organs and this could lead to confusion in the identification.

The Cichlidoqyrus sclerosus obtained from the gills of different species and hybrids of tilapia do not exhibit any distinct morphological differences. It would appear to be a consistent and uniform simple species.

The geographical distribution of C. sclerosus has extended to all parts of the world due to the introduction of its hosts, tilapias, to these regions. Though it originated from Africa, it is now found in most tropical countries, ranging from Colombia in South America to the Philippines and Malaysia in Asia. Examination

of specimens of C. sclerosus, obtained from the tilapia hybrid S. mossambicus and S. hornorum in Malaysia by the author recently, showed close identity in structure and morphology to the specimens obtained from fish examined from Israel, kept in the aquarium tanks at the University of Stirling, which were the subject of this study.

The results of the present study help to supplement the pioneering work of Paperna and Thurston (1969) on the biology of monogenetic trematodes of tilapia. It is now established that the eggs are 62 μ long and 50 μ wide and take 4-5 days to hatch at 25°C under laboratory conditions (Table VI and Table VII). The study also indicated that host mucus and urea induce egg development and hatching. The fact that eggs will develop in higher pH (Table VIII) indicates their affinity for an alkaline environment. This is in agreement with the pH (see Fryer and Iles, 1972) occurring in lakes in Africa, which is the natural habitat for the host tilapia. The author has also observed (Mohd-Shaharom, personal observations 1983) that tilapia from one pond at the Universiti Pertanian Malaysia, Serdang, Malaysia, which had a pH of 5.6, were not infected with C. sclerosus, whereas the tilapia from those ponds with a pH of greater than 7 were infected with C. sclerosus.

The behaviour of the oncomiracidium on hatching is similar to that of other free-swimming oncomiracidia which were reported earlier (Prost, 1963; Bovet, 1967; Llewellyn, 1972 and Kearm, 1981). Host mucus speeds up embryonic development and urea induces hatching though the threshold level is not quite as evident as in Acanthocotyle lobianchi (Kearm and Macdonald, 1976).

There is still considerable scope for further work of this interesting parasite. Of interest, for example, would be a

study to determine whether the secretion of the head glands of the oncomiracidia are used for weakening the operculum before hatching. It would also be interesting to determine the pH of the mucus of tilapia, possibly adapting the techniques used by Kearn and Macdonald (1976) for the marine monogeneans.

The oncomiracidium of C. sclerosus has a short life span, ranging from forty-five minutes to two hours. Prost (1963) reported that longevity of oncomiracidium of D. extensus was one-half to twenty-five hours. The short life span of the oncomiracidium of C. sclerosus may be an adaptation to the social behaviour of the host. The tilapias are not fast swimmers, they are territorial and tend to remain in one place for lengths of time. They are often found in large numbers in close contiguity. Because of this behaviour of the host, the parasites require only a short time to infect the host.

The host-specificity experiment of the study showed that C. sclerosus is not species specific (Table X). It infects both S. mossambicus and S. spilurris. Paperna and Thurston (1969) also stated that Cichlidogyrus is not species specific. This conclusion is further supported by periodic examination of other species of Sarotherodon during the study. Sarotherodon zilli and S. nilotica were also found to be infected with C. sclerosus. Thus it has a wide host range within the tilapias. This may be explained on the basis that the tilapias are closely interrelated or some of them are hybrids. Bychowsky (1933) and Hargis (1953c) pointed out that hybridization of fishes could result in non-specificity of its parasites.

The present study showed that the parasites infected

the first gill arch more heavily than the other gill arches (Fig. 13). On closer morphological examination it was found that a "fence" of spines is present on 2nd, 3rd and 4th gill arches. This "fence" of spines may act as a barrier against their infection to the other gill arches. This is considered a physical barrier to the parasites. This barrier seems to be effective as it was found that the distribution of C. sclerosus is non-random. However, earlier authors (Llewellyn, 1956; Llewellyn and Owen, 1960; Owen, 1963; Slinn, 1963; Suydam, 1971; Wooten, 1974) attributed the site-specificity of monogenetic trematodes on the gills to the direction and force of respiratory current over the gills. According to Wooten (1974) most parasites are also found on the anterior medial segment of the gill arches (or site E in the present study) which is equivalent to the distal median segment of the hemibranch of the gills. He believed that the strong current over the middle two gill arches of the ventral segment of the gills induce the parasites to settle on the sheltered sites. The present author, on several occasions, observed the juveniles of C. sclerosus making looping movements from the proximal to the distal half of the filaments. They were probably actively seeking for sites most suited to them. On the other hand, C. t. minutus was found on all gill arches and on all other sites, with little difference in distribution. In comparison, C. t. minutus are small parasites, hence occupying a smaller surface area on the gill filaments.

The present study showed that tilapia fry less than 1 cm. long are rarely infected (Fig. 14). This could probably be due to the undifferentiation of the gills (Prost, 1963). Longer

and older fish acquire more parasites till they reach a length of 17 cm. where there is a reduction in infection. This is in general agreement with other authors (Dogiel, 1961; Radha, 1971; Molnar, 1971b). The relationship between the number of parasites with size and age of the fish may be explained on the basis of the changes in the behaviour of the host (Paling, 1965) or to age resistance of the fish (Radha, 1971). Tilapia fingerlings are usually found on the sides of ponds. As they grow older they tend to move towards the centre of the pond where they become infected as the oncomiracidia are usually in the surface waters. Much older tilapia are bottom dwellers and they are less liable to get infected.

Analysis of the results of the density experiment (Chapter 5) indicate that the distributions of the parasite, from both the high density and low density tanks, are overdispersed and they fit a Negative Binomial (Table XXI) which indicates that the distribution is non-random. The overdispersion could be due to contagion or heterogeneity (Cassie, 1962). Anderson (1979), in his study of the regulation of host population growth by parasite species, also showed highly overdispersed patterns.

In the present study it was shown that the mean intensity of infection was higher in low density tanks than high density tanks. This has been explained as due to the behaviour of the tilapia (Schwanck, 1977). In high density tanks tilapia tend to be more aggressive and territorial, whereas in the low density they are closer together. Therefore, in the low density tanks, infection can take place more readily.

It would, therefore, be interesting to see if this holds true in the wild. Perhaps population studies could be carried out on two sets of ponds, one having greater numbers of tilapia than another. This study could be carried out over a longer period. Of interest is the snail model of Anderson (1978) in which he showed that increased host density results in the total number of miracidial infection reaching an asymptote and the mean number of infections/snail declines. This is because increased host density is equivalent to extending the period of exposure to infection.

So far, the only study of incidence of Cichlidogyrus on the gills of tilapia in the wild is by Thurston (1970). Her studies were based on examination of fish from several scattered sites and was not monitored over monthly or diurnal periods. She found that, from Lake Victoria, 71% of tilapia were infested with a mean number of 6.1; Lake George (6% infestation) with a mean number of 4.0 and Lake Albert (86% infestation) with a mean number of parasite of 4.8.

In the present study, under experimental conditions, the author has found that, in a regression analysis of lengths of 914 fish and the number of their parasites (Fig. 14), it was found that the mean number of parasites obtained was 4.0. The mean length of the fish was 6.1 cm. This agrees with the work of Thurston (1970) who found that, in S. mossambicus of lengths 6-7 cm. from Kajansi Experimental Station, the maximum number of parasites obtained was 4. She also found that, in S. mossambicus of lengths 3-5 cm., there was no infection of Cichlidogyrus. In this study, it was found that fish as small as 1.5 cm. became infected. This could probably be

due to the fact that, under experimental conditions, infection can take place more readily, as the fish are confined to a smaller area.

It was also seen from this study that there were periods of build-up or periods of decline of C. sclerosus at intervals of two weeks (Fig. 17-20). This has been explained as related to the life span of C. sclerosus. Of interest is the study of Ezzart (1974) on the blood characteristics of Tilapia zilli. He showed that the tilapia had a higher number of lymphocytes compared to other fish and that these lymphocytes fluctuated at mostly monthly intervals. As lymphocytes are the executive cells of the immune system (Ellis, 1978) therefore there is no doubt that there is a strong host defense in tilapia, which could be in the form of antibodies.

It was also shown that injection of hydrocortisone into tilapia led to a massive build-up of C. sclerosus (Fig. 34) According to some authors, administration of adrenal steroids inhibits formation of antibody (Batchelor, 1971) or destroy lymphocytes (Corbell, 1975). The destruction of lymphocytes in the circulatory system after injection by hydrocortisone obviously leads to the lowering of the host defense mechanism, which ultimately leads to the proliferation of C. sclerosus on the gills of tilapia.

This host defense mechanism in tilapia could be an exciting area of research for the future. To date, even the first stage, the search for antibodies in tilapia, has not been elucidated (Elis, personal communication).

Nevertheless, the immune response in fish is a relatively new field, and many authors still claim that immune response plays no part in the regulation of parasite infestation in fish (Rees, 1967; Kennedy, 1975; 1977). However, Vladimirov (1971) associated loss of D. vastator with levels of serum antibody. Vladimirov stated that the artificial immunization (vaccination of fish) against parasitic disease is possible and the level of its preventative effectiveness may be sufficiently high.

All throughout the study of C. sclerosus (Chapter 4 and Chapter 5), it was observed that the infection level of C. sclerosus was very low; occasionally greater numbers occurred but these gradually disappeared to minimal levels again. Hence there seems to be an equilibrium between parasite and host.

This thesis has attempted to look at taxonomic studies of some warm-water Ancyrocephalid Monogenea and to give a detailed study of C. sclerosus, the dominant parasite on the gills of tilapia. The literature on Monogenea of fish is scattered and, in bringing some of it together, the author hopes this thesis will be of use and serves as the basis for further, and intensive research on monogeneans from warm-water cultured fish.

APPENDIX 1AFlotation Method

This technique makes use of the fact that ova will rise to the surface in a liquid of higher specific gravity than that of the egg.

The method of choice is the zinc-sulphate flotation method, which will result in a better separation from faecal material, but is not suitable for the operculated trematode and Diphyllbothrium ova, which will burst in high gravity fluids.

Zinc-sulphate flotation

Make a 35% solution of ZnSO₄ in distilled water and then adjust to a specific gravity of 1.180, using distilled water as diluent. A car battery hydrometer is quite suitable for this and adjustment should be carried out at 20°C (room temperature). The solution should be stored in a rubber-stoppered bottle and the gravity checked periodically.

Procedure

- (1) Prepare a suspension of faeces in tap water, approximately 1 gm. in 10 ml.
- (2) Strain through a 2" diameter No. 60 sieve into a 50 ml. beaker. Add 2 ml. of ether, close the bottle and shake. Fill to the bottom of the neck with water.
- (3) Centrifuge at 2500 rpm (approx.) for 45 seconds. Loosen the plug of debris at the top and decant the supernatant liquid.
- (4) Add 3 ml. of water to the deposit and shake to suspend. Fill to the base of the neck with tap water and centrifuge for 45 seconds at 2500 rpm.

APPENDIX 1A

- (5) Decant the supernatant liquid and add 3 ml. of the $ZnSO_4$ solution. Transfer to a centrifuge tube. Add zinc-sulphate until the surface forms a "convex" meniscus at the mouth of the tube. Allow to stand for ten minutes, then gently place a coverslip onto the surface. Place on a slide with a drop of Lugols iodine solution.

APPENDIX 1B

Concentration by Formol-ether method (Ritchie)

In this method, large particles are removed by sieving and the fatty debris is separated by flotation into an ether layer. The method is reliable for operculate, as well as non-operculate, eggs.

Procedure

- (1) Emulsify a piece of faeces the size of a walnut in 10 ml. of formol saline.
- (2) Strain through a sieve (No. 60) into a universal bottle.
- (3) Add 3 ml. of ether and shake vigorously.
- (4) Centrifuge so that 200 rpm is reached in two minutes then switch off.
- (5) Loosen the fatty layer at the interface of the fluids and pour away the whole of the supernatant liquid, together with the debris.
- (6) Mix the deposit, place on a slide and examine with a low-power objective.

APPENDIX 1C

Artificial Spring Water

Adapted from Macinnes and Voge (1970).

Prepare four stock solutions in distilled water.

- (a) Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$)
0.25 g. in 1 litre H_2O
- (b) Calcium chloride (CaCl_2 , anhydrous)
11.0 g. in 1 litre H_2O
- (c) Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)
10.0 g. in 1 litre H_2O
- (d) Phosphate buffer - Dissolve 34 gm. of potassium acid phosphate (KH_2PO_4) in 500 ml. of distilled water.

Add approximately 175 ml. 1 N NaOH until a pH of 7.2 is reached. Then add 1.5 gm. $(\text{NH}_4)_2\text{SO}_4$ and dilute to 1 litre.

Combine the stock solutions as follows and dilute with distilled water.

- Solution (a) 0.5 ml.
- Solution (b) 2.5 ml.
- Solution (c) 2.5 ml.
- Solution (d) 1.25 ml.
- Distilled water 1000 ml.

The distilled water should contain less than 0.01 ppm copper and should be aerated to remove excess CO_2 and to saturate with O_2 .

APPENDIX 1D

$\text{CH}_3\text{COO Na}$, 0.20 M

Dissolve 27.2 gms of $\text{CH}_3\text{COO Na} \cdot 3\text{H}_2\text{O}$ in H_2O and dilute to 1000

ml. CH_3COOH , 0.20 M

Dilute 12 ml glacial acetic acid to 1 litre with H_2O

Standardises against NaOH with phenolphthalein as indicator and dilute to 0.20 M.

Range of pH values which may be obtained by mixing $\text{CH}_3\text{COO Na}$ and CH_3COOH solutions.

pH at 18°C	0.2 M $\text{CH}_3\text{COO Na}$ ml	0.2 M CH_3COOH ml
3.6	0.75	9.25
3.8	1.20	8.80
4.0	1.80	8.20
4.2	2.65	7.35
4.4	3.70	6.30
4.6	4.90	5.10
4.8	5.90	4.10
5.0	7.00	3.00
5.2	7.90	2.10
5.4	8.60	1.40
5.6	9.10	0.90
5.8	9.40	0.60

APPENDIX 1D

Dissolve either 35.6 gms. of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ or 71.6 gms. of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ in H_2O and dilute to 1000 ml.

NaH_2PO_4 , 0.2 M.

Dissolve either 27.6 gms. of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ or 31.2 gms. of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ in H_2O and dilute to 1000 ml.

Range of pH values which may be obtained by mixing these phosphate solutions.

pH	0.2M Na_2HPO_4 (ml.)	0.2M NaH_2PO_4 (ml.)	H_2O (ml.)
5.8	8.0	92.0	100
6.0	12.3	87.7	100
6.2	18.5	81.5	100
6.4	26.5	73.5	100
6.6	37.5	62.5	100
6.8	49.0	51.0	100
7.0	61.0	39.0	100
7.2	72.0	28.0	100
7.4	81.0	19.0	100
7.6	87.0	13.0	100
7.8	91.5	8.5	100
8.0	94.7	5.3	100

APPENDIX 2

The egg production of C. sclerosus on excised gill arches of tilapia.

S = Sarotherodon

Species of fish is S. spiluris

Length of fish is 11 cm.

Time from 12 noon to 5.30 pm. - 5.5 hours.

Number of Parasites	Number of eggs	Fecundity	Mean egg/ parasite/hour	Number of eggs/24 hours per parasite
3	12	4.00	0.73	17.45
2	8	4.00	0.73	17.45
3	14	4.67	0.85	20.38
4	8	2.00	0.36	8.73
3	12	4.00	0.73	17.45
4	17	4.25	0.77	18.55
8	43	5.38	0.98	23.48
4	8	2.00	0.36	8.73
5	6	1.20	0.22	5.24

Total number of parasites is 36

Total number of eggs = 128

Average fecundity of parasites is 3.50

Average mean egg per parasite/hour is 0.64

Average number of eggs/24 hours = 15.27

APPENDIX 2

Species of fish is S. spiluris

Length of fish is 12.5 cm.

Time from 12 noon to 5.30 p.m. - 5.5 hours

Number of Parasites	Number of Eggs	Fecundity	Mean egg/parasite/hour	Number of eggs/24 hours
2	7	3.50	0.64	15.27
4	3	0.75	0.14	3.27
1	6	6.00	1.09	26.18
2	7	3.50	0.64	15.27
3	3	1.00	0.18	4.36
3	5	1.67	0.30	7.29
2	16	8.00	1.45	34.91
2	3	1.50	0.27	6.55
2	2	1.00	0.18	4.36
4	13	3.25	0.59	14.18
2	2	1.00	0.18	4.36
4	7	1.75	0.32	7.64

Total number of parasites is 31.

Total number of eggs is 74.

Average fecundity of parasites is 2.74.

Average mean egg per parasite per hour is 0.50.

Number of egg per 24 hours is 11.97

APPENDIX 2

Species of fish is S. mossambicus

Length of fish is 11.5 cm.

Time from 10.30 a.m. to 2.45 p.m. - 4.25 hours

Number of parasites	Number of eggs	Fecundity	Mean egg/parasite/hour	Number of eggs/24 hours per parasite
1	3	3.00	0.71	16.94
1	3	3.00	0.71	16.94
1	5	5.00	1.18	28.24
1	0	-	-	0.00
1	5	5.00	1.18	28.24
2	1	0.50	0.12	2.82

Total number of egg laying parasites is 6

Total number of eggs is 17

Average fecundity of parasite is 3.3

Average mean egg/parasite/hour is 0.78

Average number of eggs per 24 hours is 18.64

APPENDIX 2

Species of fish is S. mossambicus

Length of fish is 9 cm.

Time from 11.00 a.m. to 6.30 p.m. - 7.5 hours

Number of parasites	Number of eggs	Fecundity	Mean egg/ parasite/hour	Number of eggs/24 hours
1	4	4.00	0.53	12.80
2	0	-	-	0.00
1	0	-	-	0.00
2	1	0.50	0.07	1.60
1	0	-	-	0.00
1	0	-	-	0.00
1	0	-	-	0.00

Total number of egg laying parasites is 3

Number of eggs is 5

Average fecundity of parasite is 2.25

Average mean egg per parasite per hour is 0.3

Average number of eggs per 24 hours is 7.2

APPENDIX 3

Site-specificity experiment

Spatial distribution of *C. sclerosus*.

Right side	A	B	C	D	E	F	Total
1st	4	9	9	98	327	103	550
2nd	1	10	3	85	258	87	444
3rd	3	3	6	56	254	62	384
4th	2	4	4	31	190	63	294
Left side							
1st	5	13	10	87	291	104	510
2nd	4	11	6	63	278	100	462
3rd	4	5	2	60	207	80	358
4th	1	5	2	32	190	47	277

Square-root transformation of data.

Right side	A	B	C	D	E	F	Total
1st	2.00	3.00	3.00	9.90	18.08	10.15	46.13
2nd	1.00	3.16	1.73	9.22	16.06	9.33	40.50
3rd	1.73	1.73	2.45	7.48	15.94	7.87	37.20
4th	1.41	2.00	2.00	5.57	13.78	7.94	32.70
	6.14	9.89	9.18	32.17	63.86	35.29	156.53
Left side							
1st	2.24	3.61	3.16	9.33	17.06	10.20	45.60
2nd	2.00	3.32	2.45	7.94	16.67	10.00	42.38
3rd	2.00	2.24	1.41	7.75	14.39	8.94	36.73
4th	1.00	2.24	1.41	5.66	13.78	6.86	30.95
	7.24	11.41	8.43	30.68	61.90	36.00	155.66

APPENDIX 3

Analysis of Variance for right sides of heads

- (1) Grand total = 156.53
- (2) Sum of squared observations = 1671.782
- (3) Sum of squared column totals divided by sample size of a column = $\frac{(6.14^2 + \dots + 35.29^2)}{4}$
 $= \frac{6578.176}{4} = 1644.54$
- (4) Sum of squared row totals divided by sample size of a row = $\frac{(46.13^2 + \dots + 32.7^2)}{6}$
 $= \frac{6221.3569}{6} = 1036.89$
- (5) Grand total squared and divided by total sample size
 $=$ correction term $= \frac{(1)^2}{ab}$
 $= \frac{156.53^2}{24}$
 $= 1020.90$
- (6) SS total = (2) - (5)
 $= 1671.782 - 1020.90$
 $= 650.882$
- (7) SSA (SS of columns) = (3) - (5)
 $= 1644.54 - 1020.90$
 $= 623.64$
- (8) SSB (SS of rows) = (4) - (5)
 $= 1036.89 - 1020.90$
 $= 15.99$
- (9) SS error = (6) - (7) - (8)
 $= 650.882 - 623.64 - 15.99 = 11.25$

APPENDIX 3

ANOVA TABLE

Source of variation	df	ss	ms	F
A (columns)	5	623.64	124.728	166.30
B (rows)	3	15.99	5.33	7.11
Error	15	11.25	0.75	
Total	23	650.882		

$$F_{0.05}(5, 15) = 2.90$$

$$F_{0.05}(3, 15) = 3.29$$

There was a significant difference between the sites and a significant difference between gill arches.

APPENDIX 4

Analysis of data comparing right and left gill arches for C. sclerosus distribution.

To see if there was a significant difference between right and left gill arches for total C. sclerosus distribution, a two-way Analysis of Variance was carried out on the following data from Table XI.

	Right side		Left side	
	<u>C. sclerosus</u> (total numbers)	\bar{y}	<u>C. sclerosus</u> (total numbers)	\bar{y}
1st gill arch	550	91.67	510	85.00
2nd gill arch	444	74.00	462	77.00
3rd gill arch	384	64.00	358	59.67
4th gill arch	294	49.00	277	46.17

Transformation of variates by square-root transformation gave the following:-

	Right side	Left side
1st gill arch	9.57	9.22
2nd gill arch	8.60	8.77
3rd gill arch	8.00	7.72
4th gill arch	<u>7.00</u>	<u>6.79</u>
Σy	<u>33.17</u>	<u>32.50</u>
Σy^2	278.5449	267.6238

A Model 1 two-sample Analysis of Variance was carried out.

APPENDIX 4

$$(1) \text{ Grand total} = 33.17 + 32.5 = 65.67$$

$$(2) \text{ Sum of squared observations} = (9.57^2 + \dots + 6.79^2) \\ = 546.1687$$

$$(3) \text{ Sum of squared group totals divided by } n = \frac{33.17^2 + 32.5^2}{4} \\ = \frac{1100.2489 + 1056.25}{4} \\ = 539.12$$

$$(4) \text{ Grand total squared and divided by total sample size} \\ = \text{correction term} = \frac{(1)^2}{ab} \\ = \frac{65.67^2}{8} \\ = 539.0686$$

$$(5) \text{ SS total} = (2) - (4) = 546.1687 - 539.0686 \\ = 7.1001$$

$$(6) \text{ SS groups} = (3) - (4) = 539.12 - 539.0686 \\ = 0.0514$$

$$(7) \text{ SS within} = (5) - (6) = 7.1001 - 0.0514 \\ = 7.0487$$

ANOVA TABLE

Source of variation	df	ss	ms	F
Among groups	1	0.0514	0.0514	0.04
Within groups	6	7.0487	1.17478	
Total	7	7.1001		

$$F_{.05} (1,6) = 5.99$$

There was no significant difference between the right side and the left side gill arches.

APPENDIX 5

Spatial distribution of juveniles.

Right side of the head (from Table XIV)

A	B	C	D	E	F	Total
3.16	4.36	3.61	2.65	2.83	1.73	18.34
2.24	3.61	2.65	2.83	2.24	2.00	15.57
1.73	1.73	2.00	2.45	3.16	2.45	13.52
1.00	2.24	2.00	2.83	2.45	1.00	11.52
8.13	11.94	10.26	10.76	10.68	7.18	58.95

- (1) Grand total = 58.95
- (2) Sum of squared observations = 159.1557
- (3) Sum of squared column totals divided by sample size of a column = $\frac{8.13^2 + \dots + 7.18^2}{4}$
- $$= \frac{595.32}{4}$$
- $$= 148.83$$
- (4) Sum of squared row totals divided by sample size of a row = $\frac{894.28}{6} = 149.047$
- (5) Grand total squared and divided by total sample size = correction term = $\frac{(1)^2}{ab} = \frac{58.95^2}{24}$
- $$= 144.79$$
- (6) SS total = (2) - (5) = 159.1557 - 144.79
- $$= 14.365$$
- (7) SSA (SS of columns) = (3) - (5) = 148.83 - 144.79
- $$= 4.04$$

APPENDIX 5

$$\begin{aligned}
 (8) \text{ SSB (SS of rows)} &= (4) - (5) \\
 &= 149.047 - 144.79 \\
 &= 4.257
 \end{aligned}$$

$$\begin{aligned}
 (9) \text{ SS error} &= (6) - (7) - (8) \\
 &= 14.365 - 4.040 - 4.257 \\
 &= 6.068
 \end{aligned}$$

ANOVA TABLE

Source of variation	df	ss	ms	F
A (columns)	5	4.040	0.808	1.997
B (rows)	3	4.257	1.419	3.508
Error	15	6.068	0.4045	
Total	23	14.365		

$$F_{.05} (5,15) = 2.90 \quad F_{.05} (3,15) = 3.29$$

There was no significant difference between columns but a significant difference between rows.

Duncan's Multiple Range

	A	B	C	D	E	F
	8.13	11.94	10.26	10.76	10.68	7.18
\bar{Y}	2.0325	2.985	2.565	2.69	2.67	1.795
	$p = 6$	$f_e = 15$	$s_{\bar{y}} = \sqrt{\frac{0.4045}{4}} = 0.3180$			
p	2	3	4	5	6	7
$q_{\alpha} (6,15)$	3.01	3.16	3.25	3.31	3.36	3.38
R_p	0.9584	1.0048	1.0335	1.0525	1.068	1.0748

A summary of test results using underscores were as follows:

F	A	C	E	D	B
1.79	2.03	2.56	2.67	2.69	2.98

APPENDIX 5

A summary of test results using underscores were as follows:-

F	A	C	E	D	B
7.18	8.13	<u>10.26</u>	<u>10.68</u>	<u>10.76</u>	11.94

There was a significant difference between A, B and F and these differ from C, D and E. There was no significant difference between sites C, E and D.

Duncan's Multiple Range		\bar{y}
1st gill arch	18.34	3.06
2nd gill arch	15.57	2.60
3rd gill arch	13.52	2.25
4th gill arch	11.52	1.92

$$p = 4 \quad fe = 15 \quad s\bar{y} = \sqrt{\frac{0.4045}{6}}$$

$$= 0.2596$$

p	2	3	4	5	6
q' (4,15)	3.01	3.16	3.25	3.31	3.36
Rp	0.7815	0.8203	0.8437	0.8592	0.8722

A summary of test results using underscores were as follows:-

4th	3rd	2nd	1st
<u>1.92</u>	<u>2.25</u>	<u>2.60</u>	3.06

APPENDIX 6

$\sqrt{\bar{y}}$ transformation for juvenile C. sclerosus.

Means of two groups

3.162	3.937
2.645	3.240
2.308	3.316
<u>2.039</u>	<u>2.738</u>
<u>10.154</u>	<u>13.231</u>

(1) Grand total = 23.385

(2) Sum of squared observations = 70.968

(3) Sum of squared group totals divided by n = $\frac{10.154^2 + 13.231^2}{4}$
= 69.5407

(4) Grand total squared and divided by total sample size =
correction term = $\frac{23.385^2}{8}$
= 68.357

(5) SS total = (2) - (4) = 70.968 - 68.357 = 2.611

(6) SS groups = (3) - (4) = 69.5407 - 68.357 = 1.1837

(7) SS within = (5) - (6) = 2.611 - 1.1837 = 1.4273

ANOVA TABLE

Source of variation	df	ss	ms	F
Among groups	1	1.1837	1.1837	4.977
Within groups	6	1.4273	0.2378	
Total	7	2.611		

F.05 (1,6) = 5.99

APPENDIX 7

Spatial distribution of C. t. minutus.Square-root transformation of data for right side
gill arches of C. t. minutus.

Right side	A	B	C	D	E	F	Total
1st gill arch	0.707	0.707	0.707	3.937	3.082	3.808	12.948
2nd gill arch	0.707	0.707	0.707	1.581	3.808	3.240	10.750
3rd gill arch	0.707	0.707	0.707	1.225	1.581	1.225	6.152
4th gill arch	0.707	0.707	0.707	1.225	0.707	1.225	5.278
	2.828	2.828	2.828	7.968	9.178	9.498	35.128

$$(1) \text{ Grand total} = 35.128$$

$$(2) \text{ Sum of squared observations} = (0.707^2 + \dots + 1.225^2) \\ = 81.997$$

$$(3) \text{ Sum of squared column totals divided by sample size of a} \\ \text{column} = \frac{2.828^2 + \dots + 9.498^2}{4} \\ = \frac{261.929}{4} = 65.482$$

$$(4) \text{ Sum of squared row totals divided by sample size of a row} \\ = \frac{(12.948^2 + \dots + 5.278^2)}{6} \\ = \frac{348.917}{6} = 58.1529$$

$$(5) \text{ Grand total squared and divided by total sample size} \\ = \frac{35.128^2}{24} = 51.4156$$

$$(6) \text{ SS total} = (2) - (5) = 81.997 - 51.415 = 30.582$$

$$(7) \text{ SSA (SS of columns)} = (3) - (5) = 65.482 - 51.415 \\ = 14.067$$

APPENDIX 7

$$(8) \text{ SSB (SS of rows)} = (4) - (5) = 58.1529 - 51.4156 \\ = 6.73$$

$$(9) \text{ SS error} = (6) - (7) - (8) \\ = 30.5814 - 14.0664 - 6.73 \\ = 9.785$$

ANOVA TABLE

Source of variation	df	ss	ms	F
A (columns)	5	14.0664	2.813	4.312
B (rows)	3	6.73	2.2433	3.439
Error	15	9.785	0.6523	

$$F_{0.05} (5,15) = 2.90$$

$$F_{0.05} (3,15) = 3.29$$

There was a significant difference between columns and a significant difference between rows.

Duncan's Multiple Range Analysis

$$p = 6 \quad fe = 15 \quad s\bar{y} = \sqrt{\frac{0.6523}{4}} = 0.4038$$

p	2	3	4	5	6	7
$q_{\alpha} (6,15)$	3.014	3.16	3.25	3.31	3.36	3.38
R_p	1.2171	1.2760	1.3123	1.3365	1.3567	1.3648

A summary of test results using underscores were as follows:-

A	B	C	D	E	F
<u>0.707</u>	<u>0.707</u>	<u>0.707</u>	<u>1.992</u>	<u>2.295</u>	<u>2.375</u>

There was no significant difference between A, B, C and between E and F. These sites differed from D. Sites A, B, C differed from sites E and F.

APPENDIX 8

From Table XV.

C. t. minutus

	Right side	\bar{y}	Left side	\bar{y}
1st gill arch	38	6.333	28	4.666
2nd gill arch	26	4.333	19	3.166
3rd gill arch	4	0.666	14	2.333
4th gill arch	2	0.333	1	0.166

Transformation of the means ($\sqrt{\bar{y}}$)

Right side	Left side
2.5165	2.160
2.0815	1.779
0.8160	1.527
<u>0.57706</u>	<u>0.4074</u>
<u>5.99056</u>	<u>5.8734</u>

(1) Grand total = 11.8639

(2) Sum of squared observations = 21.9924

(3) Sum of squared group totals divided by n

$$= \frac{5.99056^2}{4} + \frac{5.8734^2}{4} = \frac{70.3836}{4}$$

= 17.5959

(4) Grand total squared and divided by total sample size

$$= \text{correction term} = \frac{11.8639^2}{8}$$

= 17.5940

(5) SS total = (2) - (4) = 21.9924 - 17.5940 = 4.3984

(6) SS groups = (3) - (4) = 17.5959 - 17.5940 = 0.0019

(7) SS within = (5) - (6) = 4.3984 - 0.0019 = 4.3965

APPENDIX 8

ANOVA TABLE

Source of variation	df	ss	ms	F
Among groups	1	0.0019	0.0019	0.0025
Within groups	6	4.3984	0.7330	
Total	7	4.3965		

$$F_{0.05(1,6)} = 5.99$$

Therefore there was no significant difference between right side gill arches and left side gill arches.

APPENDIX 9Goodness of Fit Tests

General details

In order to see if the frequencies were randomly distributed or not a Poisson series was calculated for each set of observed frequency distributions. The discrepancy between the observed and the expected frequencies was then tested by means of χ^2 (Sokal and Rolf, 1973) with expected frequencies < 5 being combined and compared to the corresponding observed frequencies in one ratio. The equation used was $\chi^2 = \sum \frac{(f - \emptyset)^2}{\emptyset}$ where f is observed frequency and \emptyset is expected frequency, where χ^2 has two less degree of freedom than the number of ratios totalled (Sokal and Rolf, 1973). The smaller χ^2 was, the more closely the observed and expected frequencies agreed. A χ^2_* test was also used because the conventional χ^2 test tended to be insensitive to certain deviations because of the necessity of combining results to produce expected frequencies > 5 . Fisher (1950) has devised a test based on the function $\chi^2_* = 2 \sum [f \log_e (f/\emptyset)]$ which is distributed approximately as χ^2 . In this test pooling was only necessary to obtain observed frequencies > 0 . The smaller χ^2_* was, the more closely the observed and expected frequencies agreed.

$P = 0.005$ was taken as the limit of significance for both χ^2 and χ^2_* . For all values of $P(\chi^2)$ and $P(\chi^2_*) < 0.005$ it was assumed that the observed frequencies were not randomly distributed, according to Poisson Law. If there was any discrepancy in the results provided by the χ^2 and χ^2_* tests then the χ^2_* test was the one which was relied upon, particularly where the frequencies were small.

APPENDIX 9

Goodness of Fit Tests

Density A

Week No.	Test	Value calculated	Degrees of freedom	Critical value at P = 0.005
5	χ^2	38.307	3	12.838
	χ^2_{*}	51.598	7	20.278
6	χ^2	36.450	3	12.838
	χ^2_{*}	56.720	8	21.955
7	χ^2	12.451	2	10.597
	χ^2_{*}	20.684	6	18.548
8	χ^2	28.984	3	12.838
	χ^2_{*}	53.074	7	20.278
9	χ^2	29.028	3	12.838
	χ^2_{*}	50.940	8	21.955
10	χ^2	29.028	3	12.838
	χ^2_{*}	47.174	8	21.955
11	χ^2	18.002	1	7.879
	χ^2_{*}	45.290	5	16.750

APPENDIX 9

Goodness of Fit Tests

Density B

Week No.	Test	Value calculated	Degrees of freedom	Critical value at P = 0.005
5	χ^2	0.010	0	
	χ_{**}^2	20.768	5	16.750
6	χ^2	0.444	0	
	χ_{**}^2	21.456	4	14.860
7	χ^2	0.441	0	
	χ_{**}^2	7.862	5	16.750
8	χ^2	2.345	0	
	χ_{**}^2	16.768	4	14.860
9	χ^2	2.571	0	
	χ_{**}^2	10.508	5	16.750
10	χ^2	1.412	0	
	χ_{**}^2	9.778	4	14.860
11/12	χ^2	1.331	0	
	χ_{**}^2	17.300	4	14.860

Total Results

Density	Test	Value calculated	Degrees of freedom	Critical value at P = 0.005
A	χ^2	304.932	4	14.860
	χ_{**}^2	331.524	10	25.188
B	χ^2	72.653	4	14.860
	χ_{**}^2	102.588	9	23.589

APPENDIX 9

Poisson Distribution
Goodness of Fit Tests

TABLE 1. Combined Results - Week 5

x	Density A				Density B			
	f	θ	$\frac{(f - \theta)^2}{\theta}$	$f \log_e \left(\frac{f}{\theta} \right)$	f	θ	$\frac{(f - \theta)^2}{\theta}$	$f \log_e \left(\frac{f}{\theta} \right)$
0	23	7.854	29.208	24.713	6	1.159	0.005	9.865
1	11	15.970	1.547	-4.101	1	3.042		0.005
2	9	16.236	3.225	-5.310	1	3.993	0.005	
3	5	11.004	3.276	-3.944	2	3.494		0.005
4	4	5.593	1.051	-1.341	3	2.293	0.005	
5	3	2.275		0.830	0.830	1		1.204
6	2	0.771	1.051	1.396	0	0.527	0.005	3.876
7	0	0.224		1.396	1.396	0		
8	0	0.057	1.051	6.648	2	0.065	0.005	3.876
9	2	0.012			6.648	0		
10	0	0.003	1.051	6.908	0	0.025	0.005	3.876
11	0	0.001			6.908			
12	0		0.001	6.908	6.908	0	0	0.005
13	0	0.001				6.908	0	
14	0	0.001	6.908	6.908	0	0	0.005	3.876
15	0				0.001	6.908		
16	0	0.001	6.908	6.908	0	0	0.005	3.876
17	1				0.001	6.908		
18+	0	0	6.908	6.908	0	0	0.005	3.876
Total	60	60.000	38.307	25.799	16	16.000	0.010	10.384

APPENDIX 9

TABLE 2. Combined Results - week 6

x	Density A				Density B			
	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$
0	24	9.754	20.807	21.609	6	1.795	0.285	7.241
1	13	17.720	1.257	-4.027	1	3.927		-1.368
2	10	16.095	2.308	-4.759	5	4.295		0.760
3	1	9.747	7.850	-2.277	1	3.132		-1.578
4	4	4.427		-0.406	0	1.713		
5	2	1.608		0.436	1	0.749		-0.022
6	3	0.487		5.453	0	0.273		
7	1	0.126		2.071	0	0.085		
8	1	0.029		3.352	2	0.023		
9	0	0.006			0		0.159	
10	0		4.228		0			
11	0				0			
12	0	0.001		6.908	0	0.008		5.695
13	0				0			
14	1				0			
15+	0				0			
Total	60	60.000	36.450	28.360	16	16.000	0.444	10.728

APPENDIX 9

TABLE 3. Combined Results - Week 7

x	Density A				Density B			
	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$
0	26	15.816	6.558	12.924	5	1.795	0.285	5.122
1	13	21.088	3.102	-6.289	2	3.927		0.159
2	9	14.058	1.820	-4.014	3	4.295	0.159	
3	5	6.248	0.971	-1.114	2	3.132		0.159
4	4	2.083		2.610	2	1.713	0.159	
5	1	0.555	0.589	0.589	1	0.749		0.159
6	1	0.123	2.096	2.096	0	0.273		
7	1	0.024	3.540	3.540	0	0.085	0.159	2.154
8	0	0.005						
9+	0				0	0.008		
Total	60	60.000	12.451	10.342	16	16.000	0.444	3.931

APPENDIX 9

TABLE 4. Combined Results - Week 8

x	Density A				Density B			
	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$
0	26	11.523	18.188	21.158	4	1.398	1.032	4.205
1	11	19.013	3.377	-6.020	4	3.408		0.641
2	10	15.686	2.061	-4.502	4	4.153	-2.337	-0.150
3	4	8.627	2.482	-3.074	2	3.375		-2.337
4	2	3.559	2.876	-1.153	0	2.056	1.313	0.504
5	3	1.174		2.085	0	1.002		
6	0	0.323	5.809	2.577	0	0.407	5.521	
7	1	0.076		2.577	0	0.142		
8	2	0.016	26.537	9.657	0	0.043	8.384	
9	0	0.003		28.984	26.537	1		0.012
10	1		60.000			28.984	26.537	0
11	0	60.000		28.984	26.537			0
12	0		60.000			28.984	26.537	1
13+	0	60.000		28.984	26.537			0
Total	60		60.000			28.984	26.537	16

APPENDIX 9

TABLE 5. Combined Results - Week 9

x	Density A				Density B			
	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$
0	18	4.925	9.448	23.329	3	1.488	1.764	2.104
1	12	12.313		-0.309	5	3.535		1.734
2	6	15.391	5.730	-5.652	2	4.197		-1.482
3	6	12.826	3.633	-4.558	2	3.323		-1.015
4	5	8.016	1.135	-2.360	1	1.973		-0.680
5	4	4.008		-0.008	1	0.937		-0.268
6	4	1.670		3.494	0	0.371		
7	1	0.596		0.518	2	0.126		
8	3	0.186		8.342	0		0.807	
9	0		6.414		0			4.861
10	0				0			
11	0			2.674	0	0.050		
12	0	0.069			0			
13	1				0			
14+	0				0			
Total	60	60.000	26.360	25.470	16	16.000	2.571	5.254

APPENDIX 9

TABLE 5. Combined Results - Week 9

x	Density A				Density B			
	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$
0	18	4.925	9.448	23.329	3	1.488	1.764	2.104
1	12	12.313		-0.309	5	3.535		1.734
2	6	15.391	5.730	-5.652	2	4.197	-1.482	
3	6	12.826	3.633	-4.558	2	3.323	-1.015	
4	5	8.016	1.135	-2.360	1	1.973	-0.680	
5	4	4.008	6.414	-0.008	1	0.937	-0.268	
6	4	1.670		3.494	0	0.371		
7	1	0.596	0.518	0.518	2	0.126	0.807	
8	3	0.186	8.342	8.342	0			
9	0				0			4.861
10	0				0			
11	0	0.069	2.674	2.674	0	0.050		
12	0				0			
13	1				0			
14+	0				0			
Total	60	60.000	26.360	25.470	16	16.000	2.571	5.254

APPENDIX 9

TABLE 6. Combined Results - Week 10

x	Density A					Density B				
	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$	f	ϕ	$\frac{(f - \phi)}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$		
0	18	6.989	17.348	17.029	4	1.795	0.907	3.205		
1	16	15.026	0.063	1.005	4	3.927		0.074		
2	4	16.153	9.144	-5.583	4	4.295		-2.475		
3	8	11.577	1.105	-2.957	0	3.132				
4	4	6.222		-1.767	2	1.713		-0.416		
5	6	2.676		4.845	0	0.749				
6	1	0.959		0.042	1	0.273		1.027		
7	1	0.294		0.986	0	0.085	0.505			
8	0	0.079	1.368		0	0.023		3.474		
9	0	0.019		3.772	1	0.006				
10	1	0.004			0			0.002		
11	0				0					
12	1	0.002		6.215	0					
13+	0				0					
Total	60	60.000	29.028	23.587	16	16.000	1.412	4.889		

APPENDIX 9

TABLE 7. Combined Results - Week 11/12

x	Density A				Density B			
	f	ϕ	$\frac{(f-0)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$	f	ϕ	$\frac{(f-\phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$
0	31	17.658	10.081	17.447	7	1.686	0.520	9.965
1	6	17.658	7.697	-6.477	2	3.794		-1.281
2	2	8.829	}	-2.970	3	4.269	}	-1.058
3	3	2.943		0.058	1	3.202		-1.610
4	2	0.736	}	1.999	0	1.801	}	1.808
5	3	0.147		9.048	2	0.810		
6	1	0.025	}	}	0	}	}	}
7	0	}			}			
8	0		}	}		0	}	}
9	0	0.224			}	0		
10	0	}	}	0		}	}	}
11	0			}	}			
12	0	0.004	}			0	}	}
13	0	}		}	0	}		
14	0		}		}		0	}
15	0	}		}		1	}	
16+	0		}		}	0		}
Total	48	48.000		18.002		22.645	16	

APPENDIX 9

TABLE 8. Total Results

x	Density A				Density B			
	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$	f	ϕ	$\frac{(f - \phi)^2}{\phi}$	$f \log_e \left(\frac{f}{\phi} \right)$
0	166				35			
1	82	67.012	146.222	150.581	19	10.991	52.446	40.539
2	50	121.050	12.597	-31.938	22	25.515	1.664	-5.602
3	32	109.332	32.198	-39.118	10	29.615	1.958	-6.539
4	25	65.833	17.388	-23.084	8	22.916	7.280	-8.293
5	22	29.730	0.753	-4.332	6	13.300	2.112	-4.067
6	12	10.741		15.773	1	6.175		-0.172
7	5	3.234		15.734	2	2.389		-0.871
8	6	0.834		8.955	5	0.792		1.853
9	2	0.188		20.778	2	0.230		15.396
10	2	0.038		7.927	0	0.059		
11	0	0.007		11.310	0	0.014	7.193	6.621
12	1		95.774		1	0.003		
13	1				0			
14	1				0			
15	0	0.001		33.176	1	0.001		12.429
16	0				0			
17	1				0			
18+	0				0			
Total	408	408.000	304.932	165.762	112	112.000	72.653	51.294

APPENDIX 10

Calculation Procedures for Negative Binomial Distribution

The maximum likelihood method of calculation (Bliss and Fisher, 1953) was used to determine the negative binomial values. Firstly the parameter, K , had to be determined.

(1) Estimation of K (a) Initial estimate (\hat{K}_1)

This was determined from the mean, \bar{x} , and the variance, s^2 , of the original distribution. These were calculated from the following equations:-

$$\bar{x} = \frac{\sum(fx)}{N} \quad - \quad (1)$$

$$s^2 = \frac{\sum(fx^2) - \frac{\sum^2(fx)}{N}}{N - 1} \quad - \quad (2)$$

\hat{K}_1 was then calculated from

$$\hat{K}_1 = \frac{\bar{x}^2}{s^2 - \bar{x}} \quad - \quad (3)$$

(b) Final estimate (\hat{K}_2)

Scores (z) were calculated from trial values of K .

The scores were given by

$$z = \sum \left(\frac{Ax}{k+x} \right) - N \log_e \left(1 + \frac{\bar{x}}{K} \right) \quad - \quad (4)$$

where Ax , the accumulated frequency was

$$Ax = N - \sum f_{x-1} \quad - \quad (5)$$

Ax was simply the number of hosts having x or more parasites. This was calculated first and written opposite each x .

The value of the first trial, K_1 , was based upon the

APPENDIX 10

initial estimate of K already obtained. If the corresponding score, z_1 , was positive the next trial value selected, K_2 , was increased to obtain a negative value for z_2 . Conversely, if z_1 was negative, then K_2 was decreased to obtain a positive value for z_2 . Hence the values of K_1 and K_2 selected were such that one was larger and the other smaller than the required estimate, \hat{K}_2 , for which $z = 0$ in (4).

Interpolation between z_1 and z_2 for $z = 0$ gave a new trial value, K_3 , with which the process was continued until a value of K was obtained which was accurate to four decimal places.

APPENDIX 10

Calculation of expected frequencies

When a value for K had been calculated from the above, it was substituted in

$$p = \frac{\bar{x}}{K} \quad - \quad (6)$$

The value of p obtained was used in

$$q = 1 + p \quad - \quad (7)$$

The ratio, R , was then given by

$$R = \frac{p}{q} \quad - \quad (8)$$

Having ascertained p , q , and R , the expected frequency, ϕ , was then calculated for each value of x ,

with $x = 0$, ϕ_0 was given by

$$\phi_0 = \frac{N}{q^k} \quad - \quad (9) \text{ where } N = \text{number in sample.}$$

When $x = 1, 2, 3 \dots$ the expected frequencies were obtained from

$$\phi_x = \frac{k + x - 1}{x} \cdot R \phi_{x-1} \quad - \quad (10)$$

The final value of ϕ was obtained by subtracting the sum of the preceding expected frequencies from N . Thus, for x at its maximum value,

$$\phi_x = N - \sum \phi_{x-1} \quad (11)$$

APPENDIX 11

Negative Binomial Distribution

High Density Results Combined - Time Interval Analysis

Calculations for method of maximum likelihood.

Observed frequency distributions are shown in Table 1.

(1) Estimation of K

(a) Initial Estimate (\hat{K}_1)

\bar{x} from (1), S^2 from (2) and \hat{K}_1 from (3) are shown in Table 2.

(b) Final Estimate (\hat{K}_2)

Ax is calculated from (5) and shown in Table 1. The calculations for \hat{K}_2 using (4) are shown on pages 205-213.

(2) Calculation of Expected Frequencies (\emptyset)

The estimated values of K plus p from (6), q from (7) and R from (8) are shown for each time interval in Table 3. The expected frequencies obtained from (9), (10) and (11) are shown in Table 4.

APPENDIX 11

TABLE 1. Accumulated Frequencies (Ax)
High Density Results Combined - Time Interval Analysis

x	Week 5		Week 6		Week 7		Week 8		Week 9		Week 10		Week 11	
	f	Ax	f	Ax	f	Ax	f	Ax	f	Ax	f	Ax	f	Ax
0	23	37	24	36	26	34	26	34	18	42	18	42	31	17
1	11	26	13	23	13	21	11	23	12	30	16	26	6	11
2	9	17	10	13	9	12	10	13	6	24	4	22	2	9
3	5	12	1	12	5	7	4	9	6	18	8	14	3	6
4	4	8	4	8	4	3	2	7	5	13	4	10	2	4
5	3	5	2	6	1	2	3	4	4	9	6	4	3	1
6	2	3	3	3	1	1	0	4	4	5	1	3	1	0
7	0	3	1	2	1	0	1	3	1	4	1	2	0	0
8	0	3	1	1	0	0	2	1	3	1	0	2	0	0
9	2	1	0	1	0	0	0	1	0	1	0	2	0	0
10	0	1	0	1	0	0	1	0	0	1	1	1	0	0
11	0	1	0	1	0	0	0	0	0	1	0	1	0	0
12	0	1	0	1	0	0	0	0	0	1	1	0	0	0
13	0	1	0	1	0	0	0	0	1	0	0	0	0	0
14	0	1	1	0	0	0	0	0	0	0	0	0	0	0
15	0	1	0	0	0	0	0	0	0	0	0	0	0	0
16	0	1	0	0	0	0	0	0	0	0	0	0	0	0
17	1	0	0	0	0	0	0	0	0	0	0	0	0	0
18+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	60	122	60	109	60	80	60	99	60	150	60	129	48	48

APPENDIX 11

Negative Binomial Distribution

High Density Results Combined - Time Interval Analysis

TABLE 2.

Week No.	\bar{x}	s^2	\hat{K}_1
5	2.0333	8.5751	0.6320
6	1.8167	6.6946	0.6766
7	1.3333	7.7345	1.2687
8	1.6500	5.2144	0.7638
9	2.5000	7.6102	1.2230
10	2.1500	6.2653	1.1232
11	1.0000	2.4678	0.6813

TABLE 3.

Week No	K	p	q	R
5	0.7145	2.8458	3.8458	0.7400
6	0.7112	2.5544	3.5544	0.7187
7	1.0816	1.2327	2.2327	0.5521
8	0.7039	2.3441	3.3441	0.7010
9	1.0099	2.4755	3.4755	0.7123
10	1.0940	1.9653	2.9653	0.6628
11	0.3240	3.0864	4.0864	0.7553

APPENDIX 11

Negative Binomial Distribution

High Density Tanks Combined - Time Interval Analysis

Calculation for \hat{K}_2

(1) Week 5

In (4)

$$K_1 = 0.6000; \quad Z_1 = 92.2490130 - 88.7438062 = 3.5052068$$

$$K_2 = 0.7000; \quad Z_2 = 82.0776752 - 81.7310769 = 0.3465983$$

$$K_3 = 0.7300; \quad Z_3 = 79.5233813 - 79.8681817 = -0.3448004$$

Intrepolation for $Z = 0$ between Z_2 and Z_3 :

$$K_4 = 0.7000 + \frac{0.0300 (0.3465983)}{0.3465983 + 0.3448004} = 0.7150390$$

$$\approx 0.7150$$

In (4)

$$K_4 = 0.7150; \quad Z_4 = 80.7763103 - 80.7873165 = -0.0110062$$

$$K_5 = 0.7100; \quad Z_5 = 81.2045708 - 81.0991131 = 0.1054577$$

Interpolation for $Z = 0$ between Z_4 and Z_5 :

$$K_6 = 0.7100 + \frac{0.0050 (0.1054577)}{0.1054577 + 0.0110062} = 0.7145275$$

$$\approx 0.7145$$

In (4)

$$K_6 = 0.7145; \quad Z_6 = 80.8188925 - 80.8183724 = 0.0005201$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 0.7145$$

APPENDIX 11

Negative Binomial Distribution

High Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(2) Week 6

In (4)

$$K_1 = 0.7000; Z_1 = 77.0269981 - 76.7774078 = 0.2495903$$

$$K_2 = 0.7300; Z_2 = 74.5790373 - 74.9705489 = -0.3915116$$

Interpolation for $Z = 0$ between Z_1 and Z_2 :

$$K_3 = 0.7000 + \frac{0.0300 (0.2495903)}{0.2495903 + 0.3915116} = 0.7116794$$

$$\approx 0.7117$$

In (4)

$$K_3 = 0.7117; Z_3 = 76.0499040 - 76.0611298 = -0.0112258$$

$$K_4 = 0.7100; Z_4 = 76.1900488 - 76.1642647 = 0.0257841$$

Interpolation for $Z = 0$ between Z_3 and Z_4 :

$$K_5 = 0.7100 + \frac{0.0017 (0.0257841)}{0.0257841 + 0.0112258} = 0.7111844$$

$$\approx 0.7112$$

In (4)

$$K_5 = 0.7112; Z_5 = 76.0910596 - 76.0914308 = -0.0003712$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 0.7112$$

APPENDIX 11

Negative Binomial Distribution

High Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(3) Week 7

In (4)

$$K_1 = 1.3000; Z_1 = 41.5690359 - 42.3524129 = -0.7833770$$

$$K_2 = 1.2000; Z_2 = 44.3338472 - 44.8320746 = -0.4982274$$

$$K_3 = 1.0000; Z_3 = 51.3261905 - 50.8370145 = 0.4891760$$

Interpolation for $Z = 0$ between Z_2 and Z_3 :

$$K_4 = 1.0000 + \frac{0.2000 (0.4891760)}{0.4891760 + 0.4982274} = 1.0990833$$

$$\approx 1.0991$$

In (4)

$$K_4 = 1.0991; Z_4 = 47.5755915 - 47.6632057 = -0.0876142$$

$$K_5 = 1.0900; Z_5 = 47.8943359 - 47.9371531 = -0.0428172$$

$$K_6 = 1.0700; Z_6 = 48.6119730 - 48.5510487 = 0.0609243$$

Interpolation for $Z = 0$ between Z_5 and Z_6 :

$$K_7 = 1.0700 + \frac{0.0200 (0.0609243)}{0.0609243 + 0.0428172} = 1.0817454$$

$$\approx 1.0817$$

In (4)

$$K_7 = 1.0817; Z_7 = 48.1892585 - 48.1899246 = -0.0006661$$

$$K_8 = 1.0810; Z_8 = 48.2143176 - 48.2113711 = 0.0029465$$

Interpolation for $Z = 0$ between Z_7 and Z_8 :

APPENDIX 11

$$K_9 = 1.0810 + \frac{0.0007 (0.0029465)}{0.0029465 + 0.0006661} = 1.0815709$$

$$\approx 1.0816$$

In (4)

$$K_9 = 1.0816; Z_9 = 48.1928366 - 48.1929871 = -0.0001505$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 1.0816$$

APPENDIX 11

Negative Binomial Distribution

High Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(4) Week 8

In (4)

$$K_1 = 0.7000; Z_1 = 72.7438643 - 72.6654163 = 0.0784480$$

$$K_2 = 0.7300; Z_2 = 70.4226837 - 70.9086740 = -0.4859903$$

Interpolation for $Z = 0$ between Z_1 and Z_2 :

$$K_3 = 0.7000 + \frac{0.0300 (0.0784480)}{0.0784480 + 0.4859903} = 0.7041695$$

$$\approx 0.7042$$

In (4)

$$K_3 = 0.7042; Z_3 = 72.4080616 - 72.4136304 = -0.0055688$$

$$K_4 = 0.7020; Z_4 = 72.5835013 - 72.5452743 = 0.0382270$$

Interpolation for $Z = 0$ between Z_3 and Z_4 :

$$K_5 = 0.7020 + \frac{0.0022 (0.0382270)}{0.0382270 + 0.0055688} = 0.7039203$$

$$\approx 0.7039$$

In (4)

$$K_5 = 0.7039; Z_5 = 72.4319263 - 72.4315503 = 0.0003760$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 0.7039$$

APPENDIX 11

Negative Binomial Distribution

High Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(5) Week 9

In (4)

$$K_1 = 1.2000; Z_1 = 66.0096855 - 67.5606758 = -1.5509903$$

$$K_2 = 1.0000; Z_2 = 75.2765623 - 75.1657781 = 0.1107842$$

$$K_3 = 1.1000; Z_3 = 70.2801644 - 71.1374199 = -0.8572555$$

Interpolation for $Z = 0$ between Z_2 and Z_3 :

$$K_4 = 1.0000 + \frac{0.1000 (0.1107842)}{0.1107842 + 0.8572555} = 1.0114442$$

$$\approx 1.0114$$

In (4)

$$K_4 = 1.0114; Z_4 = 74.6639432 - 74.6807585 = -0.0168153$$

$$K_5 = 1.0100; Z_5 = 74.7385309 - 74.7399424 = -0.0014115$$

$$K_6 = 1.0050; Z_6 = 75.0063857 - 74.9521787 = 0.0542070$$

Interpolation for $Z = 0$ between Z_5 and Z_6 :

$$K_7 = 1.0050 + \frac{0.0050 (0.0542070)}{0.0542070 + 0.0014115} = 1.0098731$$

$$\approx 1.0099$$

In (4)

$$K_7 = 1.0099; Z_7 = 74.7438655 - 74.7441739 = -0.0003084$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 1.0099$$

APPENDIX 11

Negative Binomial Distribution

High Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(6) Week 10

In (4)

$$K_1 = 1.0000; Z_1 = 69.7750361 - 68.8441472 = 0.9308889$$

$$K_2 = 1.1000; Z_2 = 64.9506845 - 65.0006890 = -0.0500045$$

$$K_3 = 1.0900; Z_3 = 65.3979488 - 65.3637380 = 0.0342108$$

Interpolation for $Z = 0$ between Z_2 and Z_3 :

$$K_4 = 1.0900 + \frac{0.0100 (0.0342108)}{0.0342108 + 0.0500045} = 1.0940623$$

$$\approx 1.0941$$

In (4)

$$K_4 = 1.0941; Z_4 = 65.2137019 - 65.2143513 = -0.0006494$$

$$K_5 = 1.0940; Z_5 = 65.2181813 - 65.2179859 = 0.0001954$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 1.0940$$

APPENDIX 11

Negative Binomial Distribution

High Density Tanks Combined - Time Interval Analysis

Calculations for K_2^A

(7) Week 11

In (4)

$$K_1 = 0.7000; Z_1 = 36.7377599 - 42.5905534 = -5.8527935$$

$$K_2 = 0.6000; Z_2 = 41.3846751 - 47.0798041 = -5.6951290$$

$$K_3 = 0.3000; Z_3 = 71.9783422 - 70.3841793 = 1.5941629$$

$$K_4 = 0.4000; Z_4 = 56.9661248 - 60.1326225 = -3.1664977$$

Interpolation for $Z = 0$ between Z_3 and Z_4 :

$$K_5 = 0.3000 + \frac{0.1000 (1.5941629)}{1.5941629 + 3.1664977} = 0.3334862$$

$$\approx 0.3335$$

In (4)

$$K_5 = 0.3335; Z_5 = 65.9907946 - 66.5241350 = -0.5333404$$

$$K_6 = 0.3300; Z_6 = 66.5616957 - 66.9043952 = -0.3426995$$

$$K_7 = 0.3200; Z_7 = 68.2587684 - 68.0191690 = 0.2395994$$

Interpolation for $Z = 0$ between Z_6 and Z_7 :

$$K_8 = 0.3200 + \frac{0.0100 (0.2395994)}{0.2395994 + 0.3426995} = 0.3241147$$

$$\approx 0.3241$$

In (4)

$$K_8 = 0.3241; Z_8 = 67.5508160 - 67.5569353 = -0.0061193$$

$$K_9 = 0.3230; Z_9 = 67.7390610 - 67.6802324 = 0.0588286$$

Interpolation for $Z = 0$ between Z_8 and Z_9 :

APPENDIX 11

$$K_{10} = 0.3230 + \frac{0.0011 (0.0588286)}{0.0588286 + 0.0061193} = 0.3239964$$

$$\approx 0.3240$$

In (4)

$$K_{10} = 0.3240; z_{10} = 67.5678785 - 67.5681226 = -0.0002441$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 0.3240$$

APPENDIX 12

Negative Binomial Distribution

Low Density Results Combined - Time Interval Analysis

Calculations for Method of Maximum Likelihood Observed frequency distributions are shown in Table 1.

(1) Estimation of K

(a) Initial Estimate (\hat{K}_1)

\bar{x} from (1), s^2 from (2) and \hat{K}_1 from (3) are shown in Table 2.

(b) Final Estimate (\hat{K}_2)

Ax is calculated from (5) in Table 1.

The calculations for \hat{K}_2 using (4) are shown on pages 218-226.

(2) Calculation of Expected Frequencies (\emptyset)

The estimated values of K plus p from (6), q from (7) and R from (8) are shown for each time interval in Table 3.

The expected frequencies obtained from (9), (10) and (11) are shown in Table 4.

APPENDIX 12

TABLE 1. Accumulated Frequencies (Ax)
Low Density Results Combined - Time Interval Analysis

x	Week 5		Week 6		Week 7		Week 8		Week 9		Week 10		Week 11/12	
	f	Ax	f	Ax	f	Ax								
0	6	10	6	10	5	11	4	12	3	13	4	12	7	9
1	1	9	1	9	2	9	4	8	5	8	4	8	2	7
2	1	8	5	4	3	6	4	4	2	6	4	4	3	4
3	2	6	1	3	2	4	2	2	2	4	0	4	1	3
4	3	3	0	3	2	2	0	2	1	3	2	2	0	3
5	1	2	1	2	1	1	0	2	1	2	0	2	2	1
6	0	2	0	2	0	1	0	2	0	2	1	1	0	1
7	0	2	0	2	0	1	0	2	2	0	0	1	0	1
8	2	0	2	0	1	0	0	2	0	0	0	1	0	1
9	0	0	0	0	0	0	1	1	0	0	1	0	0	1
10	0	0	0	0	0	0	0	1	0	0	0	0	0	1
11	0	0	0	0	0	0	0	1	0	0	0	0	0	1
12	0	0	0	0	0	0	1	0	0	0	0	0	0	1
13	0	0	0	0	0	0	0	0	0	0	0	0	0	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	1
15	0	0	0	0	0	0	0	0	0	0	0	0	1	0
16+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	16	42	16	35	16	35	16	39	16	38	16	35	16	36

APPENDIX 12

Negative Binomial Distribution

Low Density Results Combined - Time Interval Analysis

TABLE 2.

Week No.	\bar{x}	s^2	\hat{K}_1
5	2.6250	7.5833	1.3897
6	2.1875	7.0958	0.9749
7	2.1875	5.0958	1.6453
8	2.4375	11.1958	0.6784
9	2.3750	5.3167	1.9175
10	2.1875	6.1625	1.2038
11/12	2.2500	14.4667	0.4144

TABLE 3.

Week No.	K	p	q	R
5	0.8430	3.1139	4.1139	0.7569
6	0.8356	2.6179	3.6179	0.7236
7	1.4057	1.5562	2.5562	0.6088
8	0.9580	2.5444	3.5444	0.7179
9	1.9572	1.2135	2.2135	0.5482
10	1.3829	1.5818	2.5818	0.6127
11/12	0.4746	4.7408	5.7408	0.8258

APPENDIX 12

Negative Binomial Distribution

Low Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(1) Week 5

In (4)

$$K_1 = 1.4000; Z_1 = 15.9858557 - 16.8968428 = -0.9109871$$

$$K_2 = 1.3000; Z_2 = 16.8233769 - 17.6800334 = -0.8566565$$

$$K_3 = 1.0000; Z_3 = 20.1357143 - 20.6056686 = -0.4699543$$

$$K_4 = 0.7000; Z_4 = 25.7118393 - 24.9303139 = 0.7815254$$

Interpolation for $Z = 0$ between Z_3 and Z_4 :

$$K_5 = 0.7000 + \frac{0.3000 (0.7815254)}{0.7815254 + 0.4699543} = 0.8873443$$

$$\approx 0.8873$$

In (4)

$$K_5 = 0.8873; Z_5 = 21.8506170 - 22.0134917 = -0.1573217$$

$$K_6 = 0.8800; Z_6 = 21.9744349 - 22.1123823 = -0.1379474$$

$$K_7 = 0.8000; Z_7 = 23.4564457 - 23.2679204 = 0.1885253$$

Interpolation for $Z = 0$ between Z_6 and Z_7 :

$$K_8 = 0.8000 + \frac{0.0800 (0.1885253)}{0.1885253 + 0.1379474} = 0.8461969$$

$$\approx 0.8462$$

In (4)

$$K_8 = 0.8462; Z_8 = 22.5713395 - 22.5839983 = -0.0126588$$

$$K_9 = 0.8400; Z_9 = 22.6852678 - 22.6730563 = 0.0122115$$

Interpolation for $Z = 0$ between Z_8 and Z_9 :

APPENDIX 12

$$K_{10} = 0.8400 + \frac{0.0062 (0.0122115)}{0.0122115 + 0.0126588} = 0.8430442$$

$$\underline{\underline{0.8430}}$$

In (4)

$$K_{10} = 0.8430; Z_{10} = 22.6299624 - 22.6298621 = 0.0001003$$

$$\underline{\underline{0}}$$

$$\therefore K_2 = 0.8430$$

APPENDIX 12

Negative Binomial Distribution

Low Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(2) Week 6

In (4)

$$K_1 = 1.0000; Z_1 = 18.0523809 - 18.5477906 = -0.4954097$$

$$K_2 = 0.9000; Z_2 = 19.4907419 - 19.7235555 = -0.2328136$$

$$K_3 = 0.8000; Z_3 = 21.2384006 - 21.0812875 = 0.1571131$$

Interpolation for $Z = 0$ between Z_2 and Z_3 :

$$K_4 = 0.8000 + \frac{0.1000 (0.1571131)}{0.1571131 + 0.2328136} = 0.8402929$$

$$\approx 0.8403$$

In (4)

$$K_4 = 0.8403; Z_4 = 20.4902312 - 20.5093215 = -0.0190903$$

$$K_5 = 0.8400; Z_5 = 20.4955671 - 20.5134493 = -0.0178822$$

$$K_6 = 0.8300; Z_6 = 20.6753677 - 20.6521320 = 0.0232357$$

Interpolation for $Z = 0$ between Z_5 and Z_6 :

$$K_7 = 0.8300 + \frac{0.0100 (0.0232357)}{0.0232357 + 0.0178222} = 0.8356509$$

$$\approx 0.8357$$

In (4)

$$K_7 = 0.8357; Z_7 = 20.5724183 - 20.5728233 = -0.0004050$$

$$K_8 = 0.8350; Z_8 = 20.5849947 - 20.5825257 = 0.002469$$

Interpolation for $Z = 0$ between Z_7 and Z_8 :

$$K_9 = 0.8350 + \frac{0.0007 (0.002469)}{0.002469 + 0.000405} = 0.8356014$$

$$\approx 0.8356$$

APPENDIX 12

In (4)

$$K_9 = 0.8356; \quad Z_9 = 20.5742138 - 20.5742087 = 0.0000051$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 0.8356$$

APPENDIX 12

Negative Binomial Distribution

Low Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(3) Week 7

In (4)

$$K_1 = 1.6000; Z_1 = 13.6292864 - 13.7872407 = -0.1579543$$

$$K_2 = 1.5000; Z_2 = 14.3049708 - 14.3917378 = -0.0867670$$

$$K_3 = 1.4000; Z_3 = 15.0617428 - 15.0557335 = 0.0060093$$

Interpolation for $Z = 0$ between Z_2 and Z_3 :

$$K_4 = 1.4000 + \frac{0.1000 (0.0060093)}{0.0060093 + 0.0867670} = 1.4064772$$

$$\approx 1.4065$$

In (4)

$$K_4 = 1.4065; Z_4 = 15.0097802 - 15.0105830 = -0.0008028$$

$$K_5 = 1.4050; Z_5 = 15.0217353 - 15.0209766 = 0.0007587$$

Interpolation for $Z = 0$ between Z_5 and Z_6 :

$$K_6 = 1.4050 + \frac{0.0015 (0.0007587)}{0.0007587 + 0.0008028} = 1.4057288$$

$$\approx 1.4057$$

In (4)

$$K_6 = 1.4057; Z_6 = 15.0161536 - 15.0161243 = 0.0000293$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 1.4057$$

APPENDIX 12

Negative Binomial Distribution

Low Density Tanks Combined - Time Interval Analysis

Calculation for \hat{K}_2

(4) Week 8

In (4)

$$K_1 = 0.7000; Z_1 = 25.7173242 - 24.0016200 = 1.7157042$$

$$K_2 = 1.0000; Z_2 = 19.5988456 - 19.7559114 = -0.1570658$$

$$K_3 = 0.9000; Z_3 = 21.2276624 - 20.9693206 = 0.2583418$$

Interpolation for $Z = 0$ between Z_2 and Z_3 :

$$K_4 = 0.9000 + \frac{0.1000 (0.2583418)}{0.2583418 + 0.1570658} = 0.9621899 \approx 0.9622$$

In (4)

$$K_4 = 0.9622; Z_4 = 20.1788980 - 20.1955223 = -0.0166243$$

$$K_5 = 0.9500; Z_5 = 20.3748937 - 20.3421677 = 0.0327260$$

Interpolation for $Z = 0$ between Z_4 and Z_5 :

$$K_6 = 0.9500 + \frac{0.0122 (0.0327260)}{0.0327260 + 0.0166243} = 0.9580903 \approx 0.9581$$

In (4)

$$K_6 = 0.9581; Z_6 = 20.2442674 - 20.2445376 = -0.0002702$$

$$K_7 = 0.9570; Z_7 = 20.2618907 - 20.2577338 = 0.0041569$$

Interpolation for $Z = 0$ between Z_6 and Z_7 :

$$K_8 = 0.9570 + \frac{0.0011 (0.0041569)}{0.0041569 + 0.0002702} = 0.9580328 \approx 0.9580$$

In (4)

$$K_8 = 0.9580; Z_8 = 20.2458680 - 20.2457364 = 0.0001316$$

 ≈ 0

$$\therefore \hat{K}_2 = 0.9580$$

APPENDIX 12

Negative Binomial Distribution

Low Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(5) Week 9

In (4)

$$K_1 = 2.0000; Z_1 = 12.5023809 - 12.5241494 = -0.0217685$$

$$K_2 = 1.9000; Z_2 = 13.0070082 - 12.9748835 = 0.0321247$$

Interpolation for $Z = 0$ between Z_1 and Z_2 :

$$K_3 = 1.900 + \frac{0.1000 (0.0321247)}{0.0321247 + 0.0217685} = 1.9596081 \approx 1.9596$$

In (4)

$$K_3 = 1.9596; Z_3 = 12.7009274 - 12.7022234 = -0.0012960$$

$$K_4 = 1.9500; Z_4 = 12.7491430 - 12.7453243 = 0.0038187$$

Interpolation for $Z = 0$ between Z_3 and Z_4 :

$$K_5 = 1.9500 + \frac{0.0096 (0.0038187)}{0.0038187 + 0.0012960} = 1.9571675 \approx 1.9572$$

In (4)

$$K_5 = 1.9572; Z_5 = 12.7129432 - 12.7129699 = -0.0000267$$

 ≈ 0

$$\therefore \hat{K}_2 = 1.9572$$

APPENDIX 12

Negative Binomial Distribution

Low Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(6) Week 10

In (4)

$$K_1 = 1.2000; Z_1 = 16.9154764 - 16.6043301 = 0.3111463$$

$$K_2 = 1.3000; Z_2 = 15.9111978 - 15.7891341 = 0.1220637$$

$$K_3 = 1.4000; Z_3 = 15.0337595 - 15.0557335 = -0.0219740$$

Interpolation for $Z = 0$ between Z_2 and Z_3 :

$$K_5 = 1.3000 + \frac{0.1000 (0.1220637)}{0.1220637 + 0.0219740} = 1.3847443 \approx 1.3847$$

In (4)

$$K_4 = 1.3847; Z_4 = 15.1608165 - 15.1631703 = -0.0023538$$

$$K_5 = 1.3800; Z_5 = 15.2003404 - 15.1965051 = 0.0038353$$

Interpolation for $Z = 0$ between Z_4 and Z_5 :

$$K_6 = 1.3800 + \frac{0.0047 (0.0038353)}{0.0038353 + 0.0023538} = 1.3829125 \approx 1.3829$$

In (4)

$$K_6 = 1.3829; Z_6 = 15.1759256 - 15.1759183 = 0.0000073$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 1.3829$$

APPENDIX 12

Negative Binomial Distribution

Low Density Tanks Combined - Time Interval Analysis

Calculations for \hat{K}_2

(7) Weeks 11/12

In (4)

$$K_1 = 0.4000; \quad Z_1 = 31.8414283 - 30.2536059 = 1.5877693$$

$$K_2 = 0.5000; \quad Z_2 = 26.8876183 - 27.2759695 = -0.3883512$$

Interpolation for $Z = 0$ between Z_1 and Z_2 :

$$K_3 = 0.4000 + \frac{0.1000 (1.5877693)}{1.5877693 + 0.3883512} = 0.4803478 \approx 0.4803$$

In (4)

$$K_3 = 0.4803; \quad Z_3 = 27.7111237 - 27.8040938 = -0.0929701$$

$$K_4 = 0.4800; \quad Z_4 = 27.7241479 - 27.8123326 = -0.0881847$$

$$K_5 = 0.4700; \quad Z_5 = 28.1671074 - 28.0904714 = 0.0766360$$

Interpolation for $Z = 0$ between Z_4 and Z_5 :

$$K_6 = 0.4700 + \frac{0.0100 (0.0766360)}{0.0766360 + 0.0881847} = 0.4746497 \approx 0.4746$$

In (4)

$$K_6 = 0.4746; \quad Z_6 = 27.9611942 - 27.9616730 = -0.0004788 \approx 0$$

$$\therefore \hat{K}_2 = 0.4746$$

APPENDIX 13

Negative Binomial Distribution

Density A and Density B Results - Overall Frequency Comparison

Calculations for Method of Maximum Likelihood

Observed frequency distributions are shown in Table 1.

(1) Estimation of K

(a) Initial Estimate (\hat{K}_1)

\bar{x} from (1), s^2 from (2) and \hat{K}_1 from (3) are shown in Table 2.

(b) Final Estimate (\hat{K}_2)

Ax is calculated from (5) and shown in Table I.

The calculations for \hat{K}_2 using (4) are shown on pages 231-232.

(2) Calculation of Expected Frequencies (\emptyset)

The estimated values of K plus p from (6), q from (7) and R from (8) are shown for each density in Table 3. The expected frequencies obtained from (9), (10) and (11) are shown in Table 4.

APPENDIX 13

TOTAL RESULTS

TABLE 1. Accumulated Frequencies

x	Density A		Density B	
	f	Ax	f	Ax
0	166	242	35	77
1	82	160	19	58
2	50	110	22	36
3	32	78	10	26
4	25	53	8	18
5	22	31	6	12
6	12	19	1	11
7	5	14	2	9
8	6	8	5	4
9	2	6	2	2
10	2	4	0	2
11	0	4	0	2
12	1	3	1	1
13	1	2	0	1
14	1	1	0	1
15	0	1	1	0
16	0	1	0	0
17	1	0	0	0
18+	0	0	0	0
Total	408	737	112	260

APPENDIX 13

Negative Binomial Distribution

Overall Comparison - Density A and Density B

TABLE 2.

	Density A	Density B
\bar{x}	1.8064	2.3214
s^2	5.9206	7.7156
\hat{K}_1	0.7931	0.9990

TABLE 3.

	Density A	Density B
K	0.7385	0.9792
p	2.4460	2.3707
q	3.4460	3.3707
R	0.7098	0.7033

APPENDIX 13

Negative Binomial Distribution

High and Low Density Tanks - Combined Comparison

Calculations for \hat{K}_2 - High Density (A)

In (4)

$$K_1 = 0.8000; \quad Z_1 = 474.9767768 - 481.8943121 = -6.9175353$$

$$K_2 = 0.7000; \quad Z_2 = 525.8505661 - 520.4131410 = 5.4374251$$

Interpolation for $Z = 0$ between Z_1 and Z_2 :

$$K_3 = 0.7000 + \frac{0.1000 (5.4374251)}{5.4374251 + 6.9175353} = 0.7440101 \approx 0.7440$$

In (4)

$$K_3 = 0.7440; \quad Z_3 = 501.9424094 - 502.6414971 = -0.6990877$$

$$K_4 = 0.7400; \quad Z_4 = 504.0092130 - 504.2005614 = -0.1913484$$

$$K_5 = 0.7370; \quad Z_5 = 505.5726376 - 505.3770154 = 0.1956222$$

Interpolation for $Z = 0$ between Z_4 and Z_5 :

$$K_6 = 0.7370 + \frac{0.0030 (0.1956222)}{0.1956222 + 0.1913484} = 0.7385166 \approx 0.7385$$

In (4)

$$K_6 = 0.7385; \quad Z_6 = 504.7894877 - 504.7880176 = 0.0014701$$

$$\approx 0$$

$$\therefore \hat{K}_2 = 0.7385$$

APPENDIX 13

Negative Binomial Distribution

High and Low Density Tanks - Combined Comparison

Calculations for \hat{k}_2 - Low Density (B)

In (4)

$$K_1 = 1.000; Z_1 = 134.0043762 - 134.4432746 = -0.4388984$$

$$K_2 = 0.9900; Z_2 = 134.9996703 - 135.2311964 = -0.2315261$$

$$K_3 = 0.9700; Z_3 = 137.0440213 - 136.8384879 = 0.2055334$$

Interpolation for $Z = 0$ between Z_2 and Z_3

$$K_4 = 0.9700 + \frac{0.0200 (0.2055334)}{0.2055334 + 0.231526} = 0.9794053 \approx 0.9794$$

In (4)

$$K_4 = 0.9794; Z_4 = 136.0740712 - 136.0777603 = -0.0036892$$

$$K_5 = 0.9790; Z_5 = 136.1150132 - 136.1099387 = 0.0050745$$

Interpolation for $Z = 0$ between Z_4 and Z_5

$$K_6 = 0.9790 + \frac{0.0004 (0.0050745)}{0.0050745 + 0.0036892} = 0.9792316 \approx 0.9792$$

In (4)

$$K_6 = 0.9792; Z_6 = 136.0945385 - 136.0938474 = 0.0006911$$

 ≈ 0

$$\therefore \hat{k}_2 = 0.9792$$

APPENDIX 13

TABLE 4.

x	Density A		Density B	
	f	ϕ	f	ϕ
0	166	163.63	35	34.08
1	82	85.77	19	23.47
2	50	52.92	22	16.33
3	32	34.29	10	11.41
4	25	22.75	8	7.98
5	22	15.30	6	5.59
6	12	10.39	1	3.92
7	5	7.10	2	2.75
8	6	4.87	5	1.93
9	2	3.36	2	1.35
10	2	2.32	0	0.95
11	0	1.61	0	0.67
12	1	1.12	1	0.47
13	1	0.78	0	0.33
14	1	0.54	0	0.23
15	0	0.38	1	0.16
16	0	0.26	0	} 0.38
17	1	0.18	0	
18+	0	0.43	0	
Total	408	408.00	112	112.00

APPENDIX 14

Interval in days	Control fish		
3	7	9	16
6	1	35	36
9	1	35	36
12	6	19	25
15	<u>2</u>	<u>38</u>	<u>40</u>
	<u>17</u>	<u>136</u>	<u>153</u>

(1) Grand total = 153

(2) Sum of squared observations = 4427.00

(3) Sum of squared column totals divided by sample size of a column = $\frac{17^2 + 136^2}{5} = 3757.00$

(4) Sum of squared row totals divided by sample size of a row
 $= \frac{16^2 + \dots + 40^2}{2} = \frac{5073}{2}$
 $= 2536.50$

(5) Grand total squared and divided by total sample size
 $= \text{correction term} = \frac{153^2}{10}$
 $= 2340.90$

(6) SS total = (2) - (5) = 4427 - 2340.90 = 2086.10

(7) SSA (SS of columns) = (3) - (5) = 3757 - 2340.90
 $= 1416.1$

(8) SSB (SS of rows) = (4) - (5) = 2536.5 - 2340.90
 $= 195.6$

(9) SS error = (6) - (7) - (8) = 2086.1 - 1416.1 - 195.6
 $= 474.4$

APPENDIX 14

ANOVA TABLE

Source of variation	df	ss	ms	F
A (columns)	1	1416.10	1416.10	11.94
B (rows)	4	195.60	48.90	0.41
Error	4	474.40	118.60	
Total	9			

$$F_{0.05}(1,4) = 7.71$$

F was highly significant.

APPENDIX 15

Number of days	Control	Treated	
11	29	57	86
12	11	196	207
13	3	196	199
14	<u>16</u>	<u>187</u>	<u>203</u>
ΣY	<u>59</u>	<u>636</u>	<u>695</u>

- (1) Grand total = 695
- (2) Sum of squared observations = 116277
- (3) Sum of squared column totals divided by the sample size of a column = $\frac{59^2}{4} + \frac{636^2}{4} = \frac{3481}{4} + \frac{404496}{4} = 101994.25$
- (4) Sum of squared row totals divided by sample size of a row = $\frac{86^2}{2} + \dots + \frac{203^2}{2} = \frac{131055}{2} = 65527.5$
- (5) Grand total squared and divided by the total sample size = correction term = $\frac{(1)^2}{ab} = \frac{695^2}{8} = 60378.125$
- (6) SS total = (2) - (5) = 116277 - 60378.125 = 55898.875
- (7) SSA (SS of columns) = (3) - (5) = 101994.25 - 60378.125 = 41616.125
- (8) SSB (SS of rows) = (4) - (5) = 65527.5 - 60378.125 = 5149.375
- (9) SS error = 55898.875 - 41616.125 - 5149.375 = 9133.375

APPENDIX 15

ANOVA TABLE

Source of variation	df	ss	ms	F
$\bar{y}_A - \bar{y}_A$ (column)	1	41616.125	41616.125	13.67
$\bar{y}_B - \bar{y}_B$ (rows)	3	5149.375	1716.4583	0.563
$\bar{y} - \bar{y}_A - \bar{y}_B + \bar{y}$ (error)	3	9133.375	3044.458	
$\bar{y} - \bar{y}$ total	7			

$$F_{.05(1,3)} = 10.1$$

∴ F was significant.

There was a significant difference between the controls and the treated groups.

REFERENCES

- ANDERSON, R.M. (1974): An analysis of the influence of host morphometric features upon the population dynamics of Diplozoon paradoxum (Nordmann, 1832). J. Anim. Ecol. 43: 873-887.
- ANDERSON, R.M. (1978): Population dynamics of snail infection by miracidia. Parasitology 77: 201-224.
- ANDERSON, R.M. (1979): The influence of parasitic infection on the dynamics of host population growth. In Population Dynamics (ed. R.M. Anderson, B.D. Turner and R.A. Taylor). British Ecological Society Symposium. Oxford: Blackwell Scientific Publications.
- ANSCOMBE, F.J. (1950): Sampling theory of the negative binomial and logarithmic series distributions. Biometrika 37: 358-382.
- BATCHELOR, J.R. (1971): Hormonal control of antibody formation in "Regulation of the Antibody Response" (edited by Bernhard Cinader) pp. 276-293. Charles C. Thomas - Publisher.
- BAUER, O.N. and NIKOLSKAYA, N.P. (1954): Dactylogyrus solidus Akhmerov its biology, development and significance in fish breeding (en. Russe). Trudy Probl. Temat. Sovestich Zool. Inst. 4: 99-109.
- BAUER, O.N., MUSSELIUS, V.A. and STRELKOV, Y.A. (1973): Diseases of Pond Fishes Jerusalem: Israel Programme for Scientific Translation.
- BLISS, C.I. and FISHER, R.A. (1953): Fitting the negative binomial distribution to biological data and note on the efficient fitting of the negative binomial. Biometrics 9: 176-200.
- BOVET, J. (1967): Contribution à la morphologie et la biologie de Diplozoon paradoxum v Nordmann, 1832. Bull. Soc. Neuchatel Sci. Nat. 90: 63-159.
- BRADLEY, D.J. (1974): Stability in host-parasite systems. In: Ecological Stability, M.B. Usher and M.H. Williamson (eds.) Chapman and Hall, London (196 pp.).
- BURN, P.R. (1980): Density dependent regulation of a fish trematode population. J. Parasitol. 66(1): 173-174.
- BYCHOWSKY, B.E. (1933): Die Bedeutung der monogenetischen Trematoden für die Erforschung der systematischen Beziehungen der Karpfenfische. Zool. Anz. 102: 243-251.
- BYCHOWSKY, B.E. (1957): Monogenetic Trematodes, their Classification and Phylogeny. Moscow: Leningrad. Academy of Sciences, U.S.S.R. (in Russian); English

Translation by W.J. Hargis and P.C. Oustinoff (1961).
Washington: American Institute of Biological Sciences.
627 pp.

- BYCHOWSKY, B.E. and NAGIBINA, L.F. (1967): On intermediate hosts in monogeneans (Monogenoidea). Parazitoloqiya I (2): 117-123 (Russian text).
- BYKHOVSKAYA-PAVLOVSKAYA, et al. (1962): Key to parasites of freshwater fish of the U.S.S.R. Israel Program for Scientific Translations. Jerusalem 1964.
- CAMERON, T.W.M. (1964): Host-specificity and the evolution of helminthic parasites. Adv. Parasitol. 2: 1-34.
- CAMPBELL, W.C. (1963): Spontaneous cure in Trichurus muris infections in albino mice and its suppression by cortisone. J. Parasitol. 49: 628-632.
- CASSIE, R.M. (1962): Frequency distribution models in the ecology of plankton and other organisms. J. Anim. Ecol. 31: 65-92.
- CHUBB, J.C. (1979): Seasonal occurrence of helminths in freshwater fishes. Part II. Trematoda. Adv. Parasitol. 17: 141-313
- CONE, D.K. (1979): Hatching of Urocleidus adspetus Mueller, 1936 (Monogenea: Ancyrocephalinae). Can. J. Zool. 57: 833-837.
- CORBELL, M.J. (1975): The immune response in fish: A review. J. Fish. Biol. 7: 539-563.
- CROFTON, H.D. (1971): A quantitative approach to parasitism. Parasitology 62: 179-193.
- DAVIS, C. (1968): Mechanisms of hatching in aquatic invertebrate eggs. Oceanogr. Mar. Biol. Annu. Rev. 6: 325-376.
- DOGIEL, V.A. (1958): Ecology of the parasites of freshwater fishes. In: V.A. Dogiel, G.K. Petrushevski and Yu I. Polyanski (Ed.). Parasitology of Fishes pp. 1-47. Oliver and Boyd: Edinburgh and London.
- DOUGHERTY, T.F. (1959): Adrenal cortical control of lymphatic tissue mass. In The Study of Kinetics of Cellular Proliferation. Stohlmann, F. (Ed.) New York, Grune. p. 264.
- DUNCAN, B.L. (1973): Cichlidoqyrus sclerosus Paperna and Thurston from cultured Tilapia mossambica. Kalikasan, Philipp. J. Biol. 2: 154-158.
- ELLIS, A.E. (1978): The immunology of teleosts. In Fish Pathology. (Ed. R.J. Roberts). Bailliere Tindall-London.

- ERGENS, R. (1975): Contribution to the knowledge of the species Gyrodactylus aphyae Malmberg, 1957 and G. pannonicus Molnar 1968 (Monogenea: Gyrodactylidae). Vestn. Cesk. Spol. Zool. 39: 1-8.
- ESCH, G.W. (1967): Some effects of cortisone and sex on the biology of coenuriasis in laboratory mice and jack rabbits. Parasitology 57: 175-179.
- ESCH, G.W., GIBBONS, J.W., BOURQUE, J.E. (1975): An analysis of the relationship between stress and parasitism. Am. Midl. Nat. 93(2): 339-353.
- EUZET, L. and RAIBAUT, A. (1960): Le développement post-larvaire de Squalonchocotyle torpedinis, (Price, 1942). (Monogenea: Hexabothridae). Bull. Soc. Neuchatel Sci. Nat. 83: 101-108.
- EZZART, A.A., SHABANA, M.B. and FARGHALY, A.M. (1974): Studies on the blood characteristics of Tilapia zilli (Gervais). 1. Blood cells. J. Fish. Biol. 6: 1-12.
- FISHER, R.A. (1941): The negative binomial distribution. Annals of Eugenics 11: 182-187.
- FLETCHER, T.C. and GRANT, P.T. (1969): Immunoglobulins in the serum and mucus of plaice Pleuronectes platessa. Biochem. J. 115: 65.
- FLETCHER, T.C. and WHITE, A. (1973a): Lysozyme activity in the plaice Pleuronectes platessa (L). Experientia 29: 1283-1285.
- FLETCHER, T.C. and WHITE, A. (1973b): Antibody production in the plaice Pleuronectes platessa (L) after oral and parental immunization with Vibrio anguillarum antigens. Aquaculture 1: 417-428.
- FRANKLAND, H.M.T. (1955): The life history and bionomics of Diclidophora denticulata (Trematoda: Monogenea). Parasitology 45: 313-351.
- FRYER, G. and ILES, T.D. (1972): The cichlid fishes of the Great Lakes of Africa. Their biology and evolution. Oliver and Boyd, Edinburgh, 641 p.
- GLAESER, H.J. (1974): Six new species of Gyrodactylus wageneri group (Monogenea, Gyrodactylidae) with remarks on preparation, determination, terminology and host-specificity. Zool. Anz. 192 (1/2): 56-76.
- GUSSEV, A.V. (1973): Monogenea of freshwater fish in India and a world-wide analysis of the group. (Abstract of thesis). Leningrad, U.S.S.R. Zoologicheskii Institute Akademiya Nauk S.S.S.R. 31 pp. (Ru).

- GUSSEV, A.V. and KULEMINA, I.V. (1971): Analysis of the variability of characters, behaviour, and development cycle of Monogenea in relation to the age of the hosts. Parazitologiya 5 (4): 320-329.
- HA KY (1968): New species of monogeneans from freshwater fishes of North Vietnam. Part I. Parazitologiya 2 (4): 297-301.
- HA KY (1971): New species of monogeneans from freshwater fishes of North Vietnam. Part II. Parazitologiya 5: 429-440. (In Russian, English summary).
- HANEK, G. and FERNANDO, C.H. (1978a): Spatial distribution of gill parasites of Lepomis gibbosus (L) and Ambloplites rupestris (Raf.). Can. J. Zool. 56: 1235-1240.
- HANEK, G. and FERNANDO, C.H. (1978b): Seasonal dynamics and spatial distribution of Urocleidus ferox Mueller 1934, a gill parasite of Lepomis gibbosus (L). Can. J. Zool. 56: 1241-1243.
- HANEK, G. and FERNANDO, C.H. (1978c): The role of season, habitat, host age and sex on gill parasites of Ambloplites rupestris (Raf.). Can. J. Zool. 56: 1251-1253.
- HANEK, G. and FURTADO, J.I. (1973): Gyrodactylus fernandoi sp. n. (Monogenea: Gyrodactylinae) from Clarias batrachus L. in Malaysia. Zool. Anz. Leipzig 191 3/4: 196-198.
- HANEK, G., KOLMAN, M. and FERNANDO, C.H. (1974): Three new genera of Dactylogyridae (Monogenea) from freshwater fishes of Trinidad. J. Parasitol. 60: 911-913.
- HARGIS, W.J. Jnr. (1953a): Chlorotone as a trematode relaxer and its use in mass collecting techniques. J. Parasitol. 39 (2): 224-225.
- HARGIS, W.J. Jnr. (1953b): Monogenetic trematodes of Westhampton Lake fishes, III. Part 1. Comparative morphology of the species encountered. J. Parasitol. 39: 88-105.
- HARGIS, W.J. Jnr. (1953c): Monogenetic trematodes of Westhampton Lake fishes, III. Part 2. A discussion of host-specificity. Proc. Helminthol. Soc. Wash. 20: 98-104.
- HARGIS, W.J. Jnr. (1957): The host-specificity of monogenetic trematodes. Expt. Parasitol. 6: 610-625.
- HARGIS, W.J. Jnr. (1959): Systematic notes on the monogenetic trematodes. Proc. Helminthol. Soc. Wash. 26: 14-31.
- HARRIS, J.E. (1973): The immune response of dace Leuciscus leuciscus (L) to injected antigenic materials. J. Fish. Biol. 5: 261-276.
- HINES, R.S. and SPIRA, D.T. (1974): Ichthyophthiriasis in the mirror carp Cyprinus carpio. V. Acquired immunity. J. Fish. Biol. 6: 373-378.

- HIRSCH, R.P. (1980): Distribution of Polymorphus minutus among its intermediate hosts. Int. J. Parasitol. 10: 243-248.
- HOFF, C.C. (1943): Improving taxonomic literature. J. Tennessee Acad. Sci. 18: 201-203.
- HOULIHAN, D.F. and MACDONALD, S. (1979): Diclidophora merlangi and Entobdella solea: egg production and oxygen consumption at different O₂ partial pressures. Expt. Parasitol. 48: 109-117.
- IMADA, R. and MUROGA, K. (1978): Pseudodactylogyryus microrchis (Monogenea) on the gills of cultured eels. II. Oviposition, hatching and development on the host. Bull. Jpn. Soc. Sci. Fish. 44 (6): 571-576.
- IMADA, R., MUROGA, K. and HIRABAYASHI, S. (1976): Dactylogyryus extensus (Monogenoidea) from cultured carp in Japan. Bull. Jpn. Soc. Sci. Fish. 42 (2): 153-158. (In Japanese).
- IZIUMOVA, N.A. (1953a): Methods of studying the specificity of monogenetic trematodes (In Russian). Akad. Nauk. S.S.S.R. Tr. Zool. Inst. Akad. Nauk. S.S.S.R. 13: 155-159.
- IZIUMOVA, N.A. (1953b): The biology of Dactylogyryus vastator Nybelin and D. solidus Achmerov in carp farms. Avtoreferat. Dissertatsii. Leningrad. pp. 1-7.
- IZIUMOVA, N.A. (1956): Materials on the biology of Dactylogyryus vastator Nybelin (en. Russe). Parazitol. Sb. 16: 229-243.
- IZIUMOVA, N.A. (1958): Oxygen tension in water as one of the factors controlling the bionomics of Dactylogyryus solidus and D. vastator. Parazitol. Sb. 18: 295-303.
- JAIN, S.L. (1952a): Monogenea of Indian freshwater fishes. Haploclleidus gontius n. sp. (sub-family: Tetraonchinae) from the gills of Wallagonia attu (Bloch) from Lucknow. Indian J. Helminthol. 4 (2): 37-42.
- JAIN, S.L. (1952b): Monogenea of Indian freshwater fishes. II. Thaparocleidus wallagonius n.g. n. sp. (sub-family: Tetraonchinae) from the gills of Wallagonia attu (Bloch) from Lucknow. Indian J. Helminthol. 4 (2): 43-48.
- JAIN, S.L. (1955): Monogenea of Indian freshwater fishes. III. Urocleidus notopterus N. sp. (sub-family: Tetraonchinae) from the gills of Notopterus notopterus (Pallas) from Lucknow. Proc. Indian Acad. Sci. Sect. B 41 (1): 31-37.
- JAIN, S.L. (1957a): Mizelleus indicus n.g. n. sp. (sub-family: Tetraonchinae) from the gill filaments of Wallagonia attu (Bloch). Ann. Zool. (Agra.) 2 (5): 57-62.

- JAIN, S.L. (1957b): Monogenea of Indian freshwater fishes. V. Dactylogyrus multispiralis n. sp. (sub-family: Dactylogyridae) from the gill filaments of Silondia silondia (Ham.) from Lucknow. Indian Proc. Natl. Acad. Sci. Sect. B. 27 (1): 26-30.
- JAIN, S.L. (1957c): Monogenea of Indian freshwater fishes. VI. Three new trematodes belonging to the genus Neodactylogyrus Price, 1938 (family Dactylogyridae) from some freshwater fishes of Lucknow. Indian Proc. Natl. Acad. Sci. Indian Sect. B. 27 (1): 53-63.
- JAIN, S.L. (1958): Monogenea of Indian freshwater fishes. VII. Bifurcophaptor a new genus of freshwater Tetraonchinae from the gill filaments of two fishes from Lucknow. J. Parasitol. 44 (4): 388-394.
- JAMES, B.L. and LLEWELLYN, L.C. (1967): A quantitative analysis of helminth infestations in some passerine birds found dead on the Island of Skomer. J. Helminthol. 41 (1): 19-44.
- JAMES, B.L. and SRIVASTAVA, L.P. (1967): The occurrence of Podocotyle atomon (Rud, 1802) (Digenea), Bothriocephalus scorpii (Muller, 1776) (Cestoda), Contracaecum clavatum (Rud, 1809) (Nematoda) and Echinorhynchus gadi Zoega, in Muller, 1776 (Acanthocephala) in the five-bearded rockling, Onos mustelus (L.). J. Nat. Hist. 1: 363-372.
- JARROL, E.L. Jnr. (1979): Population biology of Bothriocephalus rarus Thomas (1937) in the red-spotted newt, Notophthalmus viridescens Raf. Parasitology 79: 183-193.
- KEARN, G.C. (1963): The egg, oncomiracidium and larval development of Entobdella soleae a monogenean skin parasite of the common sole. Parasitology 53: 435-447.
- KEARN, G.C. (1965): The biology of Leptocotyle minor, a skin parasite of the dogfish Scyliorhinus canicula. Parasitology 55: 473-480.
- KEARN, G.C. (1967a): The life cycles and larval development of some Acanthocotylids (Monogenea) from Plymouth rays. Parasitology 57: 157-167.
- KEARN, G.C. (1967b): Experiments on host-finding and host-specificity in the monogenean skin parasite Entobdella soleae. Parasitology 57: 585-605.
- KEARN, G.C. (1968a): The development of the adhesive organs of some diplectanid, tetraonchid and dactylogyrid gill parasites (Monogenea). Parasitology 58: 149-163.
- KEARN, G.C. (1968b): The larval development of Merizocotyle sp. a monocotylid monogenean from the nasal fossae of Raia undulata. Parasitology 58: 921-928.

- KEARN, G.C. (1970): The oncomiracidia of the monocotylid monogeneans Dictyocotyle coeliaca and Calicotyle kroyeri. Parasitology 61: 153-160.
- KEARN, G.C. (1973): An endogeneous circadian hatching rhythm in the monogenean skin parasite Entobdella soleae and its relationship to the activity rhythm of host (Solea solea). Parasitology 66: 101-122.
- KEARN, G.C. (1974a): The effects of fish skin mucus on hatching in the monogenean parasite Entobdella solea from the skin of the common sole Solea solea. Parasitology 68: 173-188.
- KEARN, G.C. (1974b): Nocturnal hatching in the monogenean skin parasite Entobdella hippoglossi from the halibut Hippoglossus hippoglossus. Parasitology 68: 161-172.
- KEARN, G.C. (1975): The mode of hatching of the monogenean Entobdella soleae a skin parasite of the common sole (Solea solea). Parasitology 71: 419-431.
- KEARN, G.C. (1976): Body surface of fishes: In "Ecological Aspects of Parasitology." Edited by C.R. Kennedy. North Holland Publishing Company, Amsterdam. pp. 185-208.
- KEARN, G.C. (1980): Light and gravity responses of the oncomiracidium of Entobdella soleae and their role in host location. Parasitology 81: 71-89.
- KEARN, G.C. (1981): Behaviour of oncomiracidia. Parasitology 82: 57-89.
- KEARN, G.C. (1982): Rapid hatching induced by light intensity reduction in the monogenean Entobdella diadema. J. Parasitol. 68 (1): 171-172.
- KEARN, G.C. and MACDONALD, S. (1976): The chemical nature of host hatching factors in the monogenean skin parasites Entobdella soleae and Acanthocotyle lobianchi. Int. J. Parasitol. 6: 457-466.
- KENNEDY, C.R. (1975): Ecological Animal Parasitology. Blackwell Sci. Pub. Oxford and Edinburgh.
- KENNEDY, C.R. (1977): The regulation of fish parasite populations. In "Regulation of Parasite Populations". Edited by Gerald, W. Esch, Academic Press, New York, pp. 63-109.
- KENNEDY, C.R. and LORD, D. (1982): Habitat specificity of the acanthocephalan Acanthocephalus clavula in eels Anguilla anguilla. J. Helminthol. 56 (2): 121-129.
- KEYMER, A.E. and ANDERSON, R.M. (1979): The dynamics of infection of Tribolium confusum by Hymenolepis diminuta: the influence of infective stage density and spatial

- distribution. Parasitology 79: 195-207.
- KEYMER, A.E. (1982): The dynamics of infection of Tribolium confusum by Hymenolepis diminuta: the influence of exposure time and host density. Parasitology, 84 (1): 157-166.
- KHALIL, L.F. (1964): On the biology of Macrogyrodactylus polypteri, Malmberg, 1956, a monogenetic trematode on Polypterus senegalus in the Sudan. J. Helminthol. 38: 219-222.
- KHOTENOVSKII, I. (1975): On the structure of eggs and larvae of some Diplozoon species. Parazitologiya 9(1): 17-27.
- KINGSTON, N., DILLION, W.A. and HARGIS, W.J. Jr. (1969): Studies on larval Monogenea of fishes from the Chesapeake Bay area. J. Parasitol. 55 (3): 544-558.
- KOLLMAN, A. (1972): Dactylogyrus vastator Nybelin 1924 (Trematoda: Monogenoidea) as a pathogen on the gills of carp (Cyprinus carpio L.). Part 3. Teil-zeitschrift für Wissenschaftliche Zoologie, Leipzig 185 (1/2): 1-54.
- KOMAROVA, T.I. (1976): Parasites of some fish larvae and fingerlings in the Kremenchung Reservoir. Hydrobiol. J. (Eng. Trans. Hidrobiol. Zh.) 12 (1): 68-72.
- KRITSKY, D.C. and THATCHER, V.E. (1974a): New monogenetic trematodes from freshwater fishes of Western Colombia with the proposal of Anacanthoroides new genus Dactylogyridae. Proc. Helminthol. Soc. Wash. 43 (2): 129-134.
- KRITSKY, D.C. and THATCHER, V.E. (1974b): Monogenetic trematodes (Monopisthocotylea: Dactylogyridae) from freshwater fishes of Colombia, South America. J. Helminthol. 48: 59-66.
- KTARI, M.H. (1969): Recherches sur l'anatomie et la biologie de Microcotyle salpae Parona et Perugia, 1890, parasite de Box Salpa L. (Téléostéen). Ann. Parasitol. Hum. Comp. 44: 425-439.
- KTARI, M.H. (1971): Recherches sur la reproduction et le développement de quelques monogenes (Polyopisthocotylea) parasites de poissons marins. Univ. Sci. natur. et technique, Languedoc, Montpellier: 1-327.
- LAMBERT, A. and MAILLARD, C.L. (1975): Répartition branchiale de deux monogènes: Diplectanum aequans (Wagener, 1857) Diesing 1858 et Diplectanum lauberi Lambert et Maillard, 1974 (Monogenea: Monopisthocotylea) parasites simultanés de Dicentrarchus labrax (Téléostéen). Ann. Parasitol. Hum. Comp. 50: 691-699.
- LEBEDEV, B.I. (1978): Some aspects of monogenean existence. Folia Parasitol. (Praha) 25: 131-136.

- LESTER, R.J.G. (1972): Attachment of Gyrodactylus to Gasterosteus and host response. J. Parasitol. 58 (4): 717-722.
- LESTER, R.J.G. and ADAMS, J.R. (1974a): A simple model of a Gyrodactylus population. Int. J. Parasitol. 4: 497-506.
- LESTER, R.J.G. and ADAMS, J.R. (1974b): Gyrodactylus alexanderi: reproduction, mortality and effect on its hosts Gasterosteus aculeatus. Can. J. Zool. 52: 827-833.
- LI, S.Y. and HSU, H.F. (1951): On the frequency distributions of parasitic helminths in their naturally infected hosts. J. Parasit. 37: 32-41.
- LJAIMAN, E.M. (1951): Influence of water temperature on the reproduction of the monogenetic trematode Dactylogyrus vastator. Trud. Mosk. Tekh. Inst. Ryb. Prom. Khoz. Im. Mikoyana 4: 190-196 (Russian text).
- LLEWELLYN, J. (1956): The host-specificity, micro-ecology, adhesive attitudes and comparative morphology of some trematode gill parasites. J. Marine Biol. Assoc. U.K. 35: 113-127.
- LLEWELLYN, J. (1957): Host-specificity in monogenetic trematodes. In: First symposium on host-specificity among parasites of vertebrates. Int. Union Biol. Sci. (B) No. 32: 199-212.
- LLEWELLYN, J. (1972): Behaviour of monogeneans. In "Behavioural Aspects of Parasite Transmission." (E.U. Canning and C.A. Wright, ed.) pp. 19-30. Supplement No. 1 to the Zoological Journal of the Linnean Society, 51.
- LLEWELLYN, J. and MACDONALD, S. (1980): Host-specificity and speciation in Diclidophoran (Monogenea) gill parasites of Trisopteran (Gadoid) fishes at Plymouth. J. Marine Biol. Assoc. U.K. 60: 73-79.
- LLEWELLYN, J. and OWEN, I.L. (1960): The attachment of the monogenean gill parasite Discocotyle sagittata Leuckart to the gills of Salmo trutta. Parasitology 50: 51-59.
- LUCKY, Z. (1973): Dactylogyrus nobilis Lan et Yu 1958 on the gills of Aristichthys nobilis acclimated in Czechoslovakia. Acta Vet. Brno. 42: 271-276.
- MACDONALD, S. (1974): Host skin mucus as a hatching stimulant in Acanthocotyle lobianchi a monogenean from the skin of Raja spp. Parasitology 68: 331-338.
- MACINNES, A.J. and VOGEL, M. (1970): Experiment and Techniques in Parasitology. W.H. Freeman and Company. San Francisco.

- MAMAYEV, YU. L. and PARUKHIN, A.M. (1975): Description of a new monogenean species Bivagina-Heterospina new species and remarks on the composition of the genus Bivagina. Hydrobiol. J. (Eng. Transl. Gidrobiol. Zh) 11 (2): 69-72.
- MANFRED, M. (1982): Fecundity and host utilization of the aphid parasite, Aphelinus semiflavus (Hymenoptera: Aphelinidae) at two host densities. Can. Entomol. 114 (8): 721-726.
- MARGOLIS, L., ESCH, G.W., HOLMES, J.C., KURIS, A.M. and SCHAD, G.A. (1982): The use of ecological terms in Parasitology. (Report of an ad hoc committee of the American Society of Parasitologists. J. Parasitol. 68 (1): 131-133.
- MATTISON, J. (1976): A study of monogenetic trematodes parasitic on Conesus Lake fish. Proc. Helminthol. Soc. Wash. 40 (2): 186-191.
- MAYS, M.A. and MILLER, G.C. (1973): A new genus and three new species of monogenetic trematodes from North Carolina centrachids with a redescription of Clavunculus-Okeechobiensis new combination. Proc. Helminthol. Soc. Wash. 40 (2): 186-191.
- MCANDREW, B.J. and MAJUMBAR, K.C. (1983): Tilapia stock identification using electrophoretic markers. Aquaculture 30: 249-261.
- MILLS, C.A., ANDERSON, R.M. and WHITFIELD, P.J. (1979): Density-dependent survival and reproduction within populations of the ectoparasitic digenean Transversotrema patialense on the fish host. J. Anim. Ecol. 48: 383-399.
- MIZELLE, J.D. and PRICE, C.E. (1964): Studies on monogenetic trematodes. XXVII. Dactylogyrid species with the proposal of Urocleidoides gen. n. J. Parasitol. 50 (4): 579-584.
- MOLNAR, K. (1971a): Studies on gill parasitosis of the grass carp Ctenopharyngodon idella caused by Dactylogyrus lamellatus Achmerow, 1952. I. Morphology and biology of Dactylogyrus lamellatus. Acta Vet. Acad. Sci. Hung. 21: 267-289.
- MOLNAR, K. (1971b): Studies on gill parasitosis of the grass carp Ctenopharyngodon idella caused by Dactylogyrus lamellatus Achmerow, 1952. II. Epizootiology. Acta. Vet. Acad. Sci. Hung. 21 (4): 361-375.
- MOLNAR, K. (1976): Observations on the incidence of Dactylogyrus vastator (Monogenea). Parasitol. Hung. 9: 35-36.
- MOLNAR, K., HANEK, G. and FERNANDO, C.H. (1974): Ancyrocephalids (Monogenea) from fresh water fishes of Trinidad. J. Parasitol. 60 (6): 914-920.

- MUELLER, J.F. (1934): Parasites of Oneida Lake fishes. Part 4. Additional notes on parasites of Oneida Lake fishes including descriptions of new species. Roosevelt Wild Life Annals 3: 335-373.
- MUELLER, J.F. (1936): Studies on North American Gyrodactyloidea. Trans. Am. Micros. Soc. 55 (1): 55-72.
- MULLIGAN, W. (1968): Immunity to intestinal helminths. The "self-cure" reaction. In "Immunity to Parasites." (Ed. by Angela E.R. Taylor). Blackwell Scientific Publications. Oxford and Edinburgh.
- MUSSELIUS, V.A. and PTASHUK, S.V. (1970): On the development and specificity of Dactylogyrus lamellatus (Monogeneoidea, Dactylogyridae). Parazitologiya 4: 125-132.
- MUSSELIUS, V.A., IVANOVA, N.S., MIRZOEVA, L.M. and VOLOVIK, S.V. (1973): Influence of some gill parasites on fish organism. Verh. Internat. Verein. Limnol. 18: 1713-1722.
- NAKATSUGAWA, T. and MUROGA, K. (1977): Seasonal changes in abundance of Dactylogyrus extensus and D. minutus (Monogenea on cultured carp). Fish Pathol. 12 (2): 99-104.
- NIGRELLI, R.F. and BREDER, C.M. (1934): The susceptibility and immunity of certain marine fishes to Epibdella melleni. J. Parasitol. 20: 259-269.
- OGAWA, K. and EGUSA, S. (1977a): The first record of Dactylogyrus minutus Kulweic 1927 (Monogenea: Dactylogyridae) from the reared carp (Cyprinus carpio) in Japan. Bull. Jpn. Soc. Sci. Fish. 43 (9): 1029-1034.
- OGAWA, K. and EGUSA, S. (1977b): Redescription of Heteraxine - Heterocerca (Monogenea: Heteraxinidae). Jpn. J. Parasitol. 26 (6): 388-396.
- OLIVER, G. (1976): Nouvelles observations sur la biologie et l'ecologie de quelques Diplectanidae (Monogenea: Monopisthocotylea) (en. Russe). In: Volume à la memoire de B.E. Bychowsky. Akad. Nauk. C.C.C.P. 34 (137): 104-109.
- OLIVIER, L. (1962): Studies on natural resistance to Taenia taeniaformis in mice. II. The effect of cortisone. J. Parasitol. 48: 758-762.
- OWEN, I.L. (1963): The attachment of the monogenean Diplozoon paradoxum to the gills of Rutilus rutilus L. Parasitology 53: 455-461.
- OWEN, I.L. (1970): The oncomiracidium of the monogenean Discocotyle sagittata. Parasitology 61: 279-292.

- O'ROURKE, F.J. (1961): Presence of blood antigens in fish mucus and its possible parasitological significance. Nature 189: 943.
- PALING, J.E. (1965): The population dynamics of the monogenean gill parasite Discocotyle sagittata Leuckart on Windermere trout, Salmo trutta, L. Parasitology 55: 667-694.
- PANDEY, R.K., R. SINGH, A. KUMAR, C.M. TRIPATHI and T.B. SINHA (1982): Bionomics of Trioxys indicus, an aphidiid parasitoid of Aphis craccivora. Behavioural activities of the parasitoid associated with its functional responses. Z. Angew. Entomol. 93 (2): 164-175.
- PAPERNA, I. (1959): Studies on monogenetic trematodes in Israel. I. Three species of monogenetic trematodes of reared carp. Bamidgeh 1: 51-67.
- PAPERNA, I. (1960a): Studies on monogenetic trematodes in Israel. II. Monogenetic trematodes of Cichlids. Bamidgeh 12 (1): 20-30.
- PAPERNA, I. (1960b): The influence of monogenetic trematodes on fish breeding economy. Bamidgeh 12: 54-55.
- PAPERNA, I. (1961): Studies on monogenetic trematodes in Israel. III. Monogenetic trematodes of the Cyprinidae and Clariidae of the Lake of Galilee. Bamidgeh 13: 14-29.
- PAPERNA, I. (1963a): Some observations on the biology and ecology of Dactylogyrus vastator in Israel. Bamidgeh 15 (1): 8-28.
- PAPERNA, I. (1963b): Dynamics of D. vastator Nybelin (Monogenea) population on the gills of carp fry in fish ponds. Bamidgeh 15 (2/3): 31-50.
- PAPERNA, I. (1964a): Adaptation of Dactylogyrus extensus (Mueller and Van Cleave, 1932) to ecological conditions of artificial ponds in Israel. J. Parasitol. 50 (1): 90-93.
- PAPERNA, I. (1964b): Competitive exclusion of Dactylogyrus extensus by D. vastator (Trematoda: Monogenea) on the gills of reared carp. J. Parasitol. 50 (1): 94-98.
- PAPERNA, I. (1964c): Host reaction to infestation of carp with Dactylogyrus vastator Nybelin, 1924 (Monogenea). Bamidgeh 16 (4): 129-141.
- PAPERNA, I. (1965): Monogenetic trematodes collected from freshwater fish in Southern Ghana. Bamidgeh 17: 107-111.
- PAPERNA, I. (1968): Monogenetic trematodes collected from freshwater fish in Ghana. Second report. Bamidgeh 20: 88-100.

- PAPERNA, I. (1969): Monogenea of the sub-family Heteronchocleidinae, Price 1968 (Dactylogyridae) from African freshwater fish. Parasitology 59 (1): 557-561.
- PAPERNA, I. (1973): New species of Monogenea (Vermea) from African freshwater fish. A preliminary report. Rev. Zool. Bot. Afr. 87 (3): 505-518.
- PAPERNA, I. and THURSTON, J.P. (1968): Monogenetic trematodes (Dactylogyridae) from fish in Uganda. Rev. Zool. Bot. Afr. 78: 284-294.
- PAPERNA, I. and THURSTON, J.P. (1969): Monogenetic trematodes collected from Cichlid fish in Uganda including the description of five new species of Cichlidogyrus. Rev. Zool. Bot. Afr. 79 (1/2): 15-33.
- PENNYCUICK, L. (1971): Frequency distributions of parasites in a population of three-spined sticklebacks, Gasterosteus aculeatus L., with particular reference to the negative binomial distribution. Parasitology 63: 389-406.
- PRICE, C.E. (1967): A revised definition of the monogenetic trematode genus Dactylogyrus with descriptions of four new species. Proc. Helminthol. Soc. Wash. 34 (2): 117-124.
- PRICE, C.E. (1968): A note on the monogenetic trematode genus Cichlidogyrus Paperna 1960. Bamidgeh 20: 100.
- PRICE, C.E. and ARAI, H.P. (1967): A proposed system of anatomy for freshwater Monogenea. Can. J. Zool. 45: 1283-1285.
- PRICE, C.E. and HENDERSON, A. (1969): Monogenean parasites of Mexican freshwater fishes. I. Introductory remarks with an account of the parasite genus Dactylogyrus Diesing, 1850. Ann. Inst. Biol. Univ. Nal. Auto'n Mexico Ser. Zool. 40 (2): 195-204.
- PRICE, C.E. and MCCLELLAN, E.S. (1969): The monogenean parasites of African fishes. IX. A new genus Gussevstrema, recovered from the gills of Terapon jarbua (Forsk.) from South Africa). Proc. Biol. Soc. Wash. 82: 171-176.
- PRICE, C.E. and MCMAHON, T.E. (1967): The monogenetic trematodes of North America freshwater fishes. Riv. Parassitol. 28 (3): 177-220.
- PRICE, C.E. and PIKE, T. (1969): The monogenean parasites of African fishes. VII. Dissolution of the family Protogyrodactylidae Johnston and Tiegs, 1922. Proc. Helminthol. Soc. Wash. 36 (2): 260-264.
- PRICE, C.E., PEEBLES, H.E. and BAMFORD, T. (1969). The monogenean parasites of African fishes. IV. Two new species from

- South African hosts. Rev. Zool. Bot. Afr. 79 (1/2): 117-124.
- PROST, M. (1963): Investigations on the development and pathogenicity of Dactylogyrus anchoratus (Duj. 1845) and D. extensus Mueller et v. Cleave, 1932 for breeding carps. Acta. Parasitol. Pol. 11: 17-47.
- RADHA, E. (1971): Some notes on the population dynamics of the monogenean gill parasite Gastrocotyle indica. Marine Bio. 8 (3): 213-219.
- REES, G. (1967): Pathogenesis of adult cestodes. Helm. Abst. 36: 1-23.
- REMLEY, L.W. (1942): Morphology and life history studies of Microcotyle spinicirrus Mac Callum 1918, a monogenetic trematode parasitic on the gills of Aplodinotus grunniens. Tr. Am. Micro. Soc. 61: 141-155.
- RIZVI, S.S.H. (1974): Monogenea of Pakistan fishes. Part 3. Dactylogyrus zulfikari new species and Dactylogyrus jaini from the gills of Barbus sarana (Ham.). Pak. J. Sci. Ind. Res. 17 (6): 215-216.
- ROBINSON, E.J. Jnr. (1961): Survival of Trichinella in stressed hosts. J. Parasitol. 47 (4, Sect. 2): 16-17.
- ROHDE, K. (1976): Marine Parasitology in Australia. Search 7: 477-482.
- ROHDE, K. (1977): A non-competitive mechanism responsible for restricting niches. Zool. Anz. 199 (3-4): 164-172.
- ROHDE, K. (1978a): Latitudinal differences in host-specificity of marine Monogenea and Digenea. Marine Bio. 47: 125-134.
- ROHDE, K. (1978b): Latitudinal gradients in species diversity and their causes. II. Marine parasitological evidence for a time hypothesis. Biol. Zentralbl. 97: 405-418.
- ROHDE, K. (1980): Host-specificity indices of parasites and their application. Experientia 36 (12): 1369-1371.
- ROWAN, W.B. (1956): The mode of hatching of the egg of Fasciola hepatica. Exp. Parasit. 5: 118-137.
- SARIG, S. (1968): A review of diseases and parasites of fishes in warm water ponds in the near East and Africa. FAO Fish Cult. Bull. 44 (5): 278-289.

- SARIG, S. (1971): Diseases of fishes: the prevention and treatment of diseases of warm water fishes under sub-tropical conditions, with special emphasis on intensive fish farming. In Diseases of Fishes (Eds. Snieszko, S.F. and Axelrod, H.R.) pp. 1-127. Neptune, New Jersey: T.F.H. Publications Inc.
- SCHWANCK, ERKKI (1977): The relation between territorial behaviour, food abundance and population density in juvenile Tilapia mariae. Zool. Revy. 39 (4): 101-110.
- SHEPHERD, A.M. (1962): The emergence of larvae from cysts in the genus Heterodera. 90 pp. Techn. Commun. Commonw. Bur. Helminth. No. 32.
- SHULMAN, S.S. (1958): Specificity of fish parasites. In: Parasitology of fishes (Ed. by Dogiel, V.A., Petrushevski, G.K. and Polyanski Yu. I.). Oliver and Boyd, Edinburgh and London. pp. 104-116.
- SLINN, D.J. (1963): Occurrence of Discocotyle sagittata on sea trout. Nature 197 (4864): 306.
- SMITH, J.D. (1976): Introduction to Animal Parasitology. Hodder and Stoughton, London, Sydney, Auckland, Toronto. 466 p. (Book).
- SOKAL, R.R. and ROHLF, F.J. (1973): Introduction to Biostatistics. W.H. Freeman and Company, San Francisco, 368 p.
- SRIVASTAVA, L.P. and JAMES, B.L. (1967): The morphology and occurrence of Gyrodactylus medius Katharina, 1894 (Monogenoidea) from Onos mustellus (L). J. Nat. Hist. 4: 481-489.
- STEWART, D.F. (1950a): Studies on resistance of sheep to infestation with Haemonchus contortus and Trichostrongylus spp. and on the immunological reactions of sheep exposed to infestation. I. The preparation of antigens for the complement fixation test and the reactivity of the biochemical functions of H. contortus. Aust. J. Agric. Res. 1: 285-300.
- STEWART, D.F. (1950b): Studies on resistance of sheep to infestation with Haemonchus contortus and Trichostrongylus spp. and on the immunological reactions of sheep exposed to infestation. II. The antibody response to infestation with H. contortus. Aust. J. Agric. Res. 1: 301-321.
- STEWART, D.F. (1950c): Studies on resistance of sheep to infestation with Haemonchus contortus and Trichostrongylus spp. and on the immunological reactions of sheep exposed to infestation. IV. The antibody response to natural infestation in grazing sheep and the "self-cure" phenomenon. Aust. J. Agric. Res. 1: 427-439.

- STEWART, D.F. (1953): Studies on resistance of sheep to infestation with Haemonchus contortus and Trichostrongylus spp. and on the immunological reactions of sheep exposed to infestation. V. The nature of the "self-cure" phenomenon. Aust. J. Agric. Res. 4: 100-117.
- STEWART, D.F. (1955): "Self-cure" in nematode infestations of sheep. Nature, Lond. 176: 1273.
- STOLL, N.R. (1929): Studies with the strongyloid nematode Haemonchus contortus. I. Acquired resistance of hosts under natural reinfection conditions out of doors. Amer. J. Hyg. 10: 384 - 418.
- STONE, G.E. and CAMERON, I.L. (1964): Methods for using Tetrahymena in studies of the normal cell cycle. Methods in Cell Physiology (Prescott. ed.). Academic Press.
- SUYDAM, E.L. (1971): The micro-ecology of three species of monogenetic trematodes of fishes from the Beaufort-Cape Hatteras area. Proc. Helm. Soc. Wash. 38: 240-246.
- THURSTON, J.P. (1970): The incidence of Monogenea and parasitic Crustacea on the gills of fish in Uganda. Rev. Zool. Bot. Afr. 82 (1-2): 111-130.
- ULMER, M.J. (1970): Site-finding behaviour in helminths in intermediate and definitive hosts. In Ecology and Physiology of Parasites. (Ed. A.M. Fallis). Toronto and Buffalo. Univ. of Toronto Press, pp. 123-159.
- VLADIMIROV, V.L. (1971): Immunity of fishes during dactylogyrosis. Parazitologiya 5 (1): 51-58. Transl. by Cambridge Scientific Abstract Maryland.
- WILES, M. (1968): The occurrence of Diplozoon paradoxum Nordmann 1832 (Trematoda: Monogenea) in certain waters of Northern England and its distribution on the gills of certain Cyprinidae. Parasitology 58: 61-70.
- WILLIAMS, C.B. (1964): Patterns in the Balance of Nature. London and New York Academic Press.
- WILSON, R.A. (1968): The hatching mechanism of the egg of Fasciola hepatica L. Parasitology 58: 78-89.
- WOOTEN, R. (1974): The spatial distribution of Dactylogyrus amphibothrium on the gills of ruffe Gymnocephalus cernua and its relation to the relative amounts of water passing over the parts of the gills. J. Helminthol. 48: 167-174.
- WUNDER, W. (1929): Die Dactylogyruskrankheit der Karpfenbrut, ihre Ursache und ihre Bekämpfung. Ztchr. Fischerei 27 (4): 511-545.

ZDĀRSKĀ, Z. (1976): Selective staining of opisthaptor sclerites
of some monogeneans. Folia Parasitol. (Prague) 23: 182.

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