A COMPARATIVE FIELD AND LABORATORY INVESTIGATION OF *TYPHLOCOELUM CUCUMERINUM* (DIGENEA: CYCLOCOELIDAE) IN VARIOUS DUCK AND SNAIL HOSTS

BY

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A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF PARASITOLOGY
McGILL UNIVERSITY
MONTREAL

JULY 1980

ABSTRACT

An extensive field survey of 12 duck species yielded two morphologically distinct populations of *Typhlocoelum* (Digenea: Cyclocoelidae). Studies on the variability of these two populations in conjunction with experimental cross infections provided conclusive evidence that they must be considered as subspecies of *Typhlocoelum cucumerinum*. A laboratory comparison of their survival strategies revealed that, in the definitive host, *Typhlocoelum cucumerinum cucumerinum* develops slowly, survives for an extended time and has a high fecundity, whereas *Typhlocoelum cucumerinum sisowi* develops more quickly, has a shorter longevity and a lower fecundity. In the intermediate host, *T. c. cucumerinum* develops more slowly than *T. c. sisowi*. The almost complete segregation of these two subspecies into dabbling and diving ducks in the field may be maintained by an ecological separation of the ducks as well as intrinsic differences between the two host-parasite associations.

ABREGE

Une étude extensive, sur le terrain, de 12 espèces de canards a permis de distinguer morphologiquement deux populations de *Typhlocoelum* (Digenea: Cyclocoelidae). L'étude de la variabilité de ces deux populations, conjuguée à des infestations croisées expérimentales a permis de conclure que ces deux populations constituent des sous-espèces de *Typhlocoelum cucumerinum*. En comparant expérimentalement leurs modes de survie, on a constaté que, chez l'hôte définitive, le *Typhlocoelum cucumerinum cucumerinum* se développe lentement, se maintient pendant un certain temps et se révèle très fécond, alors que le *Typhlocoelum cucumerinum sisowi* se développe plus rapidement, et que sa longévéité et sa fécondité sont moindres. Chez l'hôte intermédiaire, le *T. c. cucumerinum* se développe plus lentement que le *T. c. sisowi*. La ségrégation presque totale de ces deux sous-espèces que l'on retrouve, en milieu naturel, l'une chez les canards barboteurs, l'autre chez les canards plongeurs est peut être entretenue par une séparation écologique des canards et par des différences intrinsèques entre les deux tandoms hôte-parasite.

Suggested Short Title:

ASPECTS OF THE LIFE HISTORY OF *TYPHLOCOELUM CUCUMERINUM*

PREFACE

This research has evolved from a field survey undertaken to determine the distribution of *Typhlocoelum* (Digenea: Cyclocoelidae) in various species of ducks. Two morphological types of *Typhlocoelum* were obtained: the round-testis form occurred almost exclusively in dabbling ducks (genus *Anas*) whereas the lobed-testis form occurred almost exclusively in diving ducks (genus *Aythya*).

Morphological variability among parasites may arise from a variety of causes ranging from fundamental genetic differences through environmentally induced differences to artifactual variation due to muscle contraction and histological preparation. The present research has succeeded in partitioning the variability of *Typhlocoelum* among these three sources of variation and has clarified the taxonomic and biological relationship between the two forms. The readily apparent morphological distinctions in these parasites are manifestations of much more far-reaching ecological differences. The present study characterizes the life cycle strategies of the two forms with a view to gaining a clearer understanding of the role of the population in the evolution of parasitic organisms.

Due to the wide-ranging nature of this research, the results have been divided into a series of chapters. Each of these deals with a specific aspect of the research, and, as a consequence, has its own introduction, literature review, materials and methods, results and discussion. An introductory chapter, a general materials and methods and an overview serve to bind the chapters into a unified comparison of aspects of the biology of the two morphological forms of *Typhlocoelum* in various duck and snail hosts.

ACKNOWLEDGEMENTS

Throughout the course of this research, assistance has been obtained from a number of sources.

I would like to thank Dr. Rodger Titman (Department of Renewable Resources, Macdonald College), Mr. Paul DuPuis (Canadian Wildlife Service, Québec Branch), Marion Rigby and Leslie Rich for their assistance with bag checks in southern Québec.

The cooperation of duck hunters from southern Québec and the duck hunters and cleaners of the Delta Marsh in southern Manitoba was indispensable for the field survey of wild ducks.

Mr. Patrick Brown (University of Missouri), Mr. Bill Hohman, Mr. Michael Tome and Mr. Rod Sayler (Delta Waterfowl Research Station) and Mr. Jerry Serie (Northern Prairie Wildlife Research Center, Jamestown, North Dakota) provided additional duck tracheas.

Mr. Paul DuBowy (Delta Waterfowl Research Station) and Mr. Rick Wishart (University of Manitoba) provided duck tracheas, assisted in bag checks in Manitoba, and provided access to their unpublished data.

Mrs. Marilyn Koob (University of North Dakota) and Mr. Emilio Rangel (Texas A&M University) assisted me in the collection of snails from the Delta Marsh.

The following persons provided specimens of *Typhlocoelum*: Dr. C. Vaucher (Musée d'Histoire naturelle, Genève), Dr. D. Gibson (British Museum (Natural History), London, England), Dr. L.K. Khalil (Commonwealth Institute of Helminthology, St. Albans, England), Mrs. Mary Pritchard (Harold W. Manter Laboratory, University of Nebraska), Dr. Ralph Lichtenfels (Animal Parasitology Institute, Beltsville Agricultural Research Center, Beltsville, Maryland), Dr. Mary Beverley-Burton (University of Guelph), Dr. Murray Lankester (Lakehead University, Thunder Bay), Miss Neiva Lisete Schafranski (Universidade Federal de Minas Gerais, Brazil) and Dr. David Skead (Barberspan Ornithological Research Station, Transvaal, Republic of South Africa).

Dr. Carl Bursey (University of New Brunswick) did an electrophoretic analysis of *Typhlocoelum* specimens.

Mrs. M.F.I. Smith (National Museums of Canada) identified the snails used in this research.

Discussion on this research with Dr. Roy M. Anderson (Imperial College of Science and Technology, London, England) and Dr. Bruce Batt (Delta Waterfowl Research Station, Manitoba) were very helpful.

The support and cooperation of the staff and students of the Delta Waterfowl Research Station, Manitoba and the Institute of Parasitology, McGill University are gratefully acknowledged.

The Canadian Wildlife Service provided permits for collection of wild ducks and duck eggs.

Financial support for this research was provided by the Natural Sciences and Engineering Research Council of Canada (J.D. McL., A6979; M.E.R., A0069), the Formation de Chercheurs et d'Action Concertée du Ministère de l'Education du Québec, the Canadian National Sportsmen's Show (M.E.S.) and the North American Wildlife Foundation through the Delta Waterfowl Research Station, Manitoba. Personal support from NSERC Postgraduate Scholarships is also acknowledged.

My two supervisors, Dr. J.D. McLaughlin, Concordia University and Dr. Manfred E. Rau, Institute of Parasitology, McGill University, have supported me throughout this study. Dr. McLaughlin has spent many hours in the field collecting ducks for this project and has taught me a great deal about field research. Dr. Rau has contributed enormously to the development of my conceptual understanding of parasites. I am particularly grateful for the time they have contributed during the preparation of this dissertation, and the tremendous patience they have shown.

Finally, I would like to thank my husband, David Gordon, who, in addition to assisting with the statistical analyses of the data, has provided the encouragement and support that has made this research all the more rewarding.

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LIST OF ABBREVIATIONS

NUMERICAL VARIABLES:

ATA	Distance from anterior testis to pharynx
ATL	Anterior testis length
ATW	Anterior testis width
BL	Body length
BW	Body width
CL	Cirrus sac length
EGGA	Anterior extent of eggs in the uterus
EYEA	Posterior extent of eyespots in the uterus
NID	Number of inner diverticula on the intestinal caeca
OA	Distance from ovary to pharynx
OES	Length of oesophagus
OL	Ovary length
OSL	Oral sucker length
OSW	Oral sucker width
OW	Ovary width
PA	Distance from pharynx to anterior of body
PL	Pharynx length
PTA	Distance from posterior testis to pharynx
PTL	Posterior testis length
PTW	Posterior testis width
PW	Pharynx width
SRA	Distance from seminal receptacle to pharynx
SRL	Seminal receptacle length
SRW	Seminal receptacle width
VSA	Distance from ventral sucker to pharynx
VSL	Ventral sucker length
VSW	Ventral sucker width

CHARACTER VARIABLES:

AT	Anterior testis shape
CS	Position of cirrus sac
EGG	Presence of eggs in uterus

EYE	Presence of eyespots in eggs
GP	Position of genital pore
ID	Presence of inner diverticula
MAT	Maturity
PT	Posterior testis shape
UBT	Number of uterine loops separating testes
UG	Position of uterus relative to the intestine
VENSUR	Topography of the ventral surface
VITA	Anterior extent of vitellaria
VITP	Posterior extent of vitellaria

DUCK SPECIES:

BLA	American Black Duck	*Anas rubripes* Brewster
BOSCHAS	(Domestic Mallard)	*Anas boschas* (Linnaeus)
BWT	Blue-winged Teal	*Anas discors* Linnaeus
BUF	Bufflehead	*Bucephala albeola* (Linnaeus)
CAN	Canvasback	*Aythya valisineria* (Wilson)
DOM	Domestic Duck	
DOM MAL	Domestic Mallard	
DUC	Duck	
GAD	Gadwall	*Anas strepera* Linnaeus
GOL	Common Goldeneye	*Bucephala clangula* (Linnaeus)
GSC	Greater Scaup	*Aythya marila* (Linnaeus)
GWT	Green-winged Teal	*Anas crecca* Linnaeus
LSC	Lesser Scaup	*Aythya affinis* (Eyton)
MAL	Mallard	*Anas platyrhynchos* Linnaeus
MUS	Muscovy Duck	*Cairina moschata* (Linnaeus)
NYROCA	Common White-eye	*Aythya nyroca* (Gmelin)
OLD	Oldsquaw	*Clangula hyemalis* (Linnaeus)
PIN	Northern Pintail	*Anas acuta* Linnaeus
RBT	Red-billed Pintail	*Anas erythrorhyncha* Gmelin
RED	Redhead	*Aythya americana* (Eyton)
RIN	Ring-necked Duck	*Aythya collaris* (Donovan)
RUD	Ruddy Duck	*Oxyura jamaicensis* (Gmelin)
SC	Scaup	

SHO	Northern Shoveler	*Anas clypeata* Linnaeus
SOP	African Pochard	*Aythya erythropthalma* (Eyton)
SSC	Surf Scoter	*Melanitta perspicillata* (Linnaeus)
SWA	Swan	
TUF	Tufted Duck	*Aythya fuligula* (Linnaeus)
WIG	American Wigeon	*Anas americana* Gmelin
WOO	North American Wood Duck	*Aix sponsa* Linnaeus
WSC	White-winged Scoter	*Melanitta deglandi* (Bonaparte)
YBT	Yellow-billed Duck	*Anas undulata* DuBois

TO DAVID

CHAPTER I. INTRODUCTION

The survival of parasitic organisms has traditionally been equated with a balance between the overall biotic potential of the species and the environmental resistance that it encounters in completing its life cycle. Parasitologists have long had an intuitive understanding of these two phenomena. Thus, it is generally accepted that the high egg-producing capacity of adult Digenea coupled with a tremendous asexual multiplication of larval stages provide sufficient individuals to successfully overcome problems of transmission between hosts and survival within hosts.

During the past ten years, a theoretical assessment of parasite survival has also emerged. This approach recognizes that the biotic potential of any species can be quantified in terms of rates of egg and larval production, and that environmental resistance can be measured in terms of survival and death rates (Anderson *et al.*, 1977). These parameters vary depending on the parasite and the host species, and are therefore useful in comparing survival strategies. As the life cycle of the parasite increases in complexity, so does the difficulty of characterizing survival strategies.

Digenean life cycles vary enormously in complexity. There may be as many as four obligatory hosts and up to eight different developmental stages. Dispersal generally involves free-swimming miracidia and cercariae, in addition to encysted metacercariae. Reduction in the complexity of the life cycle goes hand in hand with reduction in the number of obligatory hosts and the number of developmental stages. Examples of such reduced life cycles can be found in the family Cyclocoelidae Kossack, 1911. These parasites have only two hosts: a snail intermediate host and an avian definitive host. The precocial rediae give rise to a single generation of cercariae that do not emerge from the first intermediate host but remain in the snail where they encyst forming metacercariae. This life cycle has a single free-swimming dispersal stage. Transmission to the definitive host occurs *via* the ingestion of snails harbouring metacercariae.

In a preliminary survey of wild ducks from Manitoba and Québec, two morphological types of *Typhlocoelum* Stossich, 1902 (Digenea:

Cyclocoelidae) were obtained. These have been recognized in the literature although their taxonomic and biological interrelationships were unclear at the outset of this study.

Because of the reduced life cycle of *Typhlocoelum*, this parasite provided a good model for the quantification of the survival parameters in a variety of host species. Furthermore, the presence of two morphologically distinct forms of the parasite provided an opportunity to study and compare their interrelationships and variability.

Recent literature on evolutionary processes and speciation has questioned the role of the species as the primary target of selection (Gould and Johnston, 1972). The assimilation of field and laboratory data in this study, and the understanding of their ecological associations thus gained, have provided a sound basis for an examination of recent ideas on evolution and speciation as they apply specifically to *Typhlocoelum*, and generally to the Digenea.

CHAPTER II. GENERAL MATERIALS AND METHODS

Techniques described in this chapter are those used routinely throughout the study. Details of experimental designs and techniques used in specific experiments are included in the "Materials and Methods" section of the appropriate chapters.

This research was carried out at the Delta Waterfowl Research Station, located on the Delta Marsh at the southern end of Lake Manitoba ($50^{\circ}11'N$, $98^{\circ}19'W$), and at the Institute of Parasitology of McGill University in Montreal, Québec. Unless otherwise noted, techniques were similar at both locations.

Permits for collecting ducks and duck eggs from the Delta Marsh were obtained from the Canadian Wildlife Service.

A. SOURCE AND MAINTENANCE OF EXPERIMENTAL DUCKS

All duck eggs, whether collected from captive birds in the Delta flock or from nests on the Delta Marsh, were hatched in incubators at the Delta Waterfowl Research Station. Ducks maintained at Delta were kept in outdoor pens; those held in Montreal were kept in indoor pens. Duck pens were built on concrete floors and contained a wire mesh platform and a small wading pool with running water; in all cases, ducks were maintained under snail-free conditions. A brooder lamp was provided for ducklings less than four weeks of age. Ducks were fed unmedicated 18% Duck Crumbles and Layer *ad libitum* and each pen was washed daily. Standards provided by the Canadian Council of Animal Care and the Delta Waterfowl Research Station (Ward and Batt, 1973) were followed with regard to density of ducks per pen.

Throughout this thesis, the terms "dabbling ducks" and "diving ducks" will pertain to the genera *Anas* and *Aythya*, respectively. Two species of ducks were used for the majority of the experiments. Male and female mallards were used as representative dabbling ducks and male canvasbacks were used as representative diving ducks. Due to a limited availability of canvasbacks in one year, the closely related redheads were also used as representative diving ducks. In addition, a few blue-winged teal, gadwall

and lesser scaup were used for some experiments. All ducks were between one week and four months of age when exposed to *Typhlocoelum*.

B. SOURCE AND MAINTENANCE OF EXPERIMENTAL SNAILS

Three species of snails were used in this study: *Physa gyrina* Say, *Helisoma trivolvis* (Say) and *Lymnaea elodes* (Say). The majority of these snails were obtained from eggs laid by snails collected from the Delta Marsh. Additional snails were cultured from local pet store stocks. Only laboratory-reared snails were used for experimental infections.

Snails were maintained in moulded plastic aquaria (29 x 17 x 13 cm) containing either filtered marsh water (Delta) or dechlorinated water (Montreal) aerated by means of charcoal and glass wool airlift filters. Each tank contained aquarium plants, and chalk as a source of calcium carbonate, and each was covered with a transparent sheet of plastic.

C. SOURCE OF *Typhlocoelum*

Wild ducks were collected from the Delta Marsh. The trachea and larynx of each duck were removed, cut longitudinally and examined for *Typhlocoelum*. Each worm was placed in a 4 cm Petri dish half-filled with water and illuminated. Eggs hatched shortly after being shed by the adult worms and the miracidia were used to infect snails.

D. TECHNIQUE FOR EXPOSING SNAILS TO *Typhlocoelum*

Each snail was exposed to five active miracidia for two hours in a petti-cup (Fisher) filled with approximately 2 ml of water. Snails were then transferred to individual holding tanks, each identified by a label indicating the appropriate date of exposure, the adult fluke reference number, and the duck host species and reference number.

E. TECHNIQUE FOR EXPOSING DUCKS TO *Typhlocoelum*

At 30 days after exposure to miracidia, snails were examined using a stereomicroscope. The metacercariae could be clearly seen through the snail shell. Only snails harbouring ten or more metacercariae were used for duck infections. Each duck was exposed to 40 metacercariae in 1976,

to 50 in 1977, and to 100 in 1978 and 1979.

In 1976, metacercariae were dissected from the snails, placed in a watch glass containing 0.75% saline and intubated into the proventriculus of ducks. In 1977, 1978 and 1979, ducks were exposed *via* oral intubation of a sufficient number of whole snails to approximate the appropriate infective dose. Ducks were marked with numbered leg bands and by web-clipping.

F. HISTOLOGICAL PREPARATION AND MICROSCOPIC EXAMINATION OF *Typhlocoelum*

Specimens of all life cycle stages of *Typhlocoelum* were fixed in 10% phosphate buffered formalin, stained in Acetocarmine, dehydrated in an alcohol series, cleared in xylene and mounted in Permount.

Measurements were made using a Wild M5 stereomicroscope and a Wild compound microscope. In all cases, specimens were coded so that their identity and source was not known during measurements. Unless otherwise stated, all measurements are given in millimetres.

G. ANALYSIS OF DATA

Statistical analyses of data were done on a Texas Instrument Programmable 58 calculator using parametric procedures described by Sokal and Rohlf (1969) and Rohlf and Sokal (1969) and non-parametric procedures outlined by Siegel (1956), and on an IBM 370-198 computer using routines selected from the Statistical Analysis System (Barr *et al.*, 1976) and programs written in Fortran IV. The level of significance was set at 5% in all cases.

CHAPTER III. VARIABILITY OF *Typhlocoelum* WITHIN AND AMONG VARIOUS SPECIES OF DUCKS

A. INTRODUCTION

When digeneans were first classified during the eighteenth and nineteenth centuries, they were assigned to one of two genera. The genus *Monostomum* included digeneans that lacked a ventral sucker and the genus *Distoma* incorporated all Digenea with an oral and a ventral sucker. The early twentieth century saw a number of systematic monographs dealing with these two genera in which they were reorganized into a number of different families. Of specific interest in this research is the family Cyclocoelidae which includes parasites originally from the genus *Monostomum*. The family Cyclocoelidae is characterized by a circular intestine that is formed from the anastomosis of the two caeca at the posterior end of the body.

Harrah (1922) divided the family Cyclocoelidae into three subfamilies: Cyclocoelinae Stossich, 1902; Typhlocoelinae Harrah, 1922; and Ophthalmophaginae Harrah, 1922. As defined by Harrah (1922), the subfamily Typhlocoelinae is characterized by the presence of strongly developed vitellaria, and diverticula on the intestinal caeca. He included two genera in this subfamily: *Typhlocoelum* Stossich, 1902 and *Tracheophilus* Skrjabin, 1913.

Considerable controversies regarding the validity of the subfamily Typhlocoelinae as well as the validity of the generic and specific subdivisions of the subfamily exist in the literature, and these are outlined chronologically in the following few pages. In most cases, the controversies centre around differing opinions concerning the taxonomic importance and reliability of certain characteristics.

Witenberg (1926) erected a new genus, *Typhlultimum*, to contain specimens originally identified as *Monostomum sarcidiornicola* Mégnin, 1890, but this genus was synonymized with *Typhlocoelum* in the following year by Joyeux and Baer (1927). They had examined the type specimens and found that the testes were lobed, not round as indicated in the type description. In addition, Joyeux and Baer (1927) took exception to two aspects of the

system proposed by Harrah (1922). They believed that presence of inner diverticula was of generic importance, but not sufficiently important to be used as a diagnostic feature for a subfamily. As a result they did not accept the subfamily Typhlocoelinae. Subsequent authors have recognized the subfamily, however, and it is recognized in this study.

Joyeux and Baer (1927) also questioned the validity of the genus *Tracheophilus* which had been distinguished from *Typhlocoelum* on the basis of shape of the testes, the position of the uterus in relation to the testes and the position of the genital pore (Skrjabin, 1913). Joyeux and Baer (1927) believed that the testis shape was a highly variable character and therefore not sufficient for generic distinction. As a result, they synonymized *Tracheophilus* with *Typhlocoelum* and recognized two species which they separated on the basis of the size of the pharynx and the eggs, and the shape and position of the testes. They proposed the following classification:

Typhlocoelum cucumerinum (Rudolphi, 1809): syn. *Distoma cucumerinum* Rudolphi, 1809; *Monostomum flavum* Mehlis, 1831; *Monostomum sarcidiornicola* Mégnin, 1890; *Typhlocoelum obovale* Neuman, 1909; *Typhlocoelum reticulare* Johnston, 1913.

Typhlocoelum cymbium (Diesing, 1850): syn. *Monostomum cymbium* Diesing, 1850; *Haematotrephus cymbius* (Diesing, 1850); *Tracheophilus sisowi* Skrjabin, 1913.

The next major contribution to the systematics of this group of digeneans was made by Dollfus (1948). Dollfus (1948) agreed with Joyeux and Baer (1927) that the shape of the testes was not of sufficient importance to be considered at the level of the genus, but he believed that it was of more than specific value. As a consequence, Dollfus (1948) proposed that there be two subgenera, *Typhlocoelum* Stossich, 1902 and *Tracheophilus* Skrjabin, 1913, within the genus *Typhlocoelum*, separated on the basis of the shape of the testes. Dollfus (1948) acknowledged a second genus, *Typhlophilus* Lal, 1937, which had been erected to incorporate a typhlocoelid with extremely small eggs obtained from the intestine of shovelers (Lal, 1937). Dollfus (1948) separated *Typhlocoelum* from *Typhlophilus* on the basis of the position of the genital pore and the presence and size of the ventral

sucker. Dollfus (1948) proposed the following classification:

Typhlocoelum (Typhlocoelum) cucumerinum (Rudolphi, 1809): syn. *Monostomum flavum* Mehlis, 1831.

Typhlocoelum (Typhlocoelum) obovale Neuman, 1909

Typhlocoelum (Typhlocoelum) reticulare Johnston, 1913

Typhlocoelum (Typhlocoelum) americanum Manter and Williams, 1928

Typhlocoelum (Typhlocoelum) gambense Dubois, 1930

Typhlocoelum (Tracheophilus) cymbium (Diesing, 1850): syn. *Tracheophilus sisowi* Skrjabin, 1913.

Typhlocoelum (Tracheophilus) hepaticum Sugimoto, 1919

Typhlophilus shovellus Lal, 1937

Dollfus (1948) suggested that *T. (T.) obovale, T. (T.) reticulare, T. (T.) americanum* and *T. (T.) gambense* might be found to be variations of *T. (T.) cucumerinum*.

Between 1950 and 1965, four major systematic reviews were presented on the subfamily Typhlocoelinae. Three of these were contributed by Dubois (1951, 1959, 1965) and one by Macko and Buša (1960).

Dubois (1951) proposed that the genus *Tracheophilus* be re-erected. The rationale for this was based on apparent discrepancies in the description of the type specimens of *Monostomum cymbium* published by Monticelli (1892). Kossack (1911) had first expressed some doubt on the validity of including *Monostomum cymbium* in the genus *Typhlocoelum* because, in the drawings of one of the type specimens (Monticelli, 1892), no inner diverticula were present. Also, in the figure of the type specimens, the genital pore was posterior to the pharynx. If the species *cymbium* had to be removed from the genus *Typhlocoelum*, then *Tracheophilus sisowi* would have priority, and Dubois (1951) recognized this. Dubois (1951) accepted the subfamily Typhlocoelinae and expanded the description to include five characters: 1) presence of inner diverticula on the intestinal caeca; 2) oval body, two to three times as long as wide; 3) gonads in triangular position with the anterior testis opposite the ovary and the posterior testis anterior to the intestinal arc; 4) well developed follicular vitellaria extending from the pharynx to the excretory bladder, outside the diverticula; 5) preferred site, trachea, bronchi, syrinx, larynx and perhaps the intestine. He recognized three

genera, *Typhlocoelum*, *Tracheophilus* and *Typhlophilus*, which he distinguished on the basis of testis shape, the presence of a ventral sucker, the position of the genital pore and the size of the eggs.

In 1959, Dubois presented a revised classification which was the most comprehensive critique to that date. After examining the type specimens of *Monostomum cymbium*, Dubois (1959) confirmed that there were no inner diverticula on the intestinal caeca. Accordingly, this parasite could not be included in the genus *Typhlocoelum*. Dubois (1959) therefore transferred the species *cymbium* to the genus *Neivaia* Travassos, 1929.

A second change suggested by Dubois (1959) was based on a reassessment of the generic significance of the shape of the testes. In 1951, Dubois had re-erected *Tracheophilus* to contain those typhlocoelids with round testes, but in 1959, he decided that round testes were of specific value only, as originally suggested by Joyeux and Baer (1927). As a result, *Tracheophilus sisowi* became *Typhlocoelum sisowi* comb. nov.

In addition, Dubois (1959) examined the type material of *Typhlophilus shovellus*. He found that the type material consisted of immature specimens (3.5 mm long) and that the presence of a ventral sucker and of small eggs were functions of the immaturity of the specimens. Dubois (1959) therefore rejected the genus *Typhlophilus* and placed it in synonymy with *Typhlocoelum*. The result of the 1959 paper was the following classification:

Typhlocoelum cucumerinum (Rudolphi, 1809): syn. *Distoma cucumerinum* Rudolphi, 1809; *Monostomum flavum* Mehlis, 1831; *Monostomum sarcidiornicola* Mégnin, 1890; *Typhlocoelum obovale* Neuman, 1909; *Typhlocoelum reticulare* Johnston, 1913; *Typhlocoelum americanum* Manter and Williams, 1928; *Typhlophilus shovellus* Lal, 1937.

Typhlocoelum sisowi (Skrjabin, 1913): syn. *Tracheophilus sisowi* Skrjabin, 1913; *Typhlocoelum hepaticum* Sugimoto, 1919.

Neivaia cymbium (Diesing, 1850): syn. *Monostomum cymbium* Diesing, 1850; *Haematotrephus cymbius* Stossich, 1902; *Typhlocoelum neivaia* Travassos, 1921.

Macko and Buša (1960) studied the morphometrics of 66 specimens obtained from different species of ducks and found that the shape of the

testes was highly variable ranging from round with smooth margins to elongate and deeply lobed. They concluded that testis shape could not be considered as a useful character for separating species and that there should be only one genus in the subfamily Typhlocoelinae containing a single species: *Typhlocoelum cucumerinum*. They proposed that three subspecies be recognized:

Typhlocoelum cucumerinum cucumerinum (Rudolphi, 1809): testes lobed or apparently divided; vitellaria may or may not be confluent posteriorly; parasitic in *Aythya*.

Typhlocoelum cucumerinum americanum (Manter and Williams, 1928): testes entire, indented or slightly lobed, often divided; vitellaria may or may not be confluent posteriorly; parasitic in *Anas querquedula* and *Anas crecca*.

Typhlocoelum cucumerinum cymbium (Diesing, 1850): testes oval, entire or weakly lobed, often divided; vitellaria may or may not be confluent posteriorly; parasitic in *Anas platyrhynchos* and *Anas acuta*.

Dubois (1965) criticized the system proposed by Macko and Buša (1960) on the following grounds. Firstly, he objected to the inclusion of the species *cymbium* in the genus *Typhlocoelum*, due to his previous observation regarding lack of inner diverticula in the type specimens. Secondly, he objected to the separation of *Typhlocoelum cucumerinum americanum* as a distinct subspecies. Macko and Buša (1960) had suggested that round or oval testes in juvenile flukes became more or less lengthened or lobed but never branching in the adult form. Dubois (1965) therefore concluded that the slightly lobed testes in the type specimens of *Typhlocoelum americanum* reflected the age of the flukes and that they should be synonymized with *Typhlocoelum sisowi*.

In all of the above mentioned critiques, the researchers have agreed that there are at least two distinguishable parasites in the subfamily Typhlocoelinae: one with round testes and one with lobed testes. The controversy arises over the importance of testis shape in classifying the parasites.

In this study, 562 specimens of the subfamily Typhlocoelinae

obtained from the upper respiratory tract of wild ducks have been subjected to a detailed analysis of morphological variability within and among host species. This chapter presents the results of this study, and discusses the identity of the parasites.

B. MATERIALS AND METHODS

1. SOURCE OF SPECIMENS

All specimens used for this study were obtained from the upper respiratory tract (bronchi, syrinx, trachea or larynx) of wild ducks from southern Manitoba and southern Québec. Flukes were relaxed in water for six to twelve hours prior to fixation and, after staining, the flukes were dehydrated under sufficient pressure to prevent them from curling.

In addition to material obtained from wild ducks in southern Québec and southern Manitoba, specimens lent by individuals or museums throughout the world were also examined. These sources and the details on specimens provided by each are listed in Appendix 3-1.

2. NUMERICAL VARIABLES USED IN MORPHOLOGICAL ANALYSES

The measurements made from the whole mount of each specimen (Figure 3-1) included the maximum body length and width, the length (parallel to the body length axis) and width (perpendicular to the body length axis) of the pharynx, the ovary, the seminal receptacle, and the anterior and posterior testes, the length of the oesophagus from the posterior margin of the pharynx to the bifurcation of the intestine, and the length of the cirrus sac. The positions of the gonads and seminal receptacle were quantified in terms of the distance from the middle of the pharynx to the middle of the organs (see Figure 3-1). The distance from the middle of the pharynx to the anterior end of the body was also measured. The maximum number of inner diverticula on either side of each specimen was determined. The anterior extent of eggs in the uterus and the posterior extent of eggs with eyespots were quantified in relation to the distance from the intestinal bifurcation (see Figues 3-1).

When mature specimens were used as a source of eggs, the body length and width of the relaxed, live specimens, and the number of fully embryonated

Figure 3-1. Diagram showing some of the measurements made on whole mounts of specimens.

1, Distance from seminal receptacle to pharynx;

2, Distance from ovary to pharynx;

3, Distance from eyespots to intestinal bifurcation;

4, Distance from eggs to intestinal bifurcation;

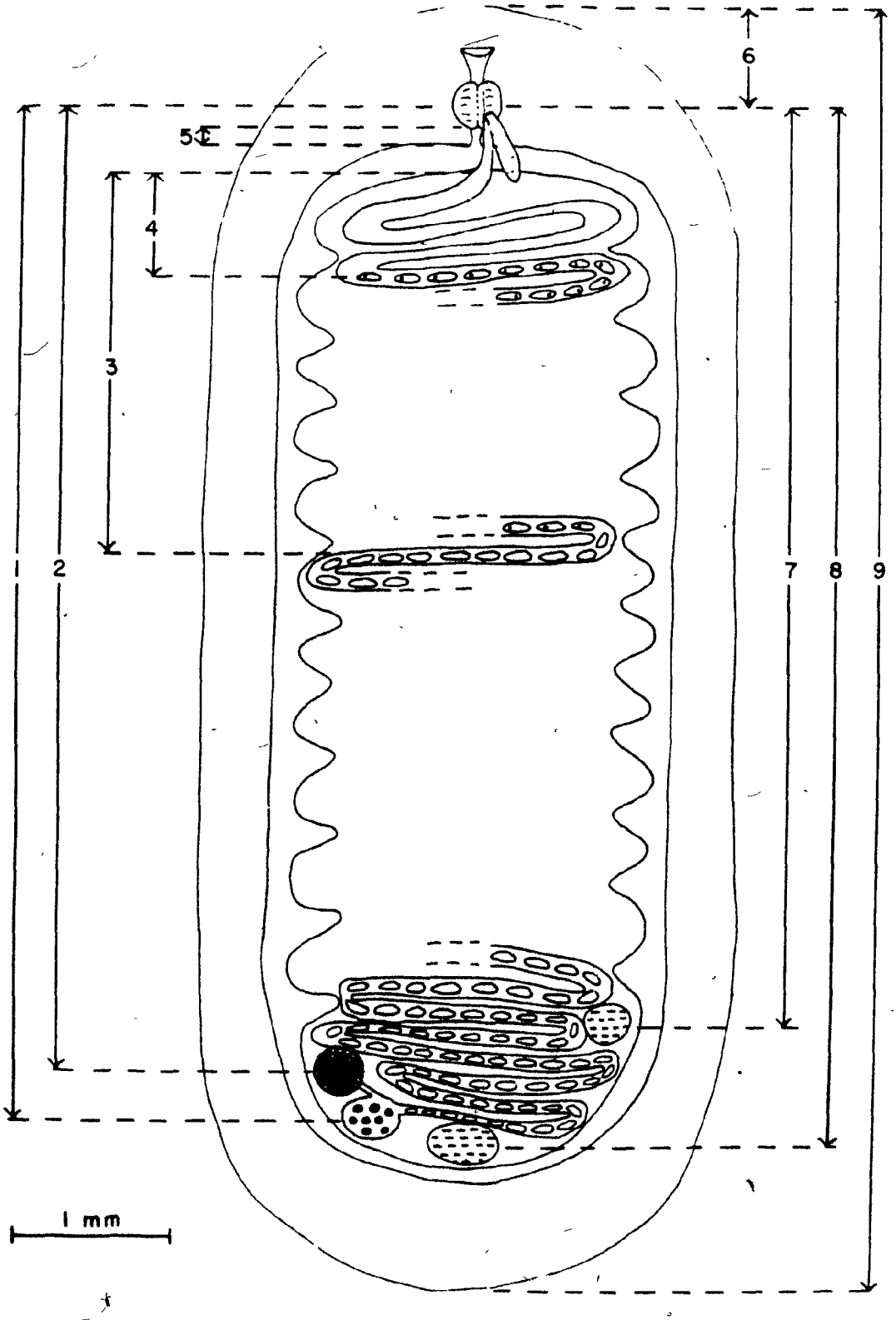
5, Oesophagus length;

6, Distance from pharynx to anterior;

7, Distance from anterior testis to pharynx;

8, Distance from posterior testis to pharynx;

9, Body length.


6
5
4
3
2
7
8
9
1 mm

eggs, determined by counting the number of miracidia used to infect snails, were recorded. The mean length and width of ten eggs prior to hatching was also determined.

3. CHARACTER VARIABLES USED IN MORPHOLOGICAL ANALYSES

In addition, 13 character variables were examined and assigned to specific character states as follows:

Anterior Testis Shape (Figure 3-2):

a - round

b - deeply lobed and branching

Posterior Testis Shape (Figure 3-2):

a - round

b - deeply lobed and branching

Eggs:

a - present

b - absent

Eyespots:

a - present

b - absent

Maturity:

a - mature if eyespots present within the eggs

b - immature if no eyespots present in eggs

Uterus and Testes:

a - several loops of the uterus separate the anterior and posterior testes

b - a single loop of the uterus separates the anterior and posterior testes

c - there are no loops of the uterus between the anterior and posterior testes

Anterior Extent of Vitellaria (Figure 3-3A):

a - to the level of the intestinal bifurcation

b - to the level of the pharynx

c - asymmetrical

Figure 3-2. Diagram showing two character states for the character variables, anterior testis shape and posterior testis shape.

A, round;

B, lobed.

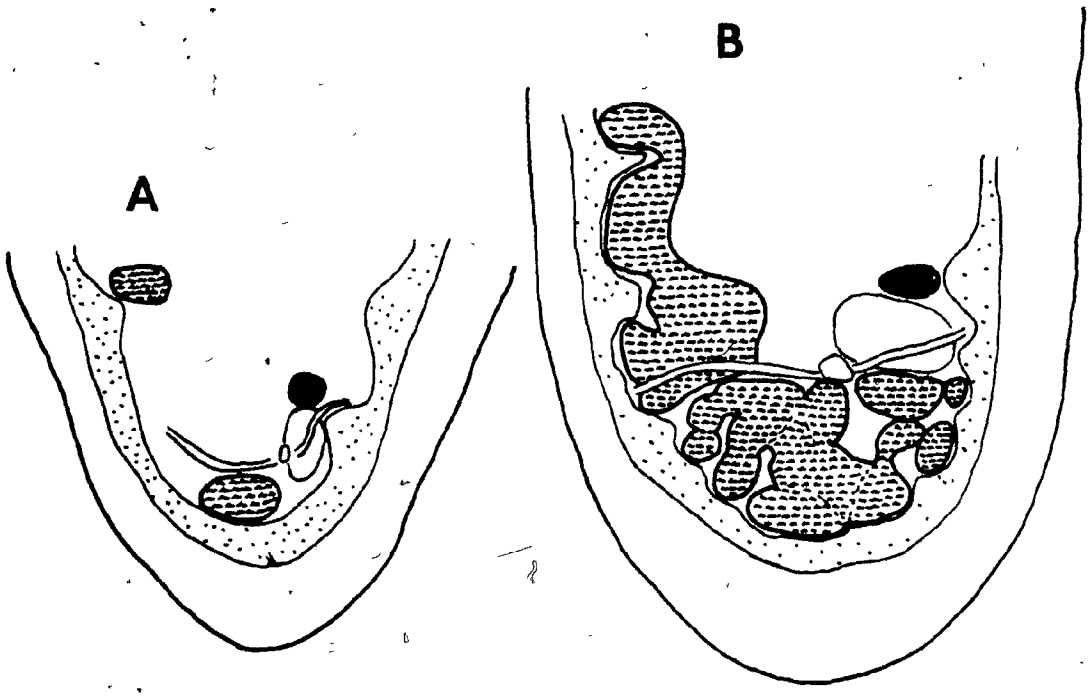

A
B

Figure 3-3. Diagram showing character states for the character variables, anterior extent of vitellaria (A) and posterior extent of vitellaria (B).

A. Anterior Extent of Vitellaria:

a, to level of intestinal bifurcation;

b, to level of pharynx;

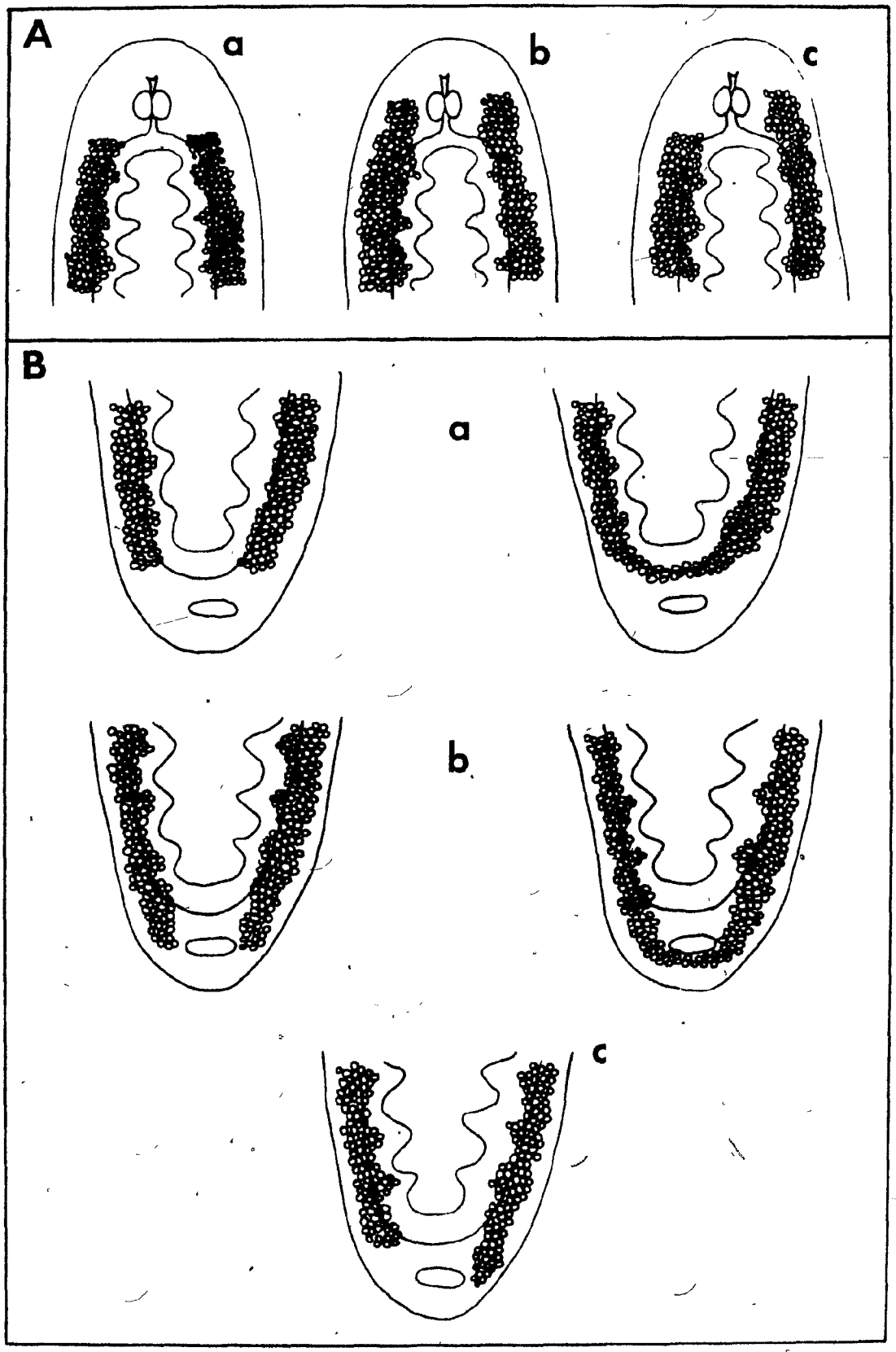
c, asymmetrical.

B. Posterior Extent of Vitellaria:

a, to level of intestinal arc or confluent around intestinal arc;

b, to level of excretory bladder or confluent around excretory bladder;

c, asymmetrical.


A
a
b
c
B
a
b
c

Posterior Extent of Vitellaria (Figure 3-3B):

a - to the level of the intestinal arc or confluent around the posterior margin of the intestinal arc

b - to the level of the excretory bladder or confluent around the posterior margin of the excretory bladder

c - asymmetrical

Ventral Surface (Figure 3-4):

a - large,deep indentations

b - small, inconspicuous indentations

c - smooth

Genital Pore:

a - in front of, or ventral to the anterior region of the pharynx

b - ventral to the middle of the pharynx

c - ventral to the posterior region, or behind the pharynx

Cirrus Sac:

a - extends past the intestinal bifurcation

b - does not extend past the intestinal bifurcation

Uterus and Intestine:

a - uterus confined to the intercaecal area

b - uterus extends laterally over the intestine

Inner Diverticula:

a - present

b - absent

4. DATA ANALYSIS

These above data were entered on computer cards along with the following data for each specimen:

Worm Reference Number - unique number assigned to each specimen

Host Reference Number - unique number assigned to each host

Host Species

Host Age - juvenile, adult

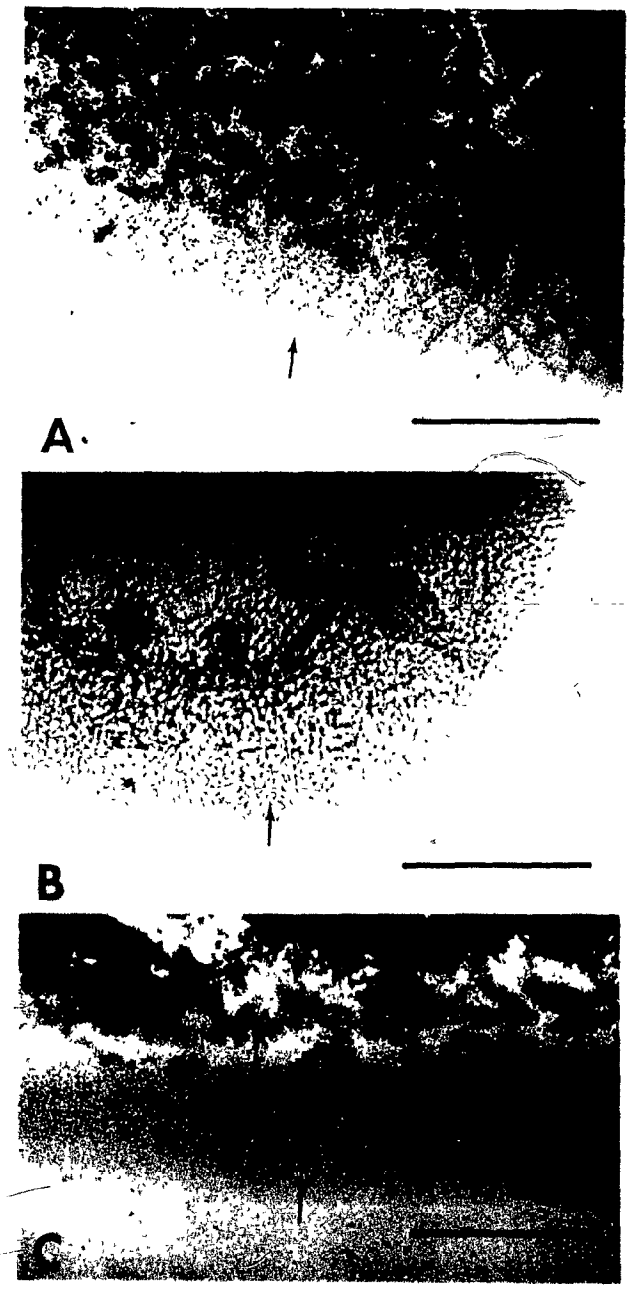
Host Sex

Host Location - southern Québec, southern Manitoba

Season of Collection - summer, fall

Figure 3-4. Photographs of the character states for the character variable, ventral surface.
A, Large, deep indentations;
B, Small, inconspicuous indentations;
C, Smooth.

Bar represents 0.5 mm.


A
B
C

Total Number of Specimens Obtained in That Host

Unless otherwise noted, only specimens obtained from wild ducks in southern Manitoba and southern Québec are included in the analyses.

C. RESULTS

1. MORPHOMETRIC VARIABILITY OF *Typhlocoelum* WITHIN HOST SPECIES

In an attempt to assess the influence of host age, sex and location on the numerical variables of *Typhlocoelum*, 95% confidence limits constructed on the mean measurements of mature flukes from each host species were compared.

In mallards and canvasbacks, significant effects of host age on the morphometrics of *Typhlocoelum* were detected. Flukes from adult mallards (Table 3-1) have a longer oesophagus, but the distances from the pharynx to the anterior testis and seminal receptacle are shorter than in flukes from juvenile mallards. Flukes from juvenile canvasbacks (Table 3-2) have a greater body length, ovary width, anterior testis width and greater distances from the pharynx to the gonads and seminal receptacle than flukes from adult canvasbacks.

In no case did the sex of the host species significantly influence the morphology of the flukes.

In mallards, significant differences between fluke populations from Québec and Manitoba were detected in seven numerical variables: body length, body width, pharynx length and distance from the pharynx to the gonads and seminal receptacle (Table 3-3). No such differences were detected in flukes from any other host species.

In addition to the specimens obtained from Canada in this study, specimens from wild ducks in the United States, Brazil, Britain, Iran and South Africa were examined. The morphometric data taken from these specimens are presented in Appendix 3-2. Although the sample sizes for these specimens are small, there are further indications of differences in measurements within host species based on their geographical location.

Table 3-1. Comparison of numerical variables of mature specimens of *Typhlocoelum* obtained from juvenile and adult mallards.

NUMERICAL VARIABLE*	JUVENILE MALLARDS n	JUVENILE MALLARDS MEAN (mm) ± 95% C.L.	ADULT MALLARDS n	ADULT MALLARDS MEAN (mm) ± 95% C.L.
BL	21	8.67 ± 0.42	47	8.20 ± 0.20
BW	22	3.63 ± 0.15	52	3.46 ± 0.10
PL	21	0.327 ± 0.014	47	0.306 ± 0.013
PW	21	0.310 ± 0.018	47	0.316 ± 0.015
OL	19	0.262 ± 0.031	46	0.305 ± 0.017
OW	19	0.317 ± 0.023	46	0.343 ± 0.019
SRL	14	0.317 ± 0.076	37	0.295 ± 0.030
SRW	14	0.326 ± 0.058	37	0.345 ± 0.035
ATL	18	0.232 ± 0.025	42	0.273 ± 0.019
ATW	18	0.261 ± 0.039	42	0.326 ± 0.026
PTL	22	0.310 ± 0.130	45	0.305 ± 0.024
PTW	22	0.431 ± 0.166	45	0.393 ± 0.024
PA	21	0.624 ± 0.036	48	0.624 ± 0.032
OA	20	6.43 ± 0.32	44	5.98 ± 0.29
SRA †	14	6.94 ± 0.33	35	6.34 ± 0.21
ATA †	18	6.19 ± 0.32	41	5.66 ± 0.17
PTA	20	7.01 ± 0.35	42	6.61 ± 0.19
NID	12	10.0 ± 0.3	12	10.3 ± 0.5
OES †	20	0.068 ± 0.031	33	0.137 ± 0.036

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

† Means are significantly different.

Table 3-2. Comparison of numerical variables of mature specimens of *Typhlocoelum* obtained from juvenile and adult canvasbacks.

NUMERICAL VARIABLE*	JUVENILE CANVASBACKS n	JUVENILE CANVASBACKS MEAN (mm) ± 95% C.L.	ADULT CANVASBACKS n	ADULT CANVASBACKS MEAN (mm) ± 95% C.L.
BL †	11	10.81 ± 0.81	15	9.16 ± 0.83
BW	12	4.27 ± 0.55	15	3.43 ± 0.52
PL	12	0.311 ± 0.032	14	0.278 ± 0.033
PW	12	0.308 ± 0.032	14	0.266 ± 0.029
OL	11	0.403 ± 0.052	15	0.315 ± 0.046
OW †	11	0.454 ± 0.054	15	0.337 ± 0.047
SRL	10	0.340 ± 0.085	7	0.274 ± 0.032
SRW	10	0.474 ± 0.106	7	0.443 ± 0.078
ATL	11	1.652 ± 0.473	14	1.174 ± 0.170
ATW †	11	0.502 ± 0.162	14	0.290 ± 0.041
PTL	11	0.777 ± 0.168	14	0.565 ± 0.102
PTW	11	1.381 ± 0.309	14	1.002 ± 0.184
PA	12	0.632 ± 0.046	14	0.544 ± 0.066
OA †	11	8.09 ± 0.61	13	6.66 ± 0.68
SRA †	11	8.49 ± 0.67	8	6.48 ± 0.47
ATA †	11	7.80 ± 0.55	12	6.46 ± 0.78
PTA †	11	9.13 ± 0.67	12	7.46 ± 0.84
NID	10	11.1 ± 0.7	11	11.5 ± 0.6
OES	11	0.091 ± 0.046	13	0.077 ± 0.030

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

† Means are significantly different.

Table 3-3. Comparison of numerical variables of mature specimens of *Typhlocoelum* obtained from mallards in southern Québec and southern Manitoba.

NUMERICAL VARIABLE*	SOUTHERN QUEBEC n	SOUTHERN QUEBEC MEAN (mm) ± 95% C.L.	SOUTHERN MANITOBA n	SOUTHERN MANITOBA MEAN (mm) ± 95% C.L.
BL †	18	8.84 ± 0.44	108	7.86 ± 0.18
BW †	18	3.63 ± 0.15	116	3.36 ± 0.08
PL †	18	0.338 ± 0.011	109	0.314 ± 0.009
PW	18	0.320 ± 0.023	109	0.293 ± 0.010
OL	17	0.272 ± 0.037	106	0.289 ± 0.011
OW	17	0.320 ± 0.030	106	0.323 ± 0.011
SRL	12	0.329 ± 0.080	83	0.277 ± 0.019
SRW	12	0.327 ± 0.063	83	0.331 ± 0.024
ATL	16	0.231 ± 0.024	97	0.237 ± 0.015
ATW	16	0.234 ± 0.041	97	0.283 ± 0.016
PTL	17	0.229 ± 0.040	106	0.274 ± 0.029
PTW	17	0.339 ± 0.041	106	0.361 ± 0.036
PA	18	0.629 ± 0.040	109	0.635 ± 0.022
OA †	18	6.56 ± 0.34	104	5.82 ± 0.17
SRA †	13	6.98 ± 0.35	81	6.24 ± 0.17
ATA †	17	6.25 ± 0.34	97	5.54 ± 0.14
PTA †	17	7.10 ± 0.40	103	6.37 ± 0.16
NID	13	10.2 ± 0.3	56	10.5 ± 0.2
OES	18	0.084 ± 0.052	88	0.096 ± 0.016

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

† Means are significantly different.

2. MORPHOMETRIC VARIABILITY OF *Typhlocoelum* AMONG HOST SPECIES

The remainder of analyses on the morphology of *Typhlocoelum* are done on the pooled data from southern Québec and southern Manitoba for each host species, regardless of the age and sex of the host.

The mean and 95% confidence limits for all numerical variables of mature worms from each host species are presented in Appendix 3-3, and the results of the analyses of variance and Duncan's multiple range tests for each numerical variable are presented in Appendix 3-4.

Worms from the various host species fell into three distinct groups with respect to anterior testis length (Table 3-4). Worms from greater scaup have the longest mean anterior testis length; those from canvasbacks, lesser scaup and redheads form an intermediate group; those from the dabbling ducks (blue-winged teal, green-winged teal, shoveler, black duck, mallard and pintail) form the group with the shortest mean anterior testis length.

The worms similarly fell into four distinct groups of host species with respect to posterior testis width (Table 3-5), although worms from green-winged teal overlap in two groups. Greater scaup worms have the largest mean posterior testis width; worms from the other three diving duck species (redhead, canvasback and lesser scaup) together with green-winged teal worms form the second group; worms from green-winged teal, shovelers and blue-winged teal form a third group; and worms from mallards, black ducks and pintails form the group with the smallest posterior testis width.

In every other numerical variable, significant differences were detected among worms from different species (Appendix 3-3), but in these cases, the overlapping was so extensive that no patterns could be detected.

In order to determine whether there were any overall trends, the total data set was subjected to a Discriminant Function Analysis (Barr *et al.*, 1976). This is a multivariate technique that computes a discriminant score using data from all numerical variables for specimens from each host species. Using the scores obtained from the data set, the computer then classifies each observation. If there are gross differences among the specimens from different host species, the computer will be able to

Table 3-4. Anterior testis length of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN ANTERIOR TESTIS LENGTH (mm) **
GSC †	7	1.337
CAN †	100	1.094
LSC †	15	0.958
RED †	24	0.951
BWT	18	0.388
GWT	5	0.380
SHO	15	0.322
BLA	6	0.262
MAL	113	0.236
PIN	17	0.217

$F = 61.85$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Table 3-5. Posterior testis width of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN POSTERIOR TESTIS WIDTH (mm) **
GSC †	7	1.471
RED †	25	1.011
CAN †	98	0.994
LSC †	16	0.851
GWT	5	0.723
SHO	15	0.594
BWT	19	0.588
MAL	123	0.358
BLA	6	0.342
PIN	18	0.301

$F = 48.20$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

correctly classify each specimen. If however, the groups are similar, the proportion of mistakes, or misclassifications, will he high.

A series of discriminant function analyses were performed where the number of variables included and the groups of host species were varied. In the first analysis, 18 numerical variables (all except the number of inner diverticula, the cirrus sac length, and the anterior extent of eggs and eyespots) were included, and worms from each host species were assigned to separate groups. Under these conditions, the computer correctly classified over 80% of green-winged teal, mallard and shoveler worms (Table 3-6).

In an attempt to increase the number of specimens in each group, a second analysis was undertaken where the eight variables with the highest number of missing values were deleted. These included the distances from the gonads and seminal receptacle to the pharynx, the length and width of the seminal receptacle, the oesophagus length and the distance from the pharynx to the anterior. Deletion of these variables did increase the sample size, but only improved the percentage of correct classifications in two host species, mallards and canvasbacks.

Careful examination of the misclassifications revealed that, when specimens from dabbling ducks were misclassified, they were most frequently classified into another dabbling duck species and, similarly, diving duck worms were frequently classified into other diving duck species. Two additional discriminant function analyses were undertaken using 18 and 10 numerical variables, but using only two groups of specimens: those from dabbling ducks and those from diving ducks. In both the 18 and the 10 variable analyses, over 90% of the specimens from both groups were correctly classified (Table 3-7).

In order to determine whether the capacity to discriminate between worms from dabbling and diving ducks was based primarily on the testes measurements, two further sets of analyses were done. In one set, all testis measurements were deleted from the analysis, and in the other, only the testis measurements were used. The results of both analyses (Table 3-8) demonstrate that discrimination is better when the testes measurements were included in the analyses.

Table 3-6. Results of discriminant function analysis of *Typhlocoelum* using 18 numerical variables and specimens from each species of wild duck.

FROM HOST SPECIES*	PERCENTAGE OF SPECIMENS CLASSIFIED INTO HOST SPECIES:										
	BLA	BWT	CAN †	GSC †	GWT	LSC †	MAL	PIN	RED †	SHO	n
BLA	50	-	-	-	-	-	50	-	-	-	4
BWT	-	57	14	-	-	14	-	-	-	14	7
CAN †	-	1	78	3	-	5	8	3	3	-	76
GSC †	-	-	57	29	-	14	-	-	-	-	7
GWT	-	-	-	-	100	-	-	-	-	-	2
LSC †	-	-	33	-	-	67	-	-	-	-	9
MAL	1	-	-	-	-	-	96	1	-	1	72
PIN	8	-	-	-	-	-	50	42	-	-	12
RED †	-	-	59	-	-	-	6	-	35	-	17
SHO	-	-	-	-	-	-	11	-	-	89	9

* The abbreviations for host species are explained in the "List of Abbreviations".

† Diving duck species.

Table 3-7. Results of discriminant function analyses of *Typhlocoelum* using 18 (A) and 10 (B) numerical variables and specimens from dabbling and diving duck species.

A.

FROM HOST GROUP	PERCENTAGE OF SPECIMENS CLASSIFIED INTO HOST GROUPS: DABBLING DUCKS	DIVING DUCKS	n
DABBLING DUCKS	96	4	106
DIVING DUCKS	6	94	109

B.

FROM HOST GROUP	PERCENTAGE OF SPECIMENS CLASSIFIED INTO HOST GROUPS: DABBLING DUCKS	DIVING DUCKS	n
DABBLING DUCKS	97	3	154
DIVING DUCKS	6	94	137

Table 3-8. Results of discriminant function analyses of *Typhlocoelum* using only testis measurements (A) and using numerical variables excluding testis measurements (B) for specimens from dabbling and diving duck species.

A.

FROM HOST GROUP	PERCENTAGE OF SPECIMENS CLASSIFIED INTO HOST GROUPS: DABBLING DUCKS	DIVING DUCKS	n
DABBLING DUCKS	95	5	171
DIVING DUCKS	8	92	144

B.

FROM HOST GROUP	PERCENTAGE OF SPECIMENS CLASSIFIED INTO HOST GROUPS: DABBLING DUCKS	DIVING DUCKS	n
DABBLING DUCKS	90	10	109
DIVING DUCKS	30	70	112

From Table 3-6, it was also observed that, within the dabbling duck species, mallard, black duck and pintail worms were frequently confused, as were blue-winged teal and shoveler worms. In the final set of analyses, specimens were divided into three groups: those from diving ducks; those from mallards, black ducks and pintails; and those from blue-winged teal, green-winged teal and shovelers. Three separate analyses were undertaken. In one, only testis measurements were included; in the second, all testis measurements were excluded; and in the third analysis, testis measurements as well as the other numerical variables were included. The results are presented in Table 3-9. The testis measurements alone were not as useful in discriminating among the three groups as were the other variables when testis measurements were excluded.

Data on the number of embryonated eggs were obtained from 76 flukes from dabbling ducks and from 5 flukes from diving ducks (Table 3-10; Figure 3-5). Although there were no significant differences in the mean number of embryonated eggs obtained from dabbling and diving duck worms, the maximum number recovered from a single dabbling duck worm was 1185 whereas as many as 3305 embryonated eggs were recovered from a single diving duck specimen. Significant positive correlations were detected between the live body length and the number of embryonated eggs in both dabbling and diving duck worms (Pearson product-moment correlation: $r = 0.385$, $P < 0.05$; $r = 0.986$, $P < 0.01$, respectively). Diving duck worms have significantly larger eggs than dabbling duck worms (0.165 and 0.137 mm, respectively).

3. VARIABILITY OF CHARACTER STATES WITHIN AND AMONG HOST SPECIES

In addition to the morphometric analysis, variability of a number of characters was also examined. Data on character variables for specimens from each host species are presented in Appendix 3-5.

The size distribution of mature and immature specimens of each host species are presented in Figure 3-6. Specimens from diving ducks attained body lengths of up to 10 mm before reaching maturity whereas the the longest immature flukes from dabbling ducks were 8 mm. Mature specimens from diving ducks also attained larger maximum body lengths than those

Table 3-9. Results of discriminant function analyses of *Typhlocoelum* using 10 numerical variables (A), using only testis measurements (B) and using numerical variables excluding testis measurements (C) for specimens from diving ducks and two groups of dabbling ducks.

A.

FROM HOST GROUP*	PERCENTAGE OF SPECIMENS CLASSIFIED INTO HOST GROUPS: MAL-PIN-BLA	BWT-GWT-SHO	DIVING DUCKS	n
MAL-PIN-BLA	100	-	-	127
BWT-GWT-SHO	-	93	7	27
DIVING DUCKS	5	1	94	137

B.

FROM HOST GROUP	PERCENTAGE OF SPECIMENS CLASSIFIED INTO HOST GROUPS: MAL-PIN-BLA	BWT-GWT-SHO	DIVING DUCKS	n
MAL-PIN-BLA	98	2	-	135
BWT-GWT-SHO	17	67	17	36
DIVING DUCKS	6	3	92	144

C.

FROM HOST GROUP	PERCENTAGE OF SPECIMENS CLASSIFIED INTO HOST GROUPS: MAL-PIN-BLA	BWT-GWT-SHO	DIVING DUCKS	n
MAL-PIN-BLA	87	3	10	89
BWT-GWT-SHO	-	100	-	20
DIVING DUCKS	25	1	74	112

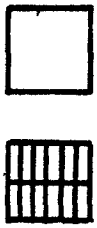
* The abbreviations for host species are explained in the "List of Abbreviations".

Table 3-10. Relationship between number of embryonated eggs and body length of specimens of *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	NUMBER OF EMBRYONATED EGGS MEAN ± 95% C.L.	r	P > r
BLA	5	407 ± 277	-	-
BWT	14	359 ± 148	-	-
MAL	57	574 ± 98	-	-
DABBLING DUCKS	44	612 ± 114	0.385	0.05
DIVING DUCKS	5	1545 ± 1531	0.986	0.01

* The abbreviations for host species are explained in the "List of Abbreviations".

Figure 3-5. Histograms of percent frequency of number of embryonated eggs contained within dabbling duck worms and diving duck worms.



Dabbling Duck Worms

Diving Duck Worms

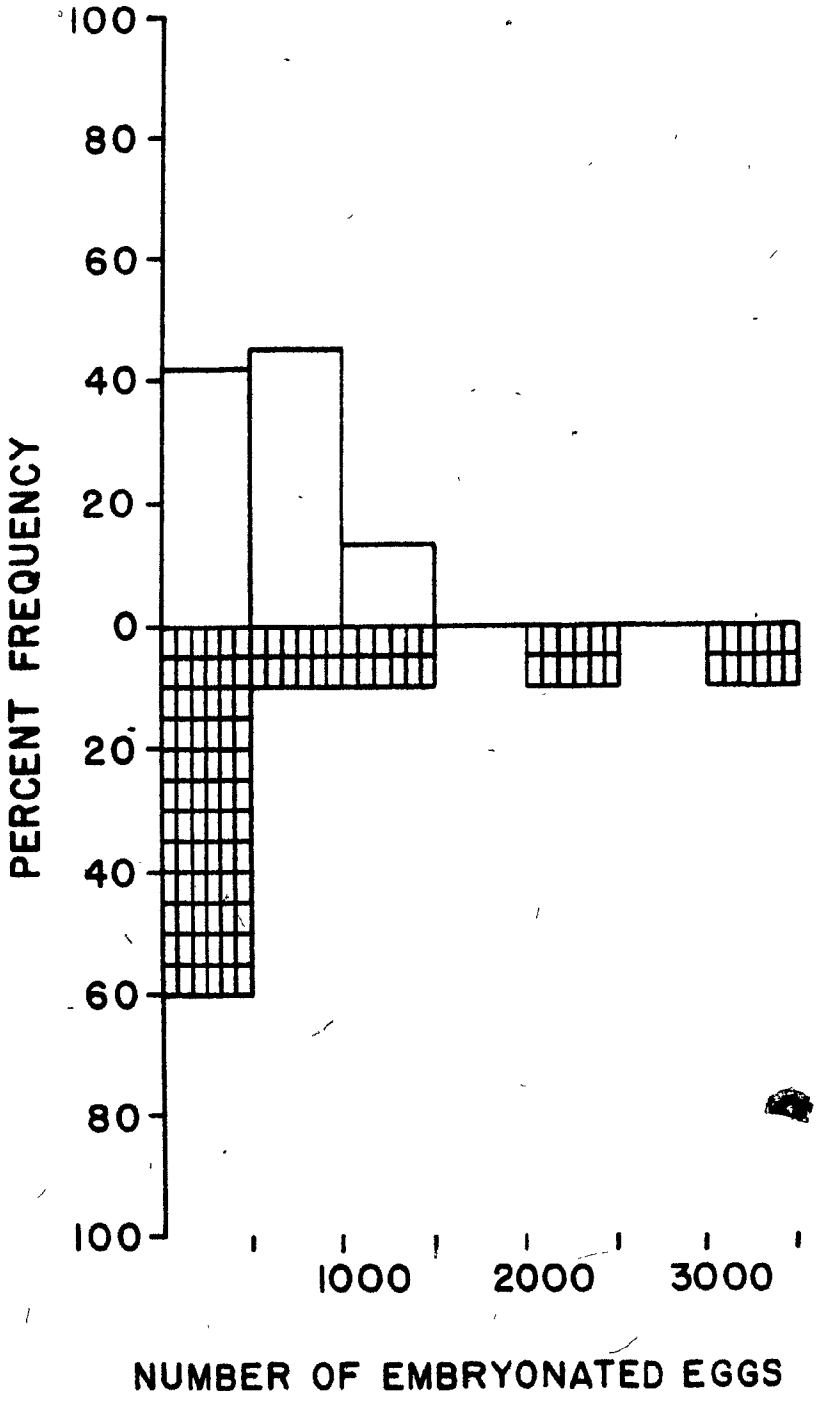
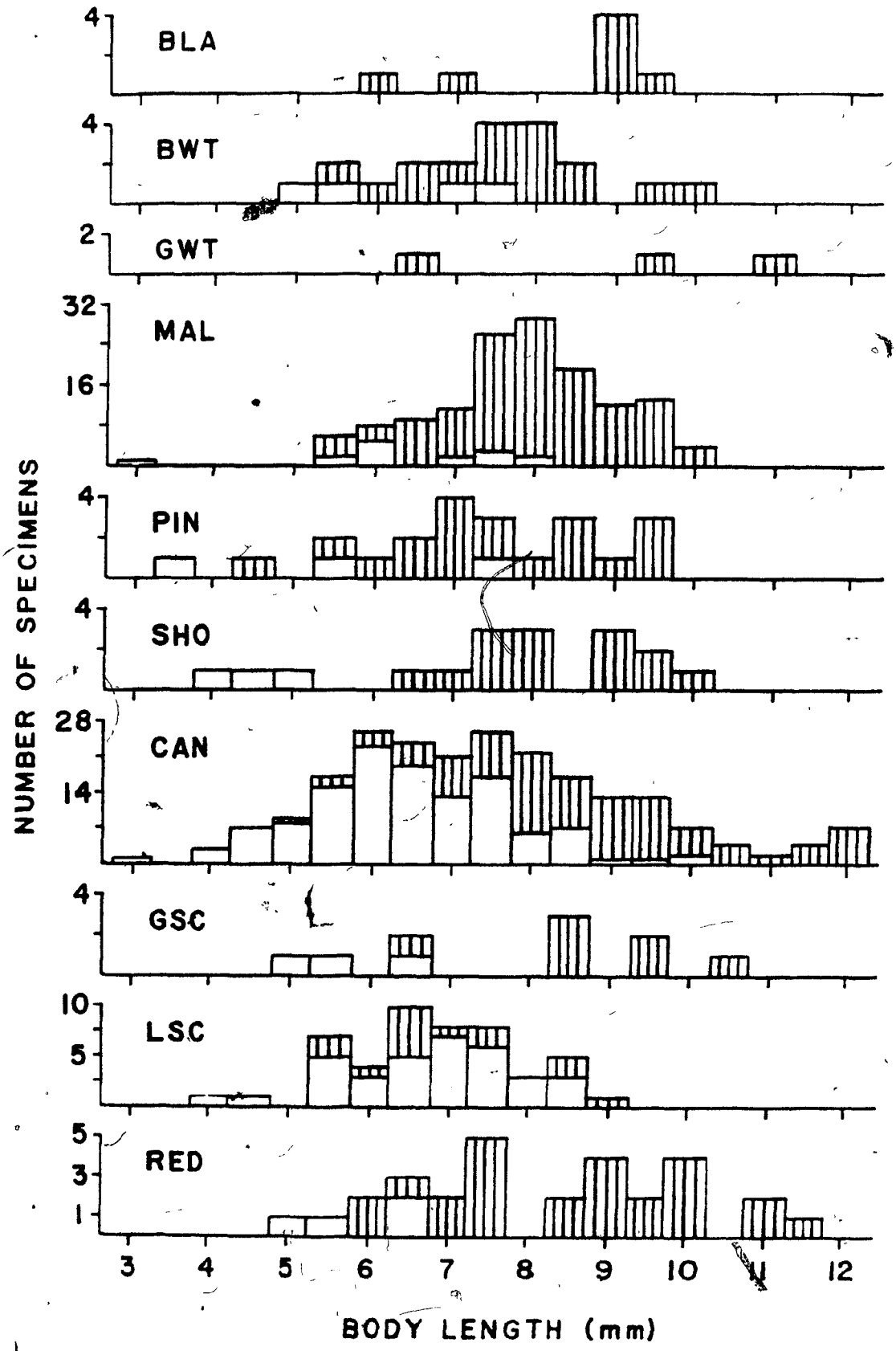

100
80
60
40
20
0
20
40
60
80
100
PERCENT FREQUENCY
1000
2000
3000
NUMBER OF EMBRYONATED EGGS

Figure 3-6. Body length histograms for mature and immature specimens of *Typhlocoelum* obtained from each host species.

Mature Specimens

Immature Specimens

The abbreviations for host species are explained in the "List of Abbreviations".


BLA
BWT
GWT
MAL
PIN
SHO
CAN
GSC
LSC
RED
4
4
2
32
16
4
4
28
14
4
10
5
5
3
1
NUMBER OF SPECIMENS
3
4
5
6
7
8
9
10
11
12
BODY LENGTH (mm)

from dabbling ducks (12 and 11 mm respectively). As a corollary to this, dabbling duck worms reached maturity at shorter body lengths than diving duck worms (4.5 and 5 mm respectively).

Over 80% of the flukes from all species of dabbling ducks and from one species of diving duck (redhead) were mature (Table 3-11). The other diving duck species had a much lower percentage of mature flukes. In all host species but two (pintail and shoveler), over 75% of the immature worms contained eggs (Table 3-11). A linear regression analysis according to host species revealed that the percentage of mature worms was not a function of the intensity of the infection.

Two of the character variables were found to be consistent in all specimens from all host species. The uterus was confined to the intercaecal region in 99.7% of the flukes. All of the specimens examined in this study had inner diverticula on the intestinal caeca, although the number of inner diverticula varied between eight and fifteen. In dabbling ducks, 85% of the worms had eleven or fewer inner diverticula whereas, in diving duck worms, only 44% had eleven or fewer inner diverticula. This difference was significant ($\chi^2 = 65.95$, $df = 1$, $P < 0.001$). There was no significant correlation between the mean number of inner diverticula and the mean body length of the flukes among the various host species.

In all other character variables, variability was found not only between dabbling and diving duck worms, but also among worms from the same host species.

Three character states were used to describe the ventral surface and in all but two host species, every character state was represented among the flukes. Among the dabbling duck worms, 83% had deep indentations over the ventral surface, whereas only 14% of the diving duck worms had a deeply indented ventral surface. Of the remaining diving duck worms, approximately half had small, inconspicuous indentations and half had a smooth ventral surface. The proportions of specimens demonstrating these three character states differed significantly between dabbling and diving duck worms ($\chi^2 = 168.49$, $df = 2$, $P = 0.0001$).

Although there was variability in the position of the genital pore among worms from the same host species, in general, the genital pore was

Table 3-11. Proportion of mature and immature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	PERCENTAGE OF MATURE *Typhlocoelum*	PERCENTAGE OF IMMATURE *Typhlocoelum* WITH EGGS
BLA	8	87.5%	100.0%
BWT	28	85.7%	75.0%
GWT	6	100.0%	-
MAL	149	88.6%	94.1%
PIN	23	87.0%	66.7%
SHO	18	83.3%	33.3%
DABBLING DUCKS	232	87.9%	82.1%
CAN	230	43.9%	98.4%
GSC	12	58.3%	100.0%
LSC	50	31.4%	88.6%
RED	31	83.9%	100.0%
RIN	3	0.0%	100.0%
DIVING DUCKS	326	45.4%	96.6%

* The abbreviations for host species are explained in the "List of Abbreviations".

located farther anterior in dabbling duck worms than in diving duck worms ($\chi^2 = 24.00$, df = 1, P = 0.0001). Similarly the posterior extent of the cirrus sac varied among specimens from the same host species. In 85% of the dabbling duck worms, the cirrus sac extended posteriorly past the intestinal bifurcation whereas this occurred in 98% of diving duck worms. The difference is significant (Fisher's exact probability test, P = 0.0477).

The vitellaria extended anteriorly to the level of the pharynx in the majority of dabbling and diving duck worms. The posterior extent of the vitellaria varied among worms from the same host species and differed significantly between dabbling and diving duck worms ($\chi^2 = 43.84$, df = 2 P = 0.0001). In dabbling duck worms, the vitellaria extended posteriorly to or around the intestinal arc in 20% of the worms whereas, in diving ducks, this occurred in 50% of the worms. In 71% of the dabbling duck worms, the vitellaria reached or passed across the excretory bladder; this state occurred in 36% of diving duck worms.

With respect to anterior and posterior testis shapes, 100% of the dabbling duck worms had round testes and 93% of the diving duck worms had lobed testes. The differences were highly significant, both for anterior testis shape ($\chi^2 = 290.71$, df = 1, P < 0.001) and posterior testis shape ($\chi^2 = 297.54$, df = 1, P < 0.001). Twelve of these specimens had testes that were classified as round, but that were slightly atypical (see Figure 3-7). This occurred in 3 of 20 (15%) blue-winged teal worms, 1 of 101 (1%) canvasback worms, 1 of 5 (20%) green-winged teal worms, 3 of 129 (2%) mallard worms and 4 of 15 (27%) shoveler worms. As can be seen from these data, this atypical shape occurred most frequently in blue-winged teal, green-winged teal and shoveler worms, although, in each of these host species, the majority of the flukes had round testes.

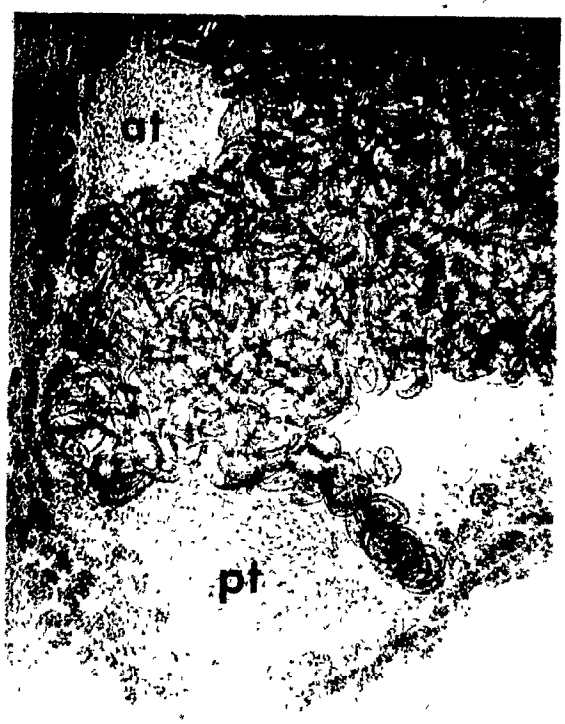
There was a significant difference between dabbling and diving duck worms in the degree to which the uterus separated the anterior and posterior testes ($\chi^2 = 258.71$, df = 2, P = 0.0001). In dabbling ducks, 97% of the worms had the testes separated by several uterine loops, whereas, in 70% of the diving duck worms, no uterine loops passed between the testes.

Figure 3-7. Photograph of specimen of *Typhlocoelum* with atypical testis shape.

at, anterior testis;

pt, posterior testis.

Bar represents 0.5 mm.


at
pt

The above analysis of character variables has demonstrated that there is considerable variability among worms from the same host species, but that, overall, the proportion of specimens demonstrating the various character states differs between worms from dabbling and diving ducks.

D. DISCUSSION

1. THE SPECIES AND ITS VARIABILITY

Historically, species have been considered to be monotypic whereby each member of the species can be identified by a set of unique characters that are present in the type specimen of that species. Furthermore, the monotypic species is indivisible; that is, it does not have distinguishable subsets. As the modern theory of evolution has developed, a second type of species has been recognized. The polytypic species concept acknowledges that there can be variation within a species, providing a possibility for subsets; and that a detailed description of one individual may not be sufficient for characterizing all other members of that species. Mayr (1970) defined species as "groups of interbreeding natural populations that are reproductively isolated from other such species". Because this definition incorporates a biological process, reproduction, Mayr refers to it as the biological species concept. Both Mayr (1970) and Roitman and Kazakov (1977) suggested that species descriptions can not be considered in the light of an individual, or single type specimen. Instead, they must be described in terms of population parameters; that is, in terms of means and variances. This type of description incorporates the inherent variability of the species.

The polytypic or biological species concept presents a parasite taxonomist with a number of logistic problems. To begin with, in order to properly describe a species, the investigator must be aware of the range of variability and the frequency with which the variants occur (Haley, 1962).

Variability of organisms is caused by a wide variety of phenomena ranging from the inherent genetic variability of populations and individuals, through phenotypic variability induced by environmental influences, through to artifactual variability.

Potential sources of artifactual variability have been described

by Stunkard (1957):

> "When an investigator is confronted with similar worms from different hosts, he has no precise way of determining whether he is dealing with one or more than one species. In such variable worms, which have no skeletal structure, and in which the shape is modified by the contraction of different sets of muscles; which may become sexually mature at one-fourth the maximum size and continue to grow as long as they live; in which the morphology is dependent on the degree of maturity; and in which the location and shape of organs are influenced by extension and contraction of the body, by accumulation of genital products (spermatozoa in the seminal vesicle or eggs in the uterus), or even by accumulation of fluid in the excretory vesicle; specific determination of a particular individual may pose an almost insoluble problem."

Artifactual variability also arises from variation in techniques used in fixing, staining, mounting and measuring specimens (Bakke, 1978b).

Variability can also be a reflection of the environment. Within any host species, the diet of the host, the age of the host, the number of parasites of the same species and the number and types of other parasites. in the host have all been shown to affect the the morphology of certain parasites (Haley, 1962). The species of host has been shown repeatedly to influence the morphology, as well as the patterns of growth and development, of Digenea (Watertor, 1967; Kinsella, 1971; Blankespoor, 1974; Bakke, 1978a; Kennedy, 1980). These sources of variability may reflect the interaction between the environment and the genotype as expressed in the phenotype, or they may reflect different genotypes which are adapted to, or selected under, certain conditions. It is often difficult to partition variability associated with environmental differences as either phenotypic or genotypic. Gould and Johnston (1972) suggested that the only way this could be accomplished is through experimental studies under uniform environmental conditions. Partitioning of this sort is discussed in detail in Chapter IV.

As a consequence of the variety of causes of variability and the relevance of the variability for parasite identification, parasitologists

must examine large samples from as many different environments as possible. Because it has been clearly demonstrated that the host species can influence the parasite, cross-infection experiments are essential in order to determine the variability resulting from host influences.

This research project has afforded an opportunity to study the variability of specimens of the genus *Typhlocoelum*, and to apply the polytypic or biological species concept to the parasites. Specimens obtained from different host species of different ages and sexes and from different geographical regions have been examined, and estimates of population parameters have been obtained.

2. MORPHOMETRIC VARIABILITY OF *Typhlocoelum* WITHIN HOST SPECIES

Morphometric comparison of *Typhlocoelum* obtained from the same host species revealed that some variability is associated with the age of the host and the geographical locality where it was collected. The sex of the host was not a source of morphometric variability in this study. In most cases, numerical variables that differed significantly were related to the body length and the distance between the gonads and the pharynx.

It is difficult to determine the biological significance of this variation within host species. Variability of *Typhlocoelum* related to the geographical location of the hosts may reflect genetically distinct populations of the parasite, although in migratory hosts, such as ducks, it is difficult to evaluate the degree to which their geographical separation reflects isolation. Variability related to the age of the host may be a consequence of the interaction between the different environments and the genotype,manifest in different phenotypes. Nevertheless, it has been shown that host age and geographical location are potential sources of variability, and to this extent they are important.

3. MORPHOMETRIC VARIABILITY OF *Typhlocoelum* AMONG HOST SPECIES

When the variability among host species was assessed, the results clearly demonstrated that there are significant differences among *Typhlocoelum* from various host species for every numerical variable. However, only

two variables characterized distinct groups: anterior testis length and posterior testis width. Flukes from all dabbling duck species examined have significantly smaller anterior and posterior testes than those from diving duck species. In addition, among the flukes from dabbling duck species, those from blue-winged teal, green-winged teal and shovelers have a larger posterior testis width than those from mallards, pintails and black ducks. The ability to group flukes from various host species was also demonstrated in the discriminant function analyses where it was not only possible to distinguish between specimens from diving and dabbling duck species, but it was also possible, within the dabbling duck species, to distinguish mallard, pintail and black duck flukes from blue-winged teal, green-winged teal and shoveler flukes. Although dabbling and diving duck worms were apparently distinguished in the discriminant function analyses primarily by the size of the testes, the two subsets within the dabbling duck species were not distinguished on the basis of testis size.

Presumably, the morphometric variability demonstrated among specimens from different host species may result from host-induced differences and/or from genetically different populations. The range of variability can be viewed as a spectrum. The greatest variation occurs between flukes from dabbling and diving ducks. Within each of these two groups there is variability among specimens from different host species, and within each host species, there is variability among the specimens. At each level, however, the extent of the variability decreases. The lowest level of natural variability would be individual variation among specimens from a single host.

4. COMPARISON OF CHARACTER VARIABLES AMONG *Typhlocoelum* WITHIN AND AMONG HOST SPECIES

Because specimens of *Typhlocoelum* from dabbling and diving duck species have been shown to fall into two distinct groups, morphometrically, it was of interest to determine whether this distinction could be detected using character variables as well.

Two of the character variables examined in this study can be

cited as potential sources of artifactual variability: position of the cirrus sac and maturity.

The posterior extent of the cirrus sac has been cited as a species-differentiating characteristic by previous researchers (see Ch. III, A). This character is a function of two variable phenomena. Firstly, the posterior extent of the cirrus sac depends on the volume of sperm contained within the cirrus sac and secondly, whether or not the cirrus sac extends posterior to the intestinal bifurcation is dependent on the volume of food contained in the intestine. Therefore, variability in the posterior extent of the cirrus sac is largely artifactual.

Stunkard (1957) noted that the degree of maturity of a parasite can influence its morphology and this was clearly demonstrated in Figure 3-6. The inclusion of immature specimens in calculations of mean body lengths would have dramatically altered the values.

In the past, researchers have used a number of criteria to distinguish a mature or gravid worm from an immature or juvenile one (Watertor, 1967; Roitman and Kazakov, 1977). Smyth (1966) considered that the most mature stage in strigeid maturation was the appearance of fully formed eggs. This is probably the best criterion to use, because it is applicable not only to digeneans with eggs that do not undergo embryonation in the uterus, but also to digeneans where the eggs become fully embryonated *in utero*.

In the case of the genus *Typhlocoelum*, complete embryonation of the eggs occurs inside the adult fluke. The miracidium which develops inside the egg has a prominent eyespot, and therefore, the presence of eyespots within the eggs was recognized as the character representative of the most fully formed *Typhlocoelum*. In order to minimize the influence of immature specimens when comparing parasites from different duck species, analyses were done using only mature flukes. Therefore this source of artifactual variability was eliminated in this study.

The variability in all of the other character variables presumably reflects either genetic and/or phenotypic differences among specimens.

A number of species of the subfamily Typhlocoelinae have been described and almost as many have been declared invalid (see Ch. III, A).

The majority of the distinguishing features used to substantiate the creation of the new species have been character variables, i.e. the extent of the vitellaria, the position of the genital pore, the position of the uterus relative to the testes and the shape of the testes. It has been shown repeatedly in this study that most of the character variables used previously to distinguish genera, species or subspecies in the subfamily Typhlocoelinae are extremely variable, even among specimens from a single host species. In fact, these character variables have been shown to exhibit all possible character states in worms from a single host species. With all these characters, the proportions of worms demonstrating each character state differed significantly between worms from dabbling and diving duck species. However, while proportions are potentially useful for distinguishing between populations of flukes, they are of limited value in distinguishing one specimen from another. Therefore, proportions should be considered only when dealing with large sample sizes.

The distinct differences observed in the percentage of mature worms obtained from dabbling and diving ducks may result from different developmental times. This hypothesis will be discussed further in the light of experimental data presented in Chapters V and VI.

Only two character variables were always found to be represented by a single character state regardless of the host species. Inner diverticula were present on the intestinal caeca of all flukes, and, in all flukes, the uterus was confined to the intercaecal region. These characters may therefore be used in a description of the genus *Typhlocoelum*.

Two character variables were useful in separating the specimens into two distinct groups: the anterior and posterior testis shapes. The first group would contain specimens with round testes, the majority of which are found in dabbling ducks; the second group would contain specimens with deeply lobed testes, the majority of which are found in diving ducks.

5. COMPARISON OF SPECIMENS OBTAINED IN THIS STUDY WITH RECORDED DESCRIPTIONS OF *Typhlocoelum*

As was indicated in the Introduction to this Chapter, researchers have agreed that round- and lobed-testis forms of *Typhlocoelum* do occur and are recognizable. The controversy arises over the taxonomic significance

of the shape of the testes.

The round- and lobed-testis forms were clearly identifiable in this study and summary statistics on all numerical and character variables for mature specimens of both forms of *Typhlocoelum* pooled from all host species are presented in Tables 3-12 and 3-13. Comparison of these data with measurements and descriptions recorded in the literature demonstrate that the round-testis *Typhlocoelum* obtained in the present study is indistinguishable from the type description of *Tracheophilus sisowi* provided by Skrjabin (1913) nor is it distinguishable from *Typhlocoelum sisowi* as described by Dubois (1959) or from *Typhlocoelum cucumerinum cymbium* as described by Macko and Buša (1960) (Table 3-14). The lobed-testis form of *Typhlocoelum* obtained in the present study agrees in all respects with *Typhlocoelum cucumerinum* as described by Dubois (1959) and with *Typhlocoelum cucumerinum cucumerinum* as described by Macko and Buša (1960) (Table 3-15).

Although Macko and Buša (1960) retained the name *cymbium* to identify the round-testis form, Dubois (1959) has demonstrated that the type material of *Monostomum cymbium* lacks inner diverticula. An attempt was made in this study to obtain the type specimens, but this was unsuccessful. Diesing (1850) found the type specimens in *Himanthopus wilsonii*, a chardriiform. In this study, specimens that had been obtained from a snipe, and identified as *Tracheophilus* sp. and deposited at the Animal Parasitology Institute in Beltsville, Maryland, were examined. These specimens also lacked inner diverticula.

In all of the specimens obtained from wild ducks in the present study, inner diverticula were present and clearly recognizable. It therefore seems most probable that the type material examined by Dubois is not representative of *Typhlocoelum* and that his removal of the species *cymbium* from the genus is justified. As has been explained in the Introduction to the Chapter, the round-testis form should hence be referred to as *sisowi*.

It has been shown that a few specimens from dabbling ducks have somewhat atypical testes. The presence of this form has caused much confusion in the past. Manter and Williams (1928) obtained a typhlocoelid

Table 3-12. Descriptive statistics on the numerical variables of the round-testis and lobed-testis forms of *Typhlocoelum*.

NUMERICAL VARIABLE*	ROUND-TESTIS *Typhlocoelum* n	MEAN (mm) ± 95% C.L.	LOBED-TESTIS *Typhlocoelum* n	MEAN (mm) ± 95% C.L.
BL	192	7.97 ± 0.16	136	8.64 ± 0.30
BW	205	3.27 ± 0.07	139	3.37 ± 0.14
PL	191	0.306 ± 0.007	131	0.274 ± 0.007
PW	191	0.291 ± 0.007	132	0.256 ± 0.008
OL	196	0.285 ± 0.008	138	0.318 ± 0.014
OW	196	0.320 ± 0.008	138	0.348 ± 0.014
SRL	151	0.266 ± 0.015	116	0.288 ± 0.021
SRW	151	0.328 ± 0.017	116	0.405 ± 0.021
ATL	184	0.271 ± 0.023	135	1.123 ± 0.068
ATW	183	0.278 ± 0.014	135	0.381 ± 0.033
PTL	196	0.267 ± 0.019	136	0.609 ± 0.037
PTW	196	0.400 ± 0.028	136	1.054 ± 0.059
EGGA	192	0.179 ± 0.059	129	0.314 ± 0.108
EYEA	192	3.22 ± 0.11	133	3.39 ± 0.24
PA	189	0.609 ± 0.016	132	0.548 ± 0.017
OA	187	5.97 ± 0.14	132	6.39 ± 0.24
SRA	150	6.38 ± 0.15	117	6.81 ± 0.26
ATA	178	5.64 ± 0.14	130	6.20 ± 0.24
PTA	188	6.52 ± 0.14	131	7.22 ± 0.27
NID	114	10.6 ± 0.2	124	11.8 ± 0.2
OES	163	0.099 ± 0.012	124	0.083 ± 0.010
CL	17	0.495 ± 0.076	14	0.667 ± 0.064
EL	20	0.137 ± 0.003	1	0.165 -
EW	20	0.078 ± 0.002	1	0.090 -

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Table 3-13. Proportions of round-testis and lobed-testis forms of *Typhlocoelum* demonstrating various character states for the character variables.

CHARACTER VARIABLE*	ROUND-TESTIS *Typhlocoelum* NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c	LOBED-TESTIS *Typhlocoelum* NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c
AT	198	0		0	138	
PT	207	0		0	139	
UBT	191	5	2	8	25	103
VITA	18	164	8	15	106	8
VITP	41	146	16	70	42	20
VENSUR	169	23	13	14	61	61
GP	25	5	5	2	10	7
CS	25	4		61	0	
UG	204	1		139	0	
ID	207	0		139	0	

* The abbreviations for the character variables are explained in the "List of Abbreviations".

** The character states for each character variable are described on pages 14 - 19.

Table 3-14. Descriptions of *Tracheophilus sisowi* recorded by Skrjabin (1913), of *Typhlocoelum sisowi* recorded by Dubois (1959) and of *Typhlocoelum cucumerinum sisowi* recorded by Macko and Busa (1960) (measurements in mm).

VARIABLE*	*Tracheophilus sisowi*	*Typhlocoelum sisowi*	*Typhlocoelum cucumerinum cucumerinum*		
HOST	BOSCHAS	-	DOM MAL	MAL	PIN
n	-	-	46	2	7
BL	7.56	5.5 - 11.5	3.9 - 11.4	9.8	7.8 - 10.0
BW	2.98	2.2 - 4.5	2.1 - 4.9	3.9	2.5 - 4.0
PL	0.29	0.270 - 0.390	0.18 - 0.42	0.308	0.205 - 0.329
PW	0.25	0.210 - 0.390	0.24 - 0.31	0.384	0.233 - 0.381
OL	0.45	-	0.135 - 0.512	0.411	0.260 - 0.432
OW	0.45	-	0.293 - 0.565	0.343	0.349 - 0.404
ATL	0.34	-	0.18 - 0.565	0.514	0.308 - 0.480
ATW	0.34	-	0.226 - 0.474	0.356	0.240 - 0.411
PTL	0.34	-	0.135 - 0.452	0.397	0.274 - 0.411
PTW	0.34	-	0.226 - 0.587	0.466	0.349 - 0.473
CL	-	-	0.361 - 0.497	0.473	0.480 - 0.926
EL	0.122	0.096 - 0.154	-	-	-
EW	0.063	0.050 - 0.081	-	-	-
PT**	a	a	a	a	a
GP**	-	a	b	b	a , b

* The abbreviations for host species and variables are explained in the "List of Abbreviations".

** The character states for each character variable are described on pages 14-19.

Table 3-15. Descriptions of *Typhlocoelum cucumerinum* recorded by Dubois (1959) and of *Typhlocoelum cucumerinum cucumerinum* recorded by Macko and Buša (1960). (measurements in mm).

VARIABLE*	*Typhlocoelum cucumerinum*	*Typhlocoelum cucumerinum cucumerinum*	
HOST	-	NYROCA	TUF
n	-	-	-
BL	4.0 - 13.0	9.5 - 12.5	9,3 - 15.1
BW	1.0 - 8.6	3.5 - 4.5	3.8 - 5.2
PL	0.190 - 0.520	0.301 - 0.360	0.329 - 0.418
PW	-	0.301 - 0.360	-
OL	-	0.381 - 0.365	0.286 - 0.617
OW	-	0.463 - 0.769	0.368 - 0.583
ATL	-	1.357 - 1.946	1.022 - 2.213
ATW	-	-	0.340 - 1.226
PTL	-	1.357 - 2.263	0.545 - 0.724
PTW	-	-	1.185 - 2.263
CL	-	0.994 - 1.335	1.090 - 1.199
EL	0.107 - 0.195	-	-
EW	0.060 - 0.095	-	-
GP**	b , c	a	a
PT**	b	b	b

* The abbreviations for host species and variables are explained in the "List of Abbreviations".

** The character states for each character variable are described on pages 14-19.

from a shoveler and found that it had slightly indented testes. They called the specimen *Typhlocoelum americanum*. Dollfus (1948) and Dubois (1959) both agreed that *T. americanum* should be synonymized with the lobed-testis *Typhlocoelum cucumerinum*, but Dubois (1965) changed his mind after examining the type specimen, and decided that *T. americanum* was synonymous with *Typhlocoelum sisowi*. Macko and Buša (1960) demonstrated quite clearly the range of variability in the shape of the testes, from round with smooth margins to oval and slightly indented to deeply lobed and branching. Although they did not believe that these three forms represented different species, they did give them subspecific recognition. They also suggested that, in some cases, the smooth, round form might become slightly indented as the flukes grew older.

Based on an examination of the specimens with slightly indented testes obtained in this study (see Figure 3-7), plus examination of the type material of *Typhlocoelum americanum* (see Table 3-16), it is clear that they resemble the round-testis form more closely than the lobed-testis form. Typically, the lobed testes are deeply indented or branched, and are also much larger than the round testes. The slightly indented testes are smaller with irregular margins and are definitely not branched. It is therefore believed that *T. americanum* is synonymous with the round-testis form of *Typhlocoelum*.

During the 1960's, two new descriptions of typhlocoelids were presented. Fotedar (1964) described a new species, *Typhlocoelum indicum*, from the air passages of a mallard in Kashmir, which he distinguished from other species on the basis of kidney-shaped testes. Jain (1966) described a new variety of typhlocoelid, *Tracheophilus sisowi acirratus*, from a pintail in India. The vitellaria of this fluke did not extend anteriorly to the pharynx, and the cirrus sac was feebly developed. The measurements for both of these parasites are presented in Table 3-17. Comparison with the present material reveals that the measurements of both *T. indicum* and *T. s. acirratus* agree with those obtained from the round-testis form of *Typhlocoelum* in this study. On the basis of the variability in the shape of the testes demonstrated by Macko and Buša (1960) and seen in the present study, there is no justification for separating Fotedar's material from

Table 3-16. Measurements made on the type specimen of *Typhlocoelum americanum* Manter and Williams, 1928 (measurements in mm).

VARIABLE*	VALUE
HOST	SHO
n	1
BL	5.99
BW	2.96
PL	0.210
PW	0.220
OL	0.210
OW	0.200
ATL	0.420
ATW	0.180
PTL	0.140
PTW	0.580
EGGA	0.000
EYEA	2.14
PA	0.370
AT**	
PT**	
VITA	b***
VITP	b***
VENSUR	a***

* The abbreviations for numerical and character variables are listed in the "List of Abbreviations".

** Drawing represents the shape of the testes.

*** The character states for the character variables are described on pages 14-19.

Table 3-17. Type descriptions of *Typhlocoelum indicum* as provided by Fotedar (1964) and of *Tracheophilus sisowi acirratus* as provided by Jain (1966) (measurements in mm).

VARIABLE*	*Typhlocoelum indicum*	*Tracheophilus sisowi acirratus*
HOST	MAL	PIN
n	7	2
BL	7.5 - 8.6	8.92
BW	2.4 - 2.6	3.16
PL	0.20 - 0.21	0.165
PW	0.193 - 0.195	0.045
OL	0.16 - 0.18	0.225
OW	0.14 - 0.15	0.480
ATL	0.40 - 0.43	0.420
ATW	0.194 - 0.197	0.510
PTL	0.60 - 0.64	0.375
PTW	0.23 - 0.26	0.420
NID	12 - 14	-
OES	0.044 - 0.046	-
EL	0.12 - 0.14	0.105
EW	0.085 - 0.090	0.075
AT	kidney-shaped	-
PT	kidney-shaped	-
VITA **	b	a
VITP **	b	b
CS **	b	feebly developed
GP **	c	a
UBT **	a	a
VENSUR **	a	c
UG **	-	a

* The abbreviations for numerical and character variables are listed in the "List of Abbreviations".

** The character states for the character variables are described on pages 14-19.

the round-testis *Typhlocoelum*. It has also been shown that the vitellaria in the round-testis form occasionally extend only to the intestinal bifurcation, and that the cirrus sac is subject to artifactual variability. Therefore, recognition of *T. s. acirratus* as a separate subspecies is not justified. Both *T. indicum* and *T. s. acirratus* are therefore considered to be synonyms of the round-testis *Typhlocoelum*.

Having studied the range of variability in specimens of *Typhlocoelum*, one fact becomes clear: there are two morphologically distinct forms of the parasite. These may be different species, or they may be different populations within the same species. Alternatively, the morphological differences may be the manifestation of a host-induced phenomenon. Data presented in the next chapter will be used to investigate these three hypotheses.

CHAPTER IV. EXPERIMENTAL ASSESSMENT OF THE TAXONOMIC RELATIONSHIP BETWEEN THE ROUND-TESTIS AND LOBED-TESTIS FORMS OF *Typhlocoelum*

A. INTRODUCTION

Three hypotheses were presented in Chapter III to explain the two different morphological forms of *Typhlocoelum*. The first hypothesis was that the round- and lobed-testis forms represent different species. Secondly, it was postulated that the two forms might be separate populations within the same species and finally it was proposed that the different morphologies might be the result of a host-induced phenomenon.

Presumably, if the two forms are true species, the progeny of each will retain the parental testis shape regardless of the host species. Alternatively, if the two forms are the manifestation of a host-induced phenomenon, the progeny will have round testes when parasitic in dabbling ducks, and lobed testes when parasitic in diving ducks, regardless of the parental morphology.

The applicability of any of the three hypotheses is intimately related to the separation of genetic and phenotypic variation. These two sources of variability have been discussed in Chapter III, but without experimental data, it is difficult to separate the two. Gould and Johnston (1972) have suggested that "either we must find a way to sort genetic from nongenetic sources of variation or we must measure genetic variation directly and not through the distorted mirror of its phenotype. There is only one satisfactory way to sort the two components ... collect live material, raise the progeny of each sample in the laboratory under uniform conditions, and assume that the resulting variation is genetic in origin". This is precisely the approach that has been used in this chapter to elucidate which of the three hypotheses best explains the two morphological forms of *Typhlocoelum*.

B. MATERIALS AND METHODS

Adult *Typhlocoelum* obtained from wild ducks in southern Manitoba were divided into three experimental groups on the basis of testis morphology

and host species. Round-testis specimens obtained from dabbling duck species (R-DAB) were used for the first set of experiments. Lobed-testis specimens obtained from diving duck species (L-DIV) were used for the second set of experiments. The final set of experiments were undertaken using a single round-testis specimen that had been obtained from a redhead (diving duck) (R-DIV). For convenience, the source of infective material will generally be referred to as R-DAB, L-DIV or R-DIV.

Miracidia obtained from the adult specimens were used to infect laboratory-reared *Physa gyrina* which were maintained until the metacercariae had developed. The whole snails were then intubated into ducklings. Three species of dabbling ducks (mallards, blue-winged teal and gadwall) and three species of diving ducks (canvasbacks, redheads and lesser scaup) were used as experimental hosts for *Typhlocoelum*. The ducks were necropsied either between 40 and 52 days post-infection or later than 55 days post-infection (Table 4-1). For convenience in presenting and discussing the results, these times will be referred to as 45 and 70 days post-infection, respectively. All ducks were necropsied according to the procedure described in Chapter V. All specimens were stained, mounted and the numerical and character variables were assessed.

The percent recovery of the flukes (100 x total number of specimens recovered divided by the total number of metacercariae used in the infections), the position of the flukes in the ducks (abdominal cavity, air sacs, lungs and trachea) and the morphology of the specimens were compared among the various host species from the three sources of *Typhlocoelum*.

C. RESULTS

1. ROUND-TESTIS *Typhlocoelum* FROM DABBLING DUCKS AS A SOURCE OF INFECTIVE MATERIAL

a) RECOVERY

Only two species of ducks (mallards and canvasbacks) were examined at 45 days post-infection. Six of nine mallards were infected with up to 12 parasites; four of nine canvasbacks were infected with up to five parasites. Five species of ducks were examined at 70 days post-infection: ten of the 29 mallards examined were infected with up to five flukes;

Table 4-1. Protocol for experimental cross-infections.

SOURCE OF *Typhlocoelum*	EXPERIMENTAL HOST	NUMBER OF DUCKS NECROPSIED AT: 45 DAYS P-I*	70 DAYS P-I
R-DAB[1]	MALLARD	9	29
	BLUE-WINGED TEAL	-	10
	GADWALL	-	6
	† CANVASBACK	9	9
	† LESSER SCAUP	-	3
L-DIV[2]	MALLARD	1	4
	† CANVASBACK	2	-
	† REDHEAD	-	4
	† LESSER SCAUP	-	2
R-DIV[3]	MALLARD	2	-
	† CANVASBACK	-	2

* Post-infection.

1 Round-testis *Typhlocoelum* from dabbling ducks.

2 Lobed-testis *Typhlocoelum* from diving ducks.

3 Round-testis *Typhlocoelum* from a redhead (diving duck).

† Diving duck species.

six of the nine canvasbacks examined were infected with up to 21 flukes; only one of the ten blue-winged teal was infected (two worms); none of the gadwall or lesser scaup were infected when necropsied at 70 days post-infection.

A comparison of the overall percent recovery of R-DAB flukes (Figure 4-1) reveals that, in mallards, the recovery decreased significantly between 45 and 70 days post-infection whereas, in canvasbacks, the percent recovery significantly increased.

Among the dabbling duck species at 70 days post-infection, the mean recovery was highest in mallards, intermediate in blue-winged teal and lowest in gadwall, although the differences were not statistically significant. Among the diving duck species, significantly more R-DAB flukes were recovered from canvasbacks than from lesser scaup at 70 days post-infection.

At 45 days post-infection, significantly more R-DAB specimens were recovered from mallards than from canvasbacks whereas, at 70 days post-infection, a significantly higher percentage of R-DAB flukes were recovered from canvasbacks than from mallards.

b) LOCATION OF SPECIMENS IN THE HOST

A higher percentage of specimens was recovered from the trachea of canvasbacks than mallards at both 45 days post-infection (100% of 8, and 66% of 35, respectively) and 70 days post-infection (94% of 32, and 29% of 21, respectively). At 45 and 70 days post-infection, specimens from mallards were also found in the abdominal cavity (6% and 24%, respectively), air sacs (20% and 38%, respectively), and lungs (9% and 10%, respectively). The remaining 6% of the 32 specimens recovered from canvasbacks at 70 days post-infection were located in the air sacs.

c) MORPHOLOGY OF SPECIMENS

Morphometric data for specimens recovered from the trachea at 45 days post-infection are presented in Table 4-2. Although mallard and canvasback specimens differed significantly with respect to ovary length, posterior testis width and cirrus sac length, the specimens recovered from

Figure 4-1. Graphs showing percentage of R-DAB *Typhlocoelum* recovered from various species of ducks at 45 and 70 days post-infection.

A, 45 days post-infection;

B, 70 days post-infection.

The abbreviations for host species are explained in the "List of Abbreviations".

● Dabbling Duck Species;

★ Diving Duck Species.

Bars represent 95% confidence limits.

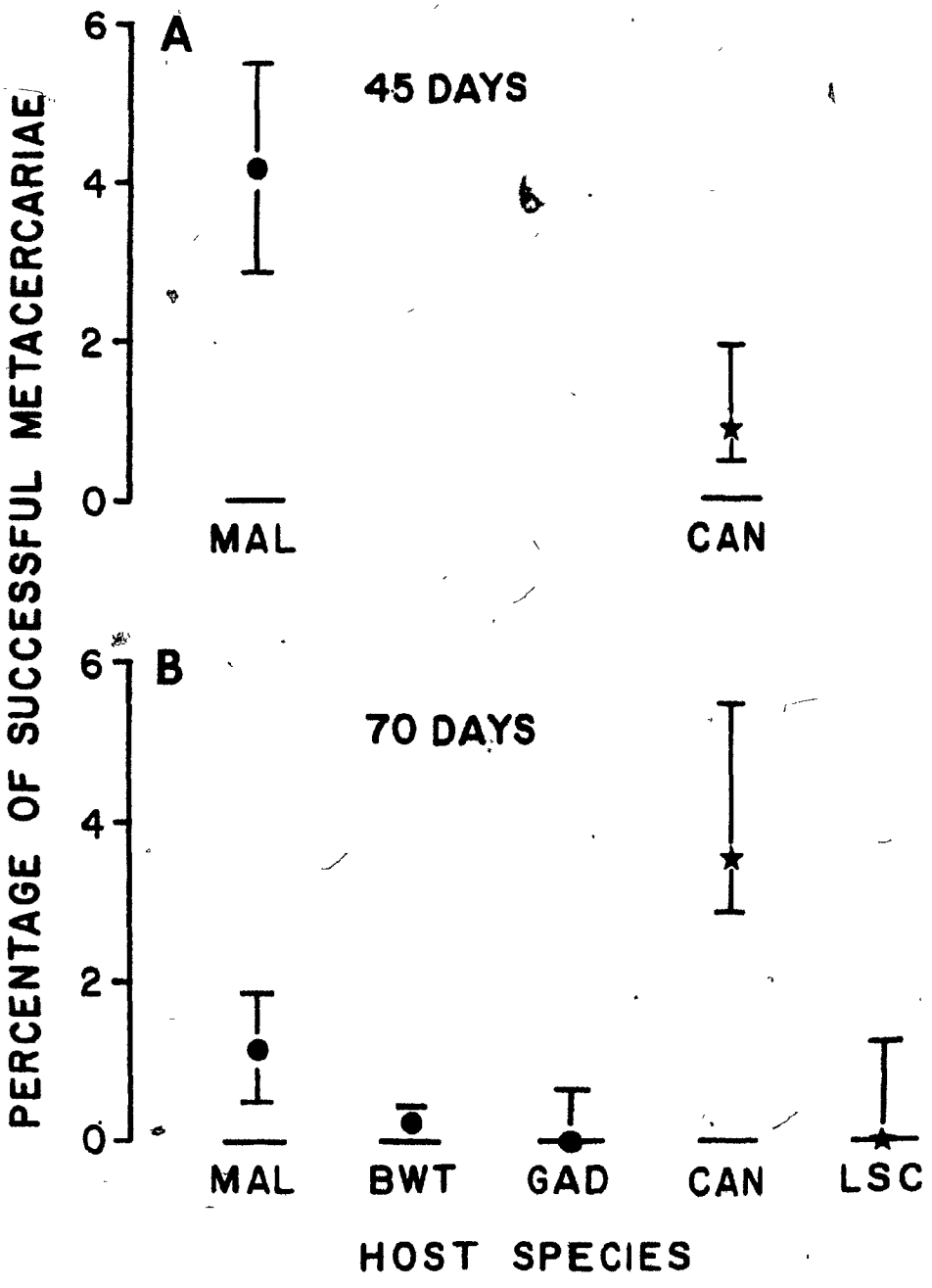

A
45 DAYS
6
4
2
0
MAL
CAN
B
70 DAYS
6
4
2
0
MAL
BWT
GAD
CAN
LSC
HOST SPECIES
PERCENTAGE OF SUCCESSFUL METACERCARIAE

Table 4-2. Descriptive statistics of the numerical variables of specimens of R-DAB *Typhlocoelum* obtained from the trachea of various species of ducks at 45 days post-infection.

NUMERICAL VARIABLE*	MALLARD n	MALLARD MEAN (mm) ± 95% C.L.	CANVASBACK n	CANVASBACK MEAN (mm) ± 95% C.L.
BL	22	7.35 ± 0.94	5	8.17 ± 0.90
BW	22	2.85 ± 0.35	5	3.28 ± 0.18
PL	22	0.241 ± 0.022	5	0.285 ± 0.029
PW	22	0.239 ± 0.023	5	0.246 ± 0.036
OL	19	0.274 ± 0.024	5	0.364 ± 0.047
OW	19	0.308 ± 0.025	5	0.358 ± 0.052
SRL	16	0.249 ± 0.048	5	0.183 ± 0.100
SRW	16	0.377 ± 0.044	5	0.305 ± 0.109
ATL	20	0.322 ± 0.061	5	0.276 ± 0.056
ATW	20	0.364 ± 0.049	5	0.285 ± 0.057
PTL	19	0.317 ± 0.033	5	0.293 ± 0.033
PTW	19	0.515 ± 0.079	5	0.364 ± 0.032
PA	22	0.544 ± 0.062	5	0.640 ± 0.060
OA	19	5.95 ± 0.25	5	5.90 ± 0.72
SRA	17	6.24 ± 0.24	5	6.17 ± 0.81
ATA	20	5.56 ± 0.22	5	5.63 ± 0.95
PTA	19	6.40 ± 0.26	5	6.45 ± 0.87
NID	17	10.0 ± 0.4	5	10.4 ± 1.4
OES	18	0.076 ± 0.048	5	0.127 ± 0.037
CL	5	0.606 ± 0.195	3	0.301 ± 0.053
OSL	1	0.108 -	-	- -
OSW	1	0.119 -	-	- -
VSL	2	0.060 -	-	- -
VSW	2	0.076 -	-	- -
VSA	2	0.402 -	-	- -

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

canvasbacks did have round testes and were otherwise identical in all character variables to the specimens from mallards (Table 4-3).

At 70 days post-infection, the specimens recovered from the trachea of mallards were smaller than those from canvasbacks (Table 4-4) and a higher proportion of mallard specimens were immature (Table 4-5). Specimens recovered from both mallards and blue-winged teal had round testes (Table 4-5). In five of the six infected canvasbacks, all of the specimens recovered had round testes. In the sixth duck, however, only two of the 21 flukes had round testes, and the remaining 19 flukes had deeply lobed testes. Associated with the change in shape of the testes was a change in the number of uterine loops separating the testes and a change in the topography of the ventral surface (Table 4-5).

Specimens recovered from the abdominal cavity, air sacs and lungs in both mallards and canvasbacks at 70 days post-infection were immature and indistinguishable (Table 4-6).

2. LOBED-TESTIS *Typhlocoelum* FROM DIVING DUCKS AS A SOURCE OF INFECTIVE MATERIAL

a) RECOVERY

At 45 days post-infection, 41% of the L-DIV metacercariae fed to two canvasbacks were recovered (Figure 4-2). Recovery from lesser scaup was significantly lower than recovery from redheads at 70 days post-infection. Although five mallards were exposed to L-DIV metacercariae, none of them were infected when necropsied at 45 or 70 days post-infection.

b) LOCATION OF SPECIMENS IN THE HOST

At 45 days post-infection, 87% of 62 specimens in canvasbacks were located in the trachea. Three of the remaining eight specimens were located in the air sacs and five were located in the lungs. At 70 days post-infection, all of the specimens recovered from redheads ($n = 83$) and lesser scaup ($n = 2$) were located in the trachea.

c) MORPHOLOGY OF SPECIMENS

The mean measurements of day 45 and day 70 specimens recovered

Table 4-3. Number of R-DAB *Typhlocoelum* recovered from the trachea of mallards and canvasbacks at 45 days post-infection demonstrating various character states for the character variables.

CHARACTER VARIABLE*	MALLARD NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c	CANVASBACK NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c
AT	20	0		5	0	
PT	20	0		5	0	
EGG	20	2		5	0	
EYE	18	4		5	0	
MAT	18	4		5	0	
UBT	19	1	0	5	0	0
VITA	0	18	0	0	5	0
VITP	3	15	2	0	5	0
VENSUR	13	8	1	5	0	0
GP	4	0	1	0	0	3
CS	1	3		3	0	
UG	22	0		5	0	

* The abbreviations for the character variables are explained in the "List of Abbreviations".

** The character states for each character variable are described on pages 14-19.

Table 4-4. Descriptive statistics of the numerical variables of specimens of R-DAB *Typhlocoelum* obtained from the trachea of various species of ducks at 70 days post-infection.

NUMERICAL VARIABLE*	MALLARD n	MALLARD MEAN (mm) ± 95% C.L.	BLUE-WINGED TEAL n	BLUE-WINGED TEAL MEAN (mm)	CANVASBACK n	CANVASBACK MEAN (mm) ± 95% C.L.
BL	5	3.189 ± 4.144	2	8.82	30	8.31 ± 0.58
BW	5	1.298 ± 1.684	2	2.88	30	3.36 ± 0.27
PL	5	0.157 ± 0.104	2	0.278	30	0.285 ± 0.021
PW	5	0.142 ± 0.110	2	0.330	30	0.255 ± 0.024
OL	2	0.180 –	2	0.230	30	0.274 ± 0.032
OW	2	0.176 –	2	0.287	30	0.294 ± 0.034
SRL	2	0.226 –	–	–	29	0.286 ± 0.035
SRW	2	0.185 –	–	–	29	0.376 ± 0.040
ATL	2	0.106 –	2	0.306	28	0.689 ± 0.179
ATW	2	0.204 –	2	0.290	28	0.290 ± 0.036
PTL	2	0.146 –	2	0.247	28	0.391 ± 0.062
PTW	2	0.162 –	2	0.515	28	0.663 ± 0.137
PA	5	0.295 ± 0.336	–	–	30	0.635 ± 0.030
OA	2	4.316 –	–	–	30	6.02 ± 0.50
SRA	2	4.431 –	–	–	30	6.24 ± 0.52
ATA	2	3.932 –	–	–	30	5.74 ± 0.42
PTA	2	4.61 –	–	–	30	6.65 ± 0.54
NID	5	10.2 ± 1.0	–	–	29	11.1 ± 0.3
OES	5	0.047 ± 0.041	–	–	30	0.048 ± 0.019

... CONTINUED ...

Table 4-4. ... CONTINUED ...

NUMERICAL VARIABLE*	MALLARD n	MALLARD MEAN (mm) ± 95% C.L.	BLUE-WINGED TEAL n	BLUE-WINGED TEAL MEAN (mm)	CANVASBACK n	CANVASBACK MEAN (mm) ± 95% C.L.
CL	-	- -	-	-	2	0.690 -
OSL	3	0.091 ± 0.069	-	-	-	- -
OSW	3	0.105 ± 0.007	-	-	-	- -
VSL	3	0.071 ± 0.030	-	-	-	- -
VSW	3	0.077 ± 0.024	-	-	-	- -
VSA	3	0.475 ± 0.180	-	-	-	- -

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Table 4-5. Number of R-DAB *Typhlocoelum* obtained from the trachea of various species of ducks at 70 days post-infection demonstrating various character states for the character variables.

CHARACTER VARIABLE*	MALLARD NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c	BLUE-WINGED TEAL NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c	CANVASBACK NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c
AT	2	0		2	0		10	20	
PT	2	0		2	0		11	19	
EGG	1	4		2	0		30	0	
EYE	1	4		2	0		14	16	
MAT	1	4		2	0		14	16	
UBT	2	0	0	2	0	0	10	0	20
VITA	0	1	0	0	2	0	2	26	2
VITP	0	1	0	0	2	0	9	18	2
VENSUR	2	0	3	2	0	0	13	3	14
GP							2	1	1
CS							3	1	
UG	5	0		2	0		30	0	

* The abbreviations for the character variables are explained in the "List of Abbreviations".

** The character states for each character variable are described on pages 14-19.

Table 4-6. Descriptive statistics of the numerical variables of specimens of R-DAB *Typhlocoelum* recovered from sites other than the trachea in mallards and canvasbacks at 70 days post-infection.

NUMERICAL VARIABLE*	MALLARD n	MALLARD MEAN (mm) ± 95% C.L.	CANVASBACK n	CANVASBACK MEAN (mm)
BL	13	1.238 ± 0.120	2	1.588
BW	13	0.674 ± 0.048	2	0.634
PL	12	0.098 ± 0.006	2	0.248
PW	12	0.088 ± 0.006	2	0.250
PA	11	0.151 ± 0.016	2	0.182
NID	10	10.3 ± 0.5	-	-
OES	12	0.026 ± 0.025	2	0.092
OSL	11	0.101 ± 0.014	2	0.130
OSW	10	0.108 ± 0.012	2	0.144
VSL	13	0.061 ± 0.006	2	0.094
VSW	13	0.077 ± 0.007	2	0.094
VSA	11	0.456 ± 0.050	2	0.585

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Figure 4-2. Graphs showing percentage of L-DIV *Typhlocoelum* recovered from various species of ducks at 45 and 70 days post-infection.

A, 45 days post-infection;

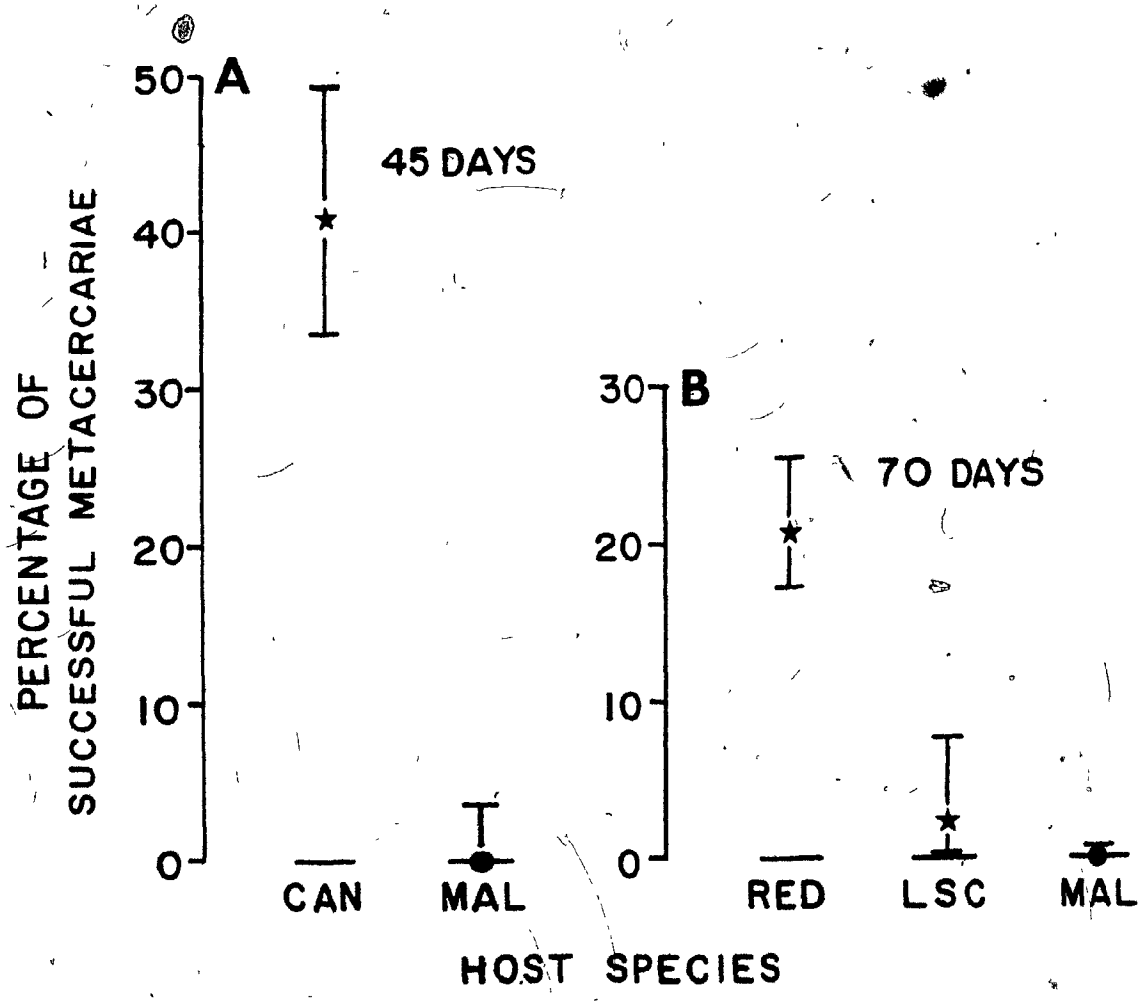
B, 70 days post-infection.

The abbreviations for host species are explained in the "List of Abbreviations".

● Dabbling Duck Species;

★ Diving Duck Species.

Bars represent 95% confidence limits.


A
50
40
30
20
10
0
45 DAYS
B
30
20
10
0
70 DAYS
PERCENTAGE OF SUCCESSFUL METACERCARIAE
CAN
MAL
RED
LSC
MAL
HOST SPECIES

from diving ducks are presented in Tables 4-7 and 4-8, respectively. In all of the specimens recovered from canvasbacks, redheads and lesser scaup, the testes were deeply lobed. The flukes resembled the lobed-testis *Typhlocoelum* with respect to other numerical and character variables as well (Tables 4-9, 4-10).

3. ROUND-TESTIS *Typhlocoelum* FROM DIVING DUCKS AS A SOURCE OF INFECTIVE MATERIAL

A single round-testis *Typhlocoelum* was obtained from a wild redhead and it was used as a source of infective material for two mallards and two canvasbacks. All four ducks became infected. The mallards were examined at 45 days post-infection; one harboured one fluke and the other harboured 15 flukes. Three flukes were recovered from the two canvasbacks examined at 70 days post-infection.

Of the flukes recovered from mallards, 50% were located in the trachea, 25% were located in the air sacs and 25% were located in the abdominal cavity. All three specimens from canvasbacks were located in the trachea.

All of the flukes recovered from the trachea of both mallards and canvasbacks had round testes and were not distinguishable from the typical round-testis *Typhlocoelum* in any numerical or character variables (Table 4-11, Table 4-12). The eight specimens recovered from the air sacs and abdominal cavity of mallards were immature and could not be distinguished from the immature R-DAB specimens recovered from mallards and canvasbacks at 70 days post-infection.

D. DISCUSSION

1. COMPARISON OF PERCENT RECOVERY

a) CONTROL INFECTIONS

Among the dabbling duck species, the recovery of R-DAB flukes decreased significantly in mallards between 45 and 70 days post-infection, and in blue-winged teal and gadwall, the recovery of R-DAB flukes at 70 days post-infection was extremely low. Sreekumaran and Peter (1973a) have suggested that the round-testis *Typhlocoelum* are coughed out of the

Table 4-7. Descriptive statistics of the numerical variables of specimens of L-DIV *Typhlocoelum* recovered from the trachea of canvasbacks at 45 days post-infection.

NUMERICAL VARIABLE*	n	CANVASBACK MEAN (mm) ± 95% C.L.
BL	53	8.49 ± 0.70
BW	53	3.32 ± 0.24
PL	48	0.247 ± 0.013
PW	50	0.223 ± 0.014
OL	51	0.297 ± 0.031
OW	51	0.323 ± 0.032
SRL	49	0.242 ± 0.031
SRW	49	0.453 ± 0.057
ATL	49	1.146 ± 0.152
ATW	48	0.500 ± 0.061
PTL	50	0.662 ± 0.079
PTW	50	1.152 ± 0.144
PA	50	0.597 ± 0.026
OA	51	6.29 ± 0.55
SRA	50	6.40 ± 0.57
ATA	49	6.14 ± 0.51
PTA	50	7.09 ± 0.62
NID	53	11.3 ± 0.2
OES	48	0.051 ± 0.021
CL	12	0.489 ± 0.082

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Table 4-8. Descriptive statistics of the numerical variables of specimens of L-DIV *Typhlocoelum* recovered from the trachea of various species of ducks at 70 days post-infection.

NUMERICAL VARIABLE*	REDHEAD n	REDHEAD MEAN (mm) ± 95% C.L.	LESSER SCAUP n	LESSER SCAUP MEAN (mm)
BL	81	9.48 ± 0.40	2	10.32
BW	81	3.78 ± 0.14	2	4.12
PL	80	0.271 ± 0.005	2	0.240
PW	80	0.252 ± 0.005	2	0.254
OL	80	0.331 ± 0.014	1	0.381
OW	80	0.371 ± 0.015	1	0.437
SRL	79	0.293 ± 0.019	1	0.212
SRW	79	0.503 ± 0.022	1	0.282
ATL	80	1.267 ± 0.086	1	1.250
ATW	80	0.470 ± 0.036	1	0.818
PTL	80	0.684 ± 0.034	1	0.874
PTW	80	1.292 ± 0.063	1	1.285
PA	80	0.619 ± 0.014	2	0.416
OA	80	7.04 ± 0.30	1	8.45
SRA	79	7.33 ± 0.31	1	8.77
ATA	80	6.75 ± 0.28	1	8.36
PTA	80	7.89 ± 0.33	1	9.51
NID	81	11.8 ± 0.2	2	12.0
OES	81	0.058 ± 0.009	2	0.000
CL	4	0.825 ± 0.203	-	-

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Table 4-9. Number of L-DIV *Typhlocoelum* recovered from the trachea of canvasbacks at 45 days post-infection demonstrating various character states for the character variables.

CHARACTER VARIABLE*	CANVASBACK NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c
AT	0	51	
PT	0	51	
EGG	46	7	
EYE	15	38	
MAT	15	38	
UBT	1	3	42
VITA	12	35	1
VITP	23	19	6
VENSUR	0	6	47
GP	2	6	4
CS	31	2	
UG	53	0	

* The abbreviations for the character variables are explained in the "List of Abbreviations".

** The character states for each character variable are described on pages 14-19.

Table 4-10. Number of L-DIV *Typhlocoelum* recovered from the trachea of redheads and lesser scaup at 70 days post-infection demonstrating various character states for the character variables.

CHARACTER VARIABLE*	REDHEAD NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c	LESSER SCAUP NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c
AT	0	81		0	2	
PT	0	81		0	2	
EGG	80	1		2	0	
EYE	70	11		0	2	
MAT	70	11		0	2	
UBT	0	1	79	0	0	2
VITA	2	73	4	0	1	0
VITP	25	37	17	2	0	0
VENSUR	0	11	70	0	1	1
GP	0	2	3			
CS	33	0		1	0	
UG	81	0		2	0	

*. The abbreviations for the character variables are explained in the "List of Abbreviations".

** The character states for each character variable are described on pages 14-19.

Table 4-11. Descriptive statistics of the numerical variables of specimens of R-DIV *Typhlocoelum* recovered from the trachea of mallards at 45 days post-infection and canvasbacks at 70 days post-infection.

NUMERICAL VARIABLE*	MALLARD n	MALLARD MEAN (mm) ± 95% C.L.	CANVASBACK n	CANVASBACK MEAN (mm) ± 95% C.L.
BL	8	6.97 ± 2.40	3	9.21 ± 1.19
BW	8	2.67 ± 0.91	3	3.29 ± 0.63
PL	8	0.249 ± 0.057	3	0.319 ± 0.020
PW	8	0.234 ± 0.056	3	0.291 ± 0.020
OL	7	0.268 ± 0.060	3	0.357 ± 0.158
OW	7	0.330 ± 0.057	3	0.367 ± 0.036
SRL	7	0.242 ± 0.092	2	0.332 -
SRW	7	0.375 ± 0.081	2	0.360 -
ATL	7	0.238 ± 0.062	3	0.240 ± 0.092
ATW	7	0.294 ± 0.068	3	0.273 ± 0.080
PTL	7	0.300 ± 0.100	3	0.306 ± 0.267
PTW	7	0.364 ± 0.050	3	0.338 ± 0.035
PA	8	0.586 ± 0.179	3	0.602 ± 0.158
OA	7	5.54 ± 1.24	3	5.56 ± 4.49
SRA	7	5.76 ± 1.28	3	5.83 ± 4.73
ATA	7	5.46 ± 1.27	3	5.11 ± 4.12
PTA	7	6.00 ± 1.36	3	6.10 ± 4.99
NID	6	10.3 ± 0.9	3	10.0 ± 2.5
OES	8	0.087 ± 0.056	3	0.061 ± 0.133

* The abbreviations for the numerical variables are explained in the "List of Abbreviations".

Table 4-12. Number of R-DIV *Typhlocoelum* recovered from the trachea of mallards at 45 days post-infection and canvasbacks at 70 days post-infection demonstrating various character states for the character variables.

CHARACTER VARIABLE*	MALLARD NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c	CANVASBACK NUMBER OF SPECIMENS IN EACH CHARACTER STATE**: a	b	c
AT	6	1		3	0	
PT	7	0		3	0	
EGG	6	2		3	0	
EYE	6	2		3	0	
MAT	6	2		3	0	
UBT	6	0	0	3	0	0
VITA	0	6	0	0	3	0
VITP	1	5	0	0	3	0
VENSUR	3	1	4	1	0	2
UG	8	0		3	0	

* The abbreviations for the character variables are explained in the "List of Abbreviations".

** The character states for each character variable are described on pages 14-19.

trachea at two months post-infection; this might account for the significant decrease in recovery from mallards between 45 and 70 days post-infection, and might also account for the low recoveries in blue-winged teal and gadwall at 70 days post-infection.

Among the diving duck species, the percent recovery of L-DIV flukes also decreased significantly between 45 and 70 days post-infection.

b) CROSS INFECTIONS

At 45 days post-infection, significantly more flukes were recovered from the control infections (R-DAB to mallards and L-DIV to canvasbacks) than from the respective cross infections (R-DAB to canvasbacks and L-DIV to mallards). Similarly, at 70 days post-infection, significantly more flukes were recovered from the control infections in redheads than in the cross infections in mallards. However, at 70 days post-infection, the recovery from the cross infection of R-DAB metacercariae into canvasbacks was significantly higher than the control, R-DAB to mallard, infection. Data at 70 days post-infection was obtained from six canvasbacks, five of which harboured five or fewer round-testis flukes, and one which harboured two round-testis flukes and 19 lobed-testis flukes. The recovery of lobed-testis flukes in control infections has been shown to be much higher than that of round-testis flukes, and it is conceivable that the unusually high recovery in the R-DAB-infected canvasbacks at 70 days post-infection was related to the presence of the lobed-testis flukes.

In both mallards and canvasbacks, the percent recovery of R-DIV and R-DAB flukes did not differ significantly (Figure 4-1, Figure 4-3).

2. LOCATION OF *Typhlocoelum*

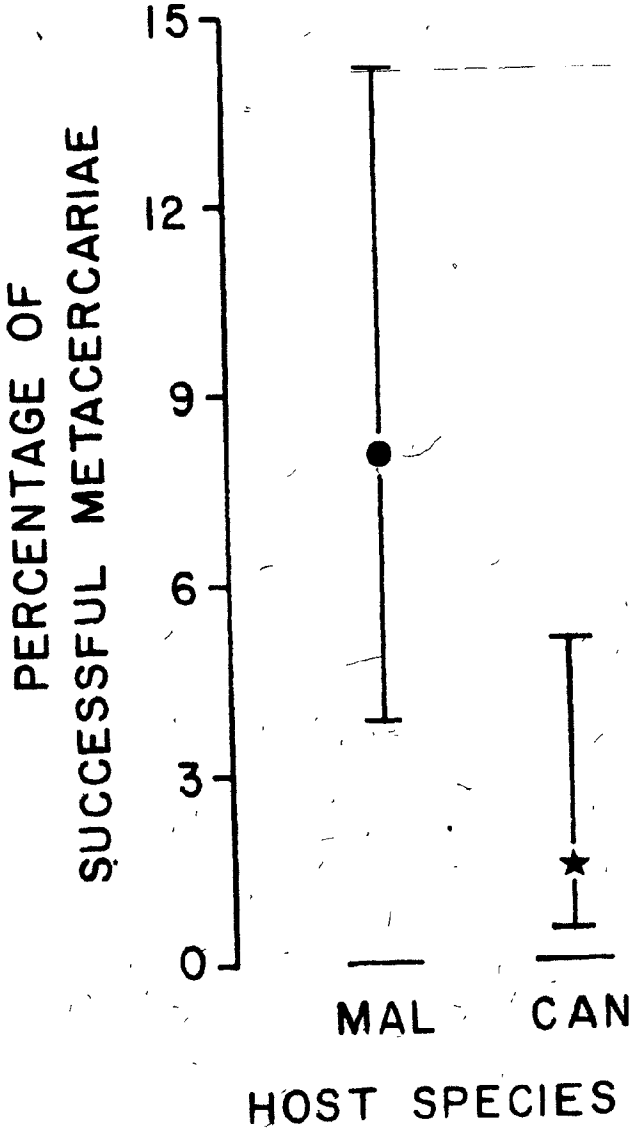
In control infections at 45 days post-infection, 66% of the R-DAB specimens and 87% of the L-DIV specimens were located in the trachea of mallards and canvasbacks, respectively. At 70 days post-infection, there was a dramatic difference in the proportion of specimens recovered from the tracheas between the control R-DAB and L-DIV infections. In L-DIV infections in redheads, all of the specimens were located in the trachea whereas, in R-DAB infections in mallards, only 29% of the flukes were located in the

Figure 4-3. Graph showing percentage of R-DIV *Typhlocoelum* recovered from mallards at 45 days post-infection and from canvasbacks at 70 days post-infection.

● Dabbling Duck Species;

★ Diving Duck Species.

Bars represent 95% confidence limits.


PERCENTAGE OF
SUCCESSFUL METACERCARIAE
15
12
9
6
3
0
MAL
CAN
HOST SPECIES

trachea. The remaining 71% of the R-DAB specimens were very small (Table 4-14) and were distributed throughout the abdominal cavity, the air sacs and the lungs. Small flukes were also recovered at 70 days post-infection from the air sacs in the cross infection of R-DAB metacercariae into canvasbacks.

3. MORPHOLOGICAL COMPARISON OF *Typhlocoelum* IN CONTROL AND CROSS INFECTIONS

In terms of clarifying the taxonomic relationship between the round- and lobed-testis forms of *Typhlocoelum*, the most important aspect of these experiments was a morphological comparison of the specimens recovered from cross infections in order to determine whether the flukes retained the morphological characteristics of the parental stock or whether their morphology reflected a host-induced phenomenon.

Canvasbacks infected with round-testis source *Typhlocoelum* were examined at 45 and 70 days post-infection, and a total of 40 specimens were recovered from ten ducks. All of the specimens recovered at 45 days post-infection had small,round testes. The mean body length was not significantly different from the control R-DAB to mallard specimens. The posterior testis was, in fact, significantly narrower in canvasback specimens than in mallard specimens. Therefore, the parental round-testis morphology had been retained in the R-DAB flukes in canvasbacks. In five of the six infected canvasbacks examined at 70 days post-infection, all specimens recovered had small round testes, but in the sixth canvasback, 19 of the 21 flukes recovered had large,deeply lobed testes and the other two had small,round testes. In this sixth duck, a fundamental change in the testis shape occurred whereby the parental round-testis form developed into the lobed-testis form in the "offspring".

When the character variables other than testis shape were examined, it was found that, in general, specimens with round testes exhibited similar character states to the control round-testis flukes in mallards. Round-testis specimens from mallards typically have deep, obvious indentations on the ventral surface, several uterine loops separating the testes, vitellaria that extend posteriorly to the excretory bladder and a genital pore that is positioned in the anterior region of the pharynx (see Chapter III). At 45 days post-infection, all specimens recovered from canvasbacks

infected with round-testis metacercariae demonstrated these typical traits. At 70 days post-infection, specimens recovered from R-DAB-infected canvasbacks fell into two groups. Those with round testes had the typical round-testis traits described above. Specimens with lobed testes demonstrated typical lobed-testis traits, i.e. no uterine loops between the testes, a smooth to slightly indented ventral surface, and a genital pore that is positioned in the posterior region of the pharynx.

It was also of interest to examine the morphology of "offspring" from a round-testis fluke that had been obtained in a diving duck. The specimens from both mallards and canvasbacks had round testes and were similar in all respects to the typical round-testis flukes.

Generally, then, the morphology of the "offspring" did not differ from that of the parental stock. However, in one case, metacercariae obtained from round-testis *Typhlocoelum* developed into 19 lobed-testis flukes and two round-testis flukes, each exhibiting all typical features for the respective testis shapes.

4. IDENTIFICATION OF THE TWO FORMS OF *Typhlocoelum*

a) TWO SPECIES HYPOTHESIS

The taxonomy of *Typhlocoelum* has been summarized in Chapter III, and three hypotheses were suggested that might explain the differences noted between the round- and lobed-testis forms. Certainly, given the morphological distinctions coupled with the fairly consistent host specificity, there would be little doubt in the minds of most digenean taxonomists that these are two separate species.

This decision would have a number of implications, if one accepts that species are "groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr, 1970). Certainly, being separate species would imply that the round-testis and lobed-testis forms would not normally cross-fertilize. Also, it would follow that each species would "breed true"; that is, the offspring would normally be expected to retain the distinguishing characteristics of the parental species. One might predict either that experimental infection of diving ducks with the round-testis "species" would not succeed, or that, if the cross infections

were successful, the morphological features of the "species" would be retained.

When the lobed-testis form was used to experimentally infect dabbling ducks (mallards) the above implications held true, as no specimens were recovered. Whether the absence of flukes was related to the inability of the L-DIV metacercariae to excyst and penetrate the intestinal wall of mallards, or whether the absence of infections indicates that the flukes were not able to establish, once inside the abdominal cavity, can not be determined from these data. When the reciprocal experiment was undertaken, and the round-testis form was used to experimentally infect diving ducks (canvasbacks), the infections were successful. At 45 days post-infection, the parasites were morphologically indistinguishable from the control specimens from mallards. To this point, then, there is no evidence for rejecting the two species hypothesis.

At day 70, however, one canvasback that had been exposed to metacercariae obtained from the round-testis form, harboured an unusually high number of flukes (21) in the trachea, compared to the control infections in mallards. Ninety percent of these flukes had lobed testes and were indistinguishable from the typical lobed-testis form. Thus, some of the eggs recovered from the round-testis "species" produced metacercariae which developed into the lobed-testis adults. This is an obvious contradiction of the two species hypothesis. This experimental host also harboured two flukes with round testes that in all ways resembled the typical round-testis form. Five other canvasbacks exposed to the round-testis form were also infected with only round-testis specimens, again indistinguishable from the typical round-testis form.

From these data it was concluded that the two species hypothesis could not be supported, because the offspring from the round-testis form did not always retain their species-diagnostic characteristics.

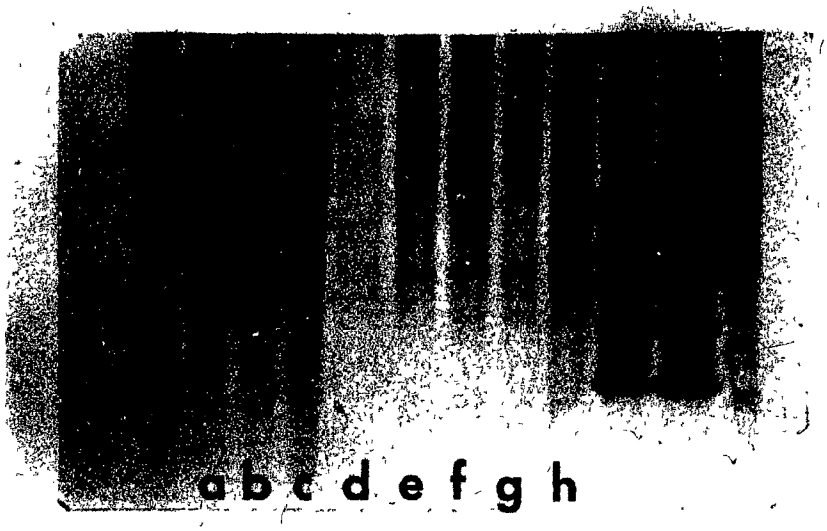
Additional support for considering the two forms to be of the same species was provided by Dr. Carl Bursey (University of New Brunswick) who kindly examined a few specimens of each morphological form using gel electrophoresis techniques. No difference in the protein bands were indicated (Figure 4-4) and Dr. Bursey therefore concluded, independently,

Figure 4-4. Photograph of a gel electrophoresis of specimens of round-testis *Typhlocoelum* obtained from mallards, of lobed-testis *Typhlocoelum* obtained from canvasbacks and of a round-testis *Typhlocoelum* obtained from a redhead.*

CODE	PARASITE	HOST	µg EXTRACT
a	Round-testis	MAL	300
b	Round-testis	MAL	250
c	Round-testis	MAL	360
d	Lobed-testis	CAN	50
e	Lobed-testis	CAN	250
f	Lobed-testis	CAN	300
g	Round-testis	RED	150
h	Round-testis	MAL	200

The abbreviations for host species are explained in the "List of Abbreviations".

* Unlabelled gels to the far left and right are control *Hymenolepis*. The second and third gels from the right are strains of Atlantic salmon.


a b c d e f g h

that the two forms could not be separated using this technique and that they represented the same species.

b) HOST-INDUCED HYPOTHESIS

A second hypothesis suggested in Chapter III was that the differentiating characteristics observed between the two forms of *Typhlocoelum* were the result of host-induced influences on the developing parasites.

Experimental evidence that the host species influences the morphology of digeneans has been rapidly accumulating during the past 20 years. Berrie (1960) experimentally infected mice, ducklings and herring-gull chicks with *Diplostomum phoxini* (Faust, 1918) and recorded differences in body length and width, length of the hindbody, ratio of body to hindbody lengths and number of eggs in the uterus among specimens from the three host species. Hsu and Hsu (1957) found that the species of definitive host influenced the arrangement and location of the testes in *Schistosoma japonicum* Katsurada, 1904. The morphology of *Telorchis bonnerensis* Waitz, 1960 varies with respect to the anterior extent of the vitellaria, the position of the ovary, the extent of the cirrus sac and the comparative sizes of the suckers, depending on the definitive host species (Watertor, 1967). A detailed study on *Quinquiserialis quinquiserialis* (Barker and Laughlin, 1911) undertaken by Kinsella (1971) revealed that the "body length, body width, oral sucker width, width of the uterine coils, testis length, ovary length, cirrus sac length, and metraterm length were all ... host-dependent to some degree". A similar study undertaken by Blankespoor (1974) demonstrated that, in *Plagiorchis noblei* Park, 1936, the host species influences not only the body size, the size of the pharynx, oesophagus and reproductive organs, but also the distribution of the vitellaria.

Palmieri (1975) studied the intraspecific variation in *Posthodiplostomum minimum* (MacCullum, 1921) and summarized: "Host induced morphological variations of adult *P. minimum* were shown to be associated with the genus and class of host employed, as well as with inherent variability of *P. minimum* exclusive of host influences. Forty-nine distinct variables were statistically analyzed and results indicate

that the major organs and structures undergoing morphological variation include oral sucker, acetabulum, holdfast organ, testes, ovary position, vitelline distribution, body shape and size, and tegumental surface and spines."

In 1978, Bakke (1978a) reported that the anterior extent of the uterus, the anterior and posterior extent of the vitellaria, the size, shape and position of the cirrus pouch, the position of the genital pore, the size of the gonads and the tegumental topography of *Leucochloridium variae* McIntosh, 1932 varied depending on the host species. Most recently, Kennedy (1980) studied morphological variability of *Haematoloechus buttensis* Ingles, 1936 in six different species of amphibians. He concluded that the size and degree of lobation of the ovary and testes, the lengths of the extracaecal uterine loops, and the vitelline gland distribution varied depending on the species of host.

Previous studies on host-induced variation have not attempted to determine whether different host species select different genotypes from a pool of metacercariae or whether the different host species influence the phenotypic expression of a common genotype. Obviously, both of these processes could be considered as host-induced and both would result in different phenotypes occurring in different host species.

If the host species were responsible for the differences observed between the round- and lobed-testis forms of *Typhlocoelum*, one would predict that the parasites would exhibit certain characteristics depending on the host species. One might expect that parasites developing in mallards would have round testes and those developing in canvasbacks would have lobed testes, regardless of the source of the infective material. This happened in only one case. As discussed above, one canvasback exposed to round-testis *Typhlocoelum* was found to harbour 19 lobed-testis and two round-testis flukes. While it is possible that the host influenced the morphology of the 19 lobed-testis flukes, this would not explain why the host did not influence the other two specimens in the same host, or all the specimens recovered from the other canvasbacks exposed to R-DAB material. In all of these cases, the parental round-testis morphology was retained and the host had no apparent effect on the morphology of the flukes.

Occasionally, in the field, the round-testis form of *Typhlocoelum* is found in diving ducks. The natural occurrence of this unusual combination provides further evidence that host-induced variation does not account for the two morphological forms of *Typhlocoelum*.

c) TWO POPULATION HYPOTHESIS

It has been demonstrated above that the two morphological forms of *Typhlocoelum* are neither separate species nor the manifestation of a host-induced phenomenon. They must, therefore, represent inter- or intra-population variants within a single species.

Mayr (1970) discussed three potential subdivisions of the species: the variety, the subspecies and the race. He believed that, historically, the variety has incorporated "two entirely different phenomena: a) individual variants within a polymorphic population; b) distinguishable populations in a polytypic species" and that it should be restricted to individual variants. Mayr (1970) suggested that, in general, the subspecies is used as a "convenient pigeonholing device of the practicing taxonomist". He defined a subspecies as "an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of the species and differing taxonomically from other populations of the species". Mayr (1970) regarded an ecological race as "a local population that is particularly conspicuously adapted to a local habitat".

There are obvious difficulties in relating these concepts to parasites. The first consideration is whether the different forms represent individual variants within a local population or whether each represents a different population. If one agrees that the habitat of the parasite is composed of its hosts and their immediate environment, then it would be difficult to accept that the round- and lobed-testis forms are individual variants within a single population. Their occurrence in different species of definitive hosts, that are presumably isolated to some extent ecologically (Godfrey, 1966) results in a degree of reproductive isolation of the two forms of parasites. This is evidenced as well by the retention of the parental morphology in the majority of the "offspring", regardless of host species. If, then, the two forms represent different

populations, neither the term "variety" nor the term "polymorphism" can be used to describe them, because both are types of within-population variability (Mayr, 1970).

From the cross-infection experiments, it has been clearly demonstrated that the round-testis form can successfully infect canvasbacks. Field data also show that the lobed-testis form occasionally develops in dabbling ducks (see Chapter VIII). It would appear, then, that the environment provided by dabbling ducks is suitable for the lobed-testis form, and similarly, diving ducks are suitable hosts for the round-testis form. Therefore, the two forms are apparently not exclusively adapted to their local habitat, and therefore the term "race" is not applicable to the two forms either.

The definition of the subspecies (Mayr, 1970) contains two important concepts. Firstly, the subspecies is a phenotypically distinct entity. This certainly applies to the adult stages of both forms of *Typhlocoelum* and it also applies to the developing stages in the duck. At 70 days post-infection R-DAB flukes recovered from sites other than the trachea in both mallards and canvasbacks were immature and phenotypically indistinguishable.

The second concept concerns the distribution of the subspecies. Mayr (1970) suggested that the subspecies would occupy a geographical subdivision of the total range of the species, or that, to some extent, it would be physically isolated from other subspecies. Application of this concept to digeneans necessarily involves some degree of interpretation. If one accepts that, to some extent, parasites that inhabit different host species are isolated, then the term "subspecies" is appropriate. In the present case, the two forms of *Typhlocoelum* are almost exclusively segregated not only into different duck species but also into two different subfamilies of ducks: Anatinae and Aythyinae. Therefore, it is concluded that the two morphological forms of *Typhlocoelum* represent different subspecies of a single species, *Typhlocoelum cucumerinum*.

d) PROPOSED CLASSIFICATION FOR THE GENUS *Typhlocoelum*

The round-testis form of *Typhlocoelum* is *Typhlocoelum cucumerinum*

sisowi and the lobed-testis form is *Typhlocoelum cucumerinum cucumerinum*. The following classification is proposed:

Typhlocoelum cucumerinum cucumerinum (Rudolphi, 1809): syn. *Distoma cucumerinum* Rudolphi, 1809; *Monostomum flavum* Mehlis, 1831; *Monostomum sarcidiornicola* Mégnin, 1890; *Typhlocoelum obovale* Neuman, 1909; *Typhlocoelum reticulare* Johnston, 1913; *Typhlocoelum gambense* Dubois, 1930.

Typhlocoelum cucumerinum sisowi (Skrjabin, 1913): syn. *Tracheophilus sisowi* Skrjabin, 1913; *Typhlocoelum hepaticum* Sugimoto, 1919; *Typhlocoelum americanum* Manter and Williams, 1928; *Typhlophilus shovellus* Lal, 1937; *Typhlocoelum indicum* Fotedar, 1964; *Typhlocoelum sisowi acirratus* Jain, 1966.

5. SIGNIFICANCE OF THE SUBSPECIES

The biological significance of the species is recognized almost universally, but the biological significance of the subspecies has been questioned by a number of researchers (Chitwood, 1957; Stunkard, 1957). Mayr (1970) stated that "it must be realized ... that in many cases the subspecies is an artifact rather than a unit of evolution".

It has been shown in this chapter that, not only do the two subspecies retain their parental morphological characteristics, but they also retain other characteristics. In particular, at 70 days post-infection, a proportion of the round-testis flukes are very small and are not located in the trachea regardless of the host species whereas all lobed-testis flukes are large and are located in the trachea. The morphological distinctions between the two subspecies may be the manifestation of other biologically significant differences.

In the next three chapters, *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* will be compared to determine whether their subspecific status is merely a convenience or whether it has biological significance.

CHAPTER V. COMPARATIVE STUDY ON THE MIGRATION OF *Typhlocoelum cucumerinum sisowi* IN MALLARDS AND *Typhlocoelum cucumerinum cucumerinum* IN CANVASBACKS

A. INTRODUCTION

Survival of both subspecies of *T. cucumerinum* is dependent on the ability of the metacercariae to excyst and penetrate the definitive host, and of the flukes to reach the trachea and remain at least long enough to mature and produce eggs.

The metacercariae of both subspecies excyst in the digestive tract of ducks, but the route of migration to the trachea has not been elucidated. Szidat (1933) postulated that metacercariae penetrate the intestinal wall and migrate, either *via* the circulatory system or *via* the abdominal cavity and air sacs, to the lungs and trachea.

Data from field surveys include records of immature *T. cucumerinum* from various areas of the body: in hepatic cystoma (Morishita, 1929), in the small intestine (Lal, 1937), associated with the viscera (Krull, 1940; Fotedar, 1964; Cornwell, 1966) and in the air sacs and lungs (Kinsella and Forrester, 1972; Rind, 1974). On the basis of these observations it is probable that the abdominal cavity and respiratory system may be important in the migration of juvenile typhlocoelids. McLaughlin (1980) has found that both *Cyclocoelum mutabile* (Zeder, 1800) and *Cyclocoelum oculeum* (Kossack, 1911) migrate *via* the abdominal cavity and respiratory tract to the air sacs and nasal passages of coots (*Fulica americana* (Gmelin)), respectively. In the case of *C. mutabile*, a distinct liver phase is evident in the migration (McLaughlin, 1977) whereas *C. oculeum* apparently do not travel through the liver (McLaughlin, 1980).

The respiratory system of ducks has been described in detail by King (1966), Lasiewski (1972) and King (1975). The terminology proposed by Lasiewski (1972) has been adopted in the present study. There are nine major air sacs (Figure 5-1). These include: the paired cervical air sacs positioned in the neck, ventral to the vertebral column and surrounding the oesophagus; a single clavicular air sac, resulting from the embryonic fusion of two pairs of air sacs and positioned at the base of the neck;

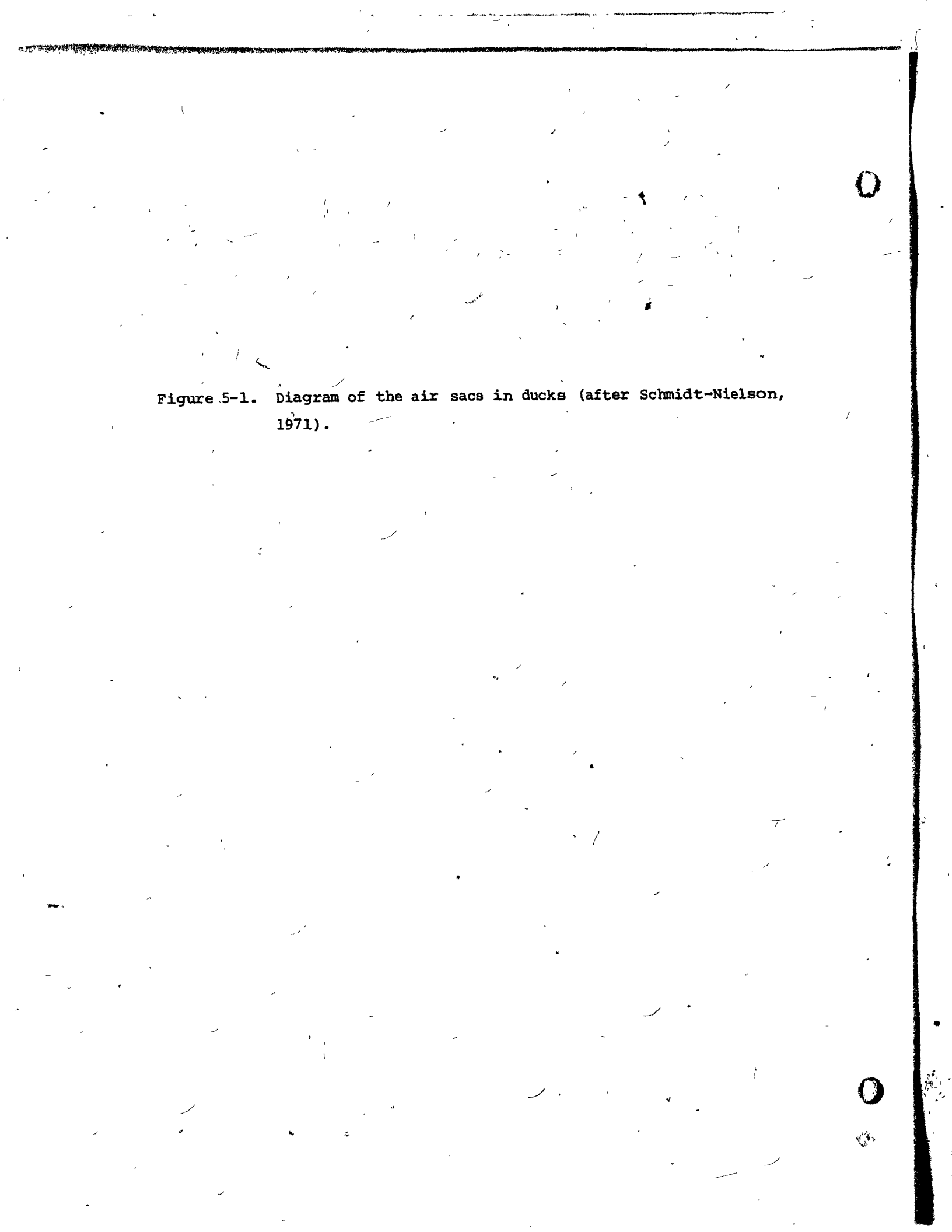
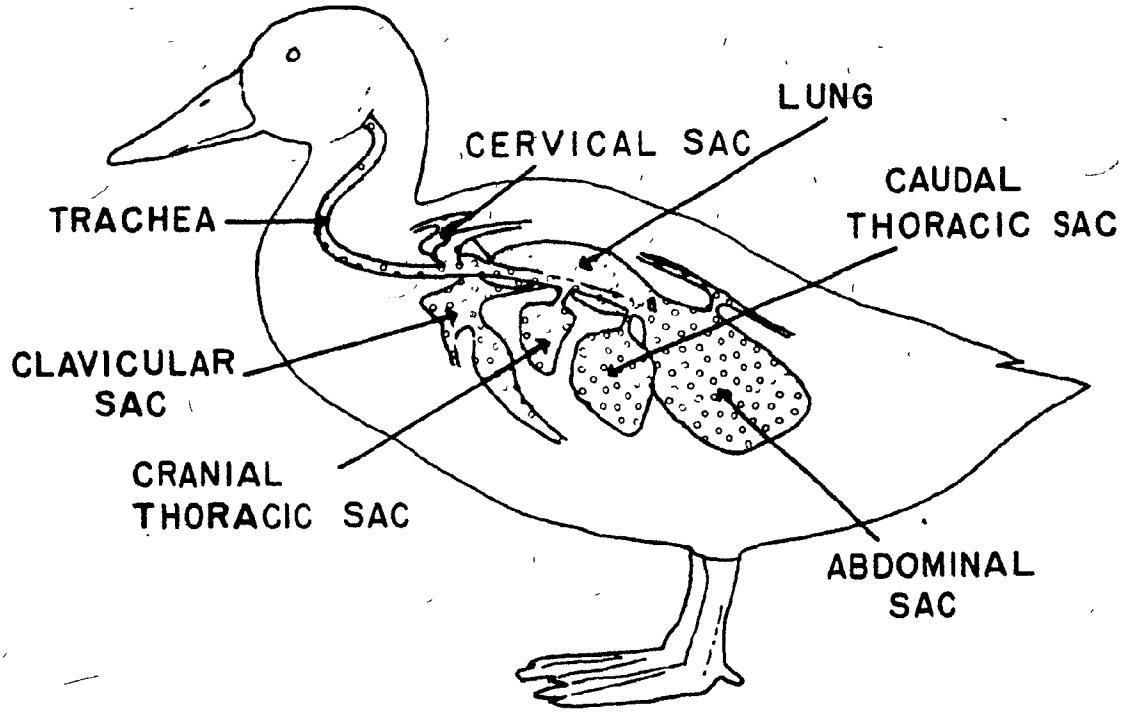


Figure 5-1. Diagram of the air sacs in ducks (after Schmidt-Nielson, 1971).


LUNG
CERVICAL SAC
CAUDAL
THORACIC SAC
TRACHEA
CLAVICULAR
SAC
CRANIAL
THORACIC SAC
ABDOMINAL
SAC

a pair of cranial thoracic air sacs and a pair of caudal thoracic air sacs, both of which lie against the dorsal surface of the sternum and pass posteriorly toward the sternoribs; and a pair of abdominal air sacs that encompass the liver, gizzard, intestine and extend posteriorly to the level of the kidneys.

Each air sac is connected directly to either a primary or secondary bronchus *via* openings called ostia. The air sac walls are ciliated only in the vicinity of the ostia.

Lasiewski (1972) divided the air sacs into two groups based on the location of the ostia connecting the air sacs to the lungs. The anterior air sacs include the cervical, clavicular and cranial thoracic air sacs, and the posterior air sacs include the caudal thoracic and abdominal air sacs. This distinction has been adopted in this study.

In this chapter, data are presented on the infectivity of metacercariae, the route and timing of migration and the duration of *T. c. sisowi* infections in dabbling ducks and *T. c. cucumerinum* infections in diving ducks.

B. MATERIALS AND METHODS

1. EXPERIMENTAL PROTOCOL

Mallards were used as the experimental host for *T. c. sisowi*, and canvasbacks and redheads were used as the experimental hosts for *T. c. cucumerinum*. A sufficient number of canvasbacks were available for the early part of the migration study (days 1 through 52), and redheads were substituted for the later days post-infection. Redhead data is included only in the longevity and survivorship analyses.

A total of 72 mallards were exposed to *T. c. sisowi*-infected *Physa gyrina via* oral intubation of whole snails. Similarly, 18 canvasbacks and 4 redheads were exposed to *T. c. cucumerinum*-infected *P. gyrina*. The number of metacercariae given to each duck varied from 40 to 100, as indicated in Chapter II. The necropsy schedule for these ducks is shown in Table 5-1.

2. NECROPSY PROCEDURE

The necropsy of each duck was undertaken in three stages. After

Table 5-1. Results of experimental infections of mallards with *Typhlocoelum cucumerinum sisowi* (A) and canvasbacks and redheads with *Typhlocoelum cucumerinum cucumerinum* (B).

A.

PARAMETER	DAYS POST-INFECTION 1	3	6	12	21	30	40	45[1]	60[2]	70[3]	90	120	150	180	210
NUMBER EXPOSED	9	7	4	7	3	3	3	6	5	8	1	3	5	4	4
NUMBER INFECTED	7	4	2	6	3	3	3	3	4	3	1	0	0	1	1
PERCENT INFECTED	78	57	50	86	100	100	100	50	80	38	100	0	0	25	25
TOTAL NUMBER OF FLUKES RECOVERED	99	70	30	38	26	38	14	21	7	9	3	0	0	3	1
MEAN NUMBER OF FLUKES PER INFECTED DUCK	14.1	17.5	15.0	6.3	8.7	12.7	4.7	7.0	1.8	3.0	3.0	0.0	0.0	3.0	1.0

B.

PARAMETER	DAYS POST-INFECTION 1	3	6	12	21	30	52[4]	60[2]
NUMBER EXPOSED	1	3	3	3	3	3	2	4
NUMBER INFECTED	1	3	3	3	3	3	2	4
PERCENT INFECTED	100	100	100	100	100	100	100	100
TOTAL NUMBER OF FLUKES RECOVERED	10	83	73	81	63	135	62	83
MEAN NUMBER OF FLUKES PER INFECTED DUCK	10.0	27.7	24.3	27.0	21.0	45.0	31.0	20.8

[1] Days 45 and 46.
[2] Days 59, 60 and 61.
[3] Days 69 and 70.
[4] Days 51 and 52.

folding back the skin and breast muscles from the sternum, small punctures were made through the abdominal wall and the sternum, and the abdominal cavity and each of the individual air sacs were flushed with 0.75% saline. The abdominal wall and sternum were then cut and the viscera exposed. The entire body cavity was rinsed and the organs were removed and rinsed in 0.75% saline in the following order: 1) gizzard, liver and intestine; 2) kidneys; 3) heart; 4) lungs; 5) external bronchi, syrinx, trachea and larynx. Each rinse was retained separately. After each organ was removed, the entire body cavity was rinsed again. Each rinse was examined using a stereomicroscope, and the number of specimens recovered was recorded. The specimens from each rinse were retained separately throughout all histological proceudres.

In addition to rinsing the surface of each organ, the liver, kidney and lungs were also cut into small pieces, squashed between glass plates and examined using a stereomicroscope. No specimens were recovered from the liver or kidney tissues during necropsies in 1976 and 1977. Therefore, these tissues were not examined during necropsies in 1978 and 1979.

To facilitate analyses, five potential sites were selected and the data were pooled accordingly: 1) those flukes recovered from the initial abdominal cavity rinse as well as those associated with the gizzard, liver, intestine and kidney rinses were considered to have been in the abdominal cavity; 2) specimens recovered from the initial abdominal and caudal thoracic air sac rinses were grouped into the posterior air sac class; 3) those specimens recovered from the cranial thoracic and clavicular air sac rinses as well as those associated with the heart rinse were classed as being in the anterior air sacs; 4) flukes removed from lung rinses were grouped into the lung class; and 5) specimens removed from the upper respiratory tract were pooled into the trachea class.

3. ANALYSIS OF SURVIVORSHIP DATA

Parasite survival at each day post-infection was determined by calculating the proportion of surviving flukes (total number of worms recovered divided by the total number of metacercariae used to infect

the ducks) from all mallards exposed to *T. c. sisowi* and from all redheads and canvasbacks exposed to *T. c. cucumerinum*. In an attempt to decrease the variability, only ducks that were given approximately 100 metacercariae were included.

The survivorship curve was obtained from a least-squares regression line fitted to the natural log of the proportion of survivors for each subspecies (Barr *et al.*, 1976). The slope of the regression line indicates the death rate of the flukes and the inverse of the slope provides the average expected life span (Dr. Roy M. Anderson, Imperial College, personal communication).

C. RESULTS

1. *Typhlocoelum cucumerinum sisowi* IN MALLARDS

Of 72 mallards exposed to *T. c. sisowi*, 40 (55.6%) became infected. The highest recovery from a single duck (52 worms) occurred at 1 day post-infection. The proportion of *T. c. sisowi* survivors (Figure 5-2) decreased significantly as the infection progressed (Pearson product-moment correlation: $r = -0.886$, $P < 0.01$). The regression line that best fits these data is described by the equation:

ln (proportion of survivors) = ln (22.02) - 0.04 (days post-infection).

This predicted survivorship curve estimates that only 22.02% of the metacercariae are successful in excysting and penetrating the intestinal wall into the abdominal cavity. The subsequent death rate is 0.04 worms per day, and the average expected life span of those juveniles that reach the abdominal cavity is 25 days.

At each day post-infection, the migration data from all mallards were pooled. The percentage of specimens recovered from each of the five sites was determined, and the results are presented in Figure 5-3. Recovery of *T. c. sisowi* from the abdominal cavity was highest at 1 day post-infection, although flukes persisted in this site throughout the duration of the infection. *Typhlocoelum cucumerinum sisowi* were recovered from the posterior air sacs between 12 and 30 days post-infection whereas specimens were found in the anterior air sacs and lungs throughout the entire experiment. The proportions of flukes recovered from the abdominal

Figure 5-2. Graph of observed and predicted mean recoveries of *Typhlocoelum cucumerinum sisowi* in mallards at various days post-infection.

★ observed recovery;

● predicted recovery;

▯ 95% confidence limits on predicted recovery.

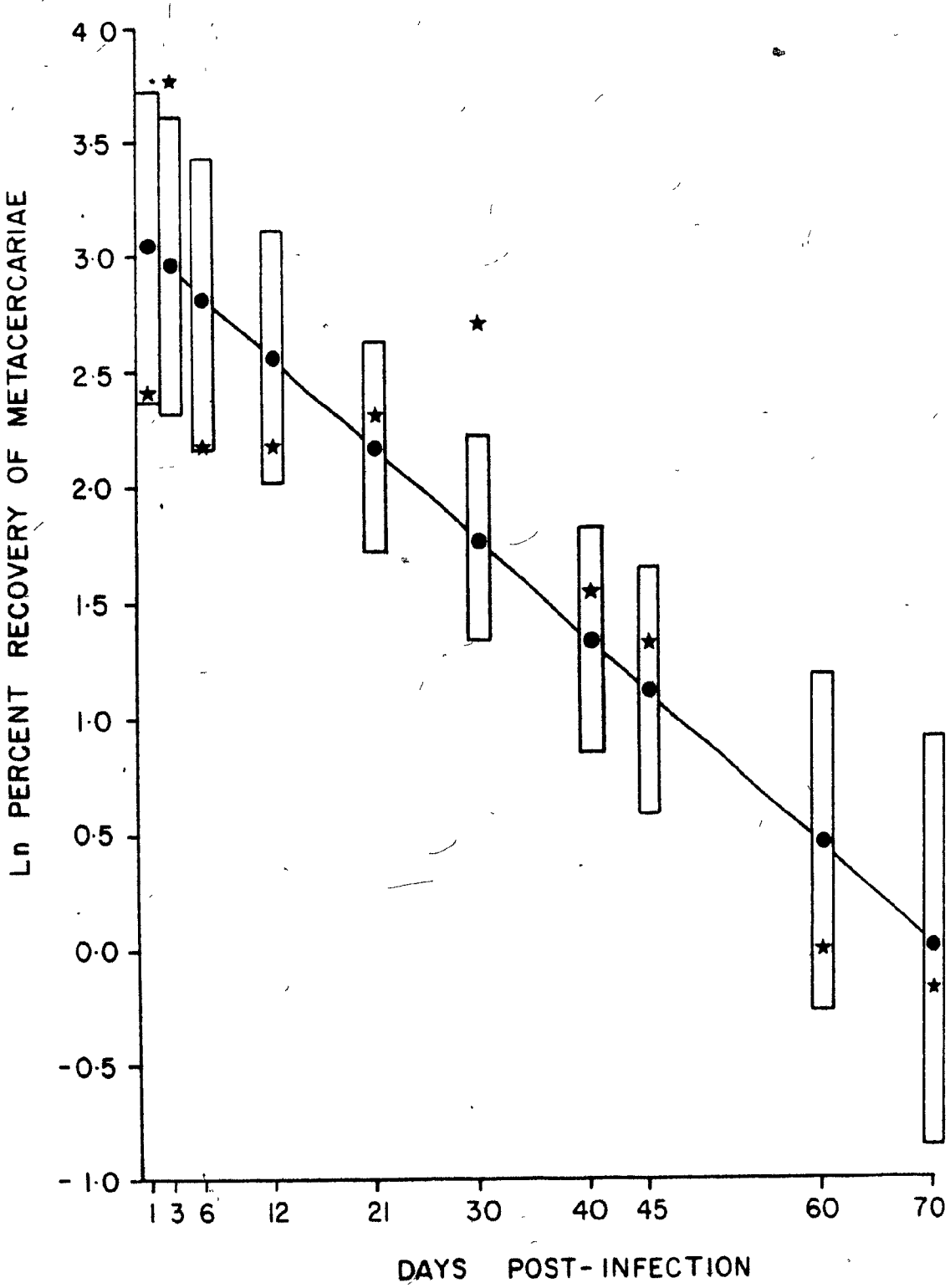
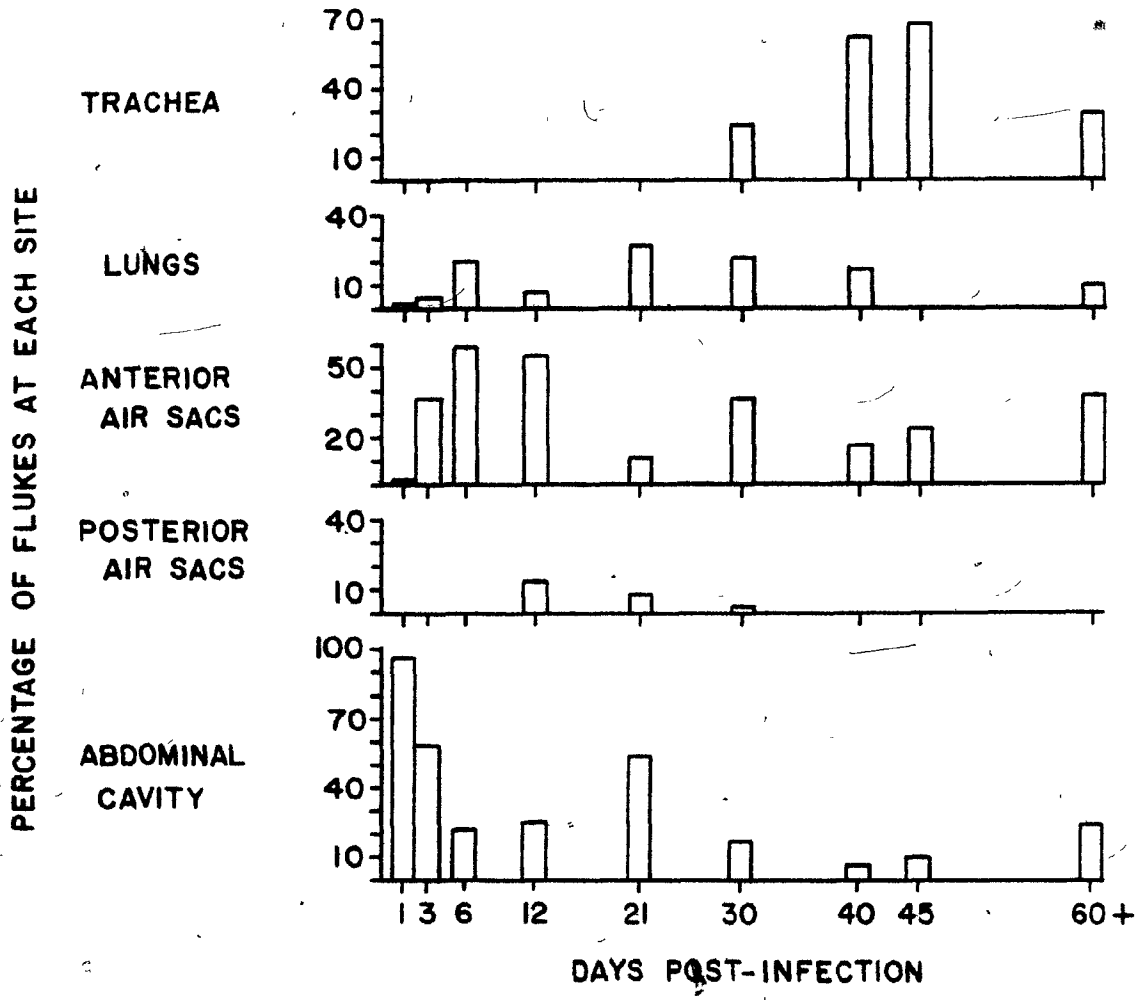

4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0·0
-0·5
-1·0
Ln PERCENT RECOVERY OF METACERCARIAE
1 3 6 12 21 30 40 45 60 70
DAYS POST-INFECTION

Figure 5-3. Graph of percent recovery of *Typhlocoelum cucumerinum sisowi* at each site in mallards, at various days post-infection.


PERCENTAGE OF FLUKES AT EACH SITE
TRACHEA
LUNGS
ANTERIOR AIR SACS
POSTERIOR AIR SACS
ABDOMINAL CAVITY
70
40
10
40
10
50
20
40
10
100
70
40
10
1 3 6 12 21 30 40 45 60+
DAYS POST-INFECTION

cavity (6% to 96%) and anterior air sacs (2% to 59%) were much higher than the proportions recovered from the posterior air sacs (3% to 14%) and lungs (2% to 27%). *Typhlocoelum cucumerinum siscowi* were recovered from the trachea as early as 30 days post-infection. The proportion of specimens recovered from the trachea increased between 30 and 45 days post-infection, but decreased dramatically between 45 and 60 days post-infection.

There were no indications of gross pathology in any of the mallards, and all flukes recovered were alive.

2. *Typhlocoelum cucumerinum cucumerinum* IN CANVASBACKS AND REDHEADS

All of the 18 canvasbacks and 4 redheads exposed to *T. c. cucumerinum* became infected (Table 5-1). The maximum recovery was 66 worms from a day 30 infection.

The data for the observed recoveries of *T. c. cucumerinum* at various days post-infection are presented in Figure 5-4. Based on these data, a statistically significant survivorship curve was not obtained.

Summary data on the proportions of *T. c. cucumerinum* recovered from the various sites in canvasbacks (Figure 5-5) suggest a relatively synchronous migration. The flukes quickly leave the abdominal cavity (majority gone by day 6). Between days 6 and 12, most of the *T. c. cucumerinum* were in the posterior air sacs. Recovery from the anterior air sacs peaked at 21 days post-infection, and the highest percent recovery from the lungs occurred at 30 days post-infection. *Typhlocoelum cucumerinum cucumerinum* were recovered from the trachea as early as 12 days post-infection and from then until day 52, the percentage continually increased.

Between 6 and 21 days post-infection, the air sac walls of infected canvasbacks appeared thickened, opaque and yellowed, and the air sacs contained thick viscous mucus which frequently surrounded the flukes. At 30 days post-infection, the air sac walls were thin and transparent, and little mucus was observed in the air sacs. Excessive mucus was present, however, in the trachea at 12 and 21 days post-infection and, in heavy infections at 30, 52 and 60 days post-infection.

Typhlocoelum cucumerinum cucumerinum were alive and moving at

Figure 5-4. Graph of observed mean recoveries of *Typhlocoelum cucumerinum cucumerinum* in canvasbacks and redheads at various days post-infection.

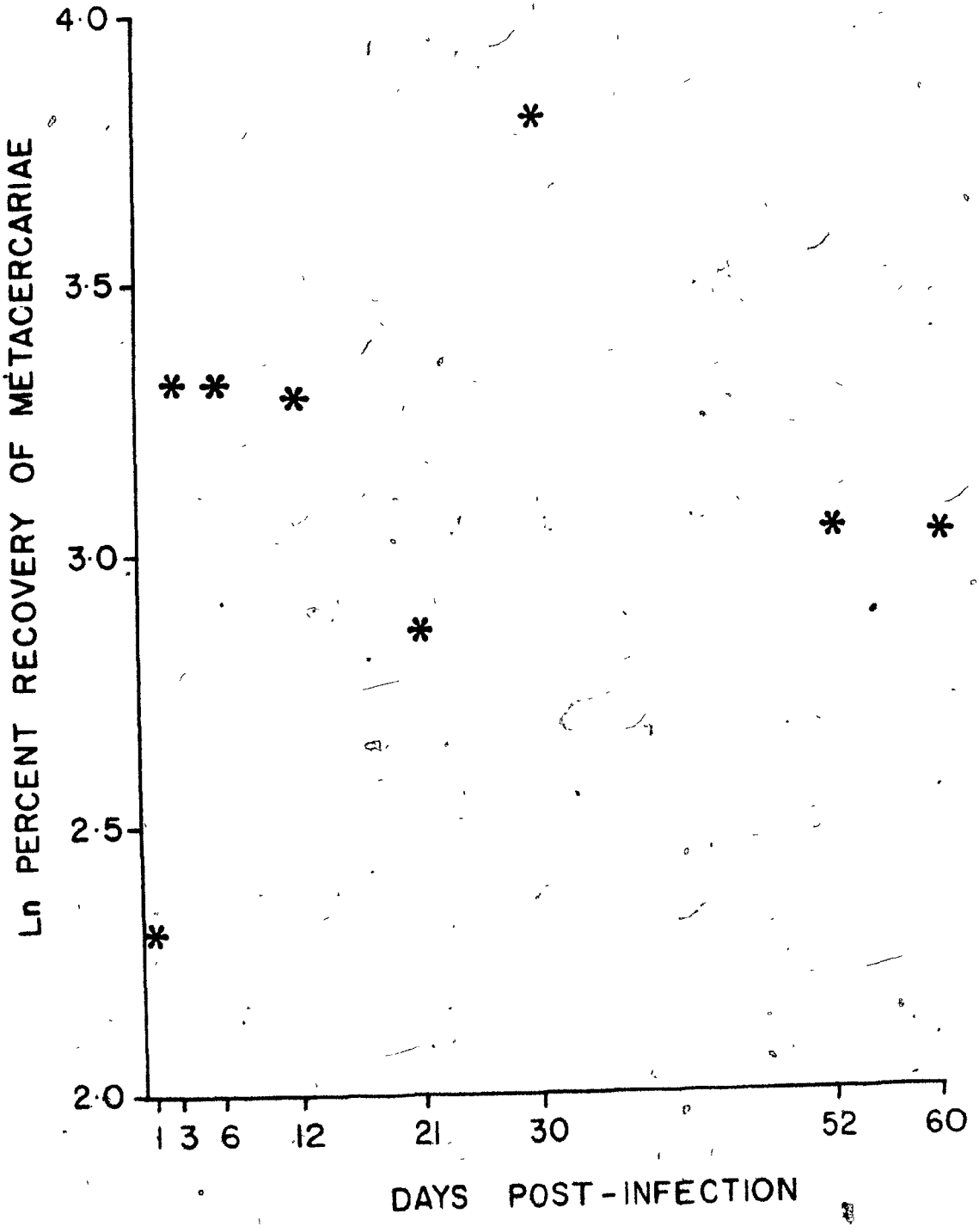
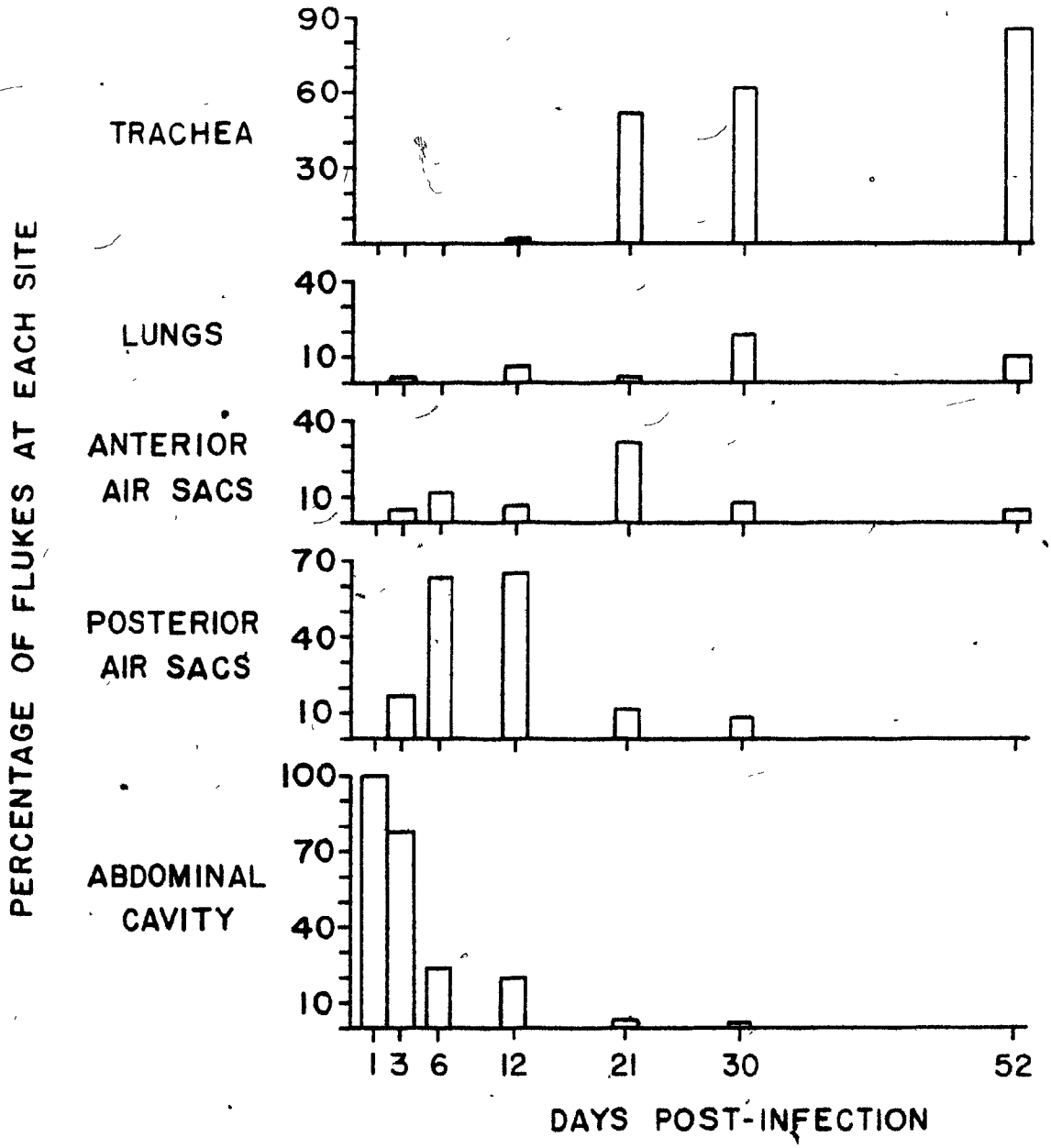

4·0
3·5
3·0
2·5
2·0
Ln PERCENT RECOVERY OF METACERCARIAE
1 3 6 12 21 30 52 60
DAYS POST-INFECTION

Figure 5-5. Graph of percent recovery of *Typhlocoelum cucumerinum cucumerinum* at each site in canvasbacks, at various days post-infection.


PERCENTAGE OF FLUKES AT EACH SITE
TRACHEA
LUNGS
ANTERIOR AIR SACS
POSTERIOR AIR SACS
ABDOMINAL CAVITY
90
60
30
40
10
40
10
70
40
10
100
70
40
10
1 3 6 12 21 30 52
DAYS POST-INFECTION

6 and 12 days post-infection, but, of the 63 specimens recovered at 21 days post-infection, 31 (49%) were dead, as indicated by the lack of movement and the unusually pale color of the flukes. All specimens recovered after 21 days post-infection were alive.

D. DISCUSSION

Data on the survival and route and timing of migration of *T. c. sisowi* and *T. c. cucumerinum* have been presented. They will now be compared, and any biological distinctions between the two subspecies will be noted.

1. SURVIVORSHIP

The first phase of a *T. cucumerinum* infection in the definitive host involves the excystment of the metacercariae. Factors that are important in excystment of digenean metacercariae have been summarized by Smyth (1966) and Lackie (1975). Once the metacercariae have excysted, they must successfully penetrate the intestinal wall and enter the abdominal cavity. Although the maximum recovery of specimens was higher for *T. c. cucumerinum* (45.0%) than for *T. c. sisowi* (43.75%), the differences were not significant. This suggests that there are no differences in the ability of the two subspecies to excyst and penetrate the intestinal wall in the two species of ducks, mallards and canvasbacks.

Two researchers have attempted experimental infections in ducks using *T. c. sisowi*. Szidat (1933) recovered only two flukes from a duck exposed to between 40 and 60 metacercariae, and Stunkard (1934b) failed to recover any flukes in a duck infected with six metacercariae. He suggested that the low recovery obtained in Szidat's infections together with the absence of infection in his study indicated that *T. c. sisowi* may become lost during migration and die.

The average expected life span of *T. c. sisowi* (25 days), calculated from the survivorship curve, was shorter than the time required for the flukes to reach the trachea (30 days). This suggests that a high mortality of flukes occurs early in the infection, and lends support to Stunkard's (1934b) suggestion. Although the expected life span of *T. c. cucumerinum*

could not be determined, it is presumably much longer than that of *T. c. sisowi* because, by 60 days post-infection, the percent recovery had not yet begun to decline. Therefore, early mortality of specimens may not be an important factor in *T. c. cucumerinum* infections.

Differences in the life spans of the two subspecies might also be a function of the duration of the infections in the trachea. Sreekumaran and Peter (1973a) reported on the duration of *T. c. sisowi* infections in ducks: "The birds experimentally infected with *T. cymbium* are observed to throw off the infection by coughing out the worms in about two months time." Two observations made in this study would lend support to this comment. The recovery of *T. c. sisowi* from the trachea peaked between days 40 and 45 post-infection, and decreased dramatically at 60 days post-infection. In fact, the only mature specimen recovered from the trachea after 45 days post-infection was obtained at 60 days post-infection. As will be shown in Chapter VI, all other specimens recovered from the trachea at or after 60 days post-infection were immature.

In addition, *T. c. sisowi* eggs were collected from the drinking water of an experimentally infected mallard at 48 days post-infection but this duck had no *T. c. sisowi* when necropsied at 133 days post-infection. If *T. c. sisowi* are expelled at two months post-infection, this would account for the low recovery of flukes at and after 60 days post-infection (Table 5-1).

In contrast, *T. c. cucumerinum* adults were obtained from canvasbacks as late as 107 days post-infection in this study (duck exposed to metacercariae from *Lymnaea elodes*) and from a lesser scaup at 180 days post-infection. Schafranski (1974) obtained adult *T. c. cucumerinum* at 150 days post-infection. These observations indicate that *T. c. cucumerinum* adults remain in the trachea longer than *T. c. sisowi*. Evidence was obtained to suggest that they do eventually leave the trachea, however. A single redhead, exposed to *T. c. cucumerinum*, was examined at 70 days post-infection using fiberoptics to illuminate the throat of the duck, and two adult *T. c. cucumerinum* were observed in the larynx. At necropsy on day 317, no worms were found.

2. MIGRATION OF *T. c. sisowi* AND *T. c. cucumerinum*

a) ROUTE OF MIGRATION

In migration studies, it is often difficult to determine exactly where a parasite was located before the host died. In this study, ducks were necropsied in a manner than would most accurately reflect the true location of the specimens in the ducks.

Szidat (1932) proposed that *T. c. sisowi* may travel to the lungs *via* the circulatory system. In this study, the surface of the heart was examined and the heart was then opened, but in no case were specimens recovered from the heart chambers. Also, there have been no previous reports of *T. c. sisowi* or *T. c. cucumerinum* recovered from the circulatory system. It therefore appears that *T. c. sisowi* and *T. c. cucumerinum* do not migrate *via* the circulatory system, or that if they do, it only occurs rarely.

Morishita (1929) cited a case where typhlocoelids were found in hepatic cystoma; neither *T. c. sisowi* nor *T. c. cucumerinum* were ever found in the livers of ducks examined in this study. McLaughlin (1977) has shown that *Cyclocoelum mutabile* have a distinct liver phase in their migration to the air sacs in coots, but this does not appear to be the case in either *T. c. sisowi* or *T. c. cucumerinum*.

In this study, both *T. c. sisowi* and *T. c. cucumerinum* were recovered from the abdominal cavity, the posterior and anterior air sacs, the lungs (Figure 5-6) and the trachea. In the case of *T. c. sisowi*, a low percentage of specimens was recovered from the posterior air sacs (maximum 14% at 12 days post-infection) whereas a large proportion of *T. c. sisowi* was recovered from the anterior air sacs (up to 59%) throughout the duration of the experiment. It is conceivable that those *T. c. sisowi* found in the posterior air sacs between days 12 and 30 represent flukes that would have reached the trachea had their host remained alive longer. The persistence of flukes in the anterior air sacs may indicate that *T. c. sisowi* have greater difficulty in reaching the lungs from the anterior air sacs than from the posterior air sacs.

A large proportion of *T. c. cucumerinum* was recovered from the posterior air sacs on days 6 and 12 post-infection (64% and 65%, respectively)

Figure 5-6. Photograph of *Typhlocoelum cucumerinum sisowi* in the lung of a mallard at 30 days post-infection.

Bar represents 3.0 mm.

COLOURED PICTURE

and few were found in the anterior air sacs (maximum 32%). Perhaps most of the *T. c. cucumerinum* travel directly from the posterior air sacs into the lungs. Flukes did persist in the anterior air sacs of canvasbacks until 52 days post-infection, and these specimens may also have had difficulty in reaching the lungs.

This hypothesis is supported to some extent by the results of an experiment in which artificially excysted *T. c. sisowi* metacercariae were implanted into the clavicular air sacs of four mallards and four canvasbacks. When the ducks were necropsied at 21 days post-infection, only three (1%) of the flukes were recovered from the mallards, and only one fluke (0.4%) was recovered from the canvasbacks. Of these, two flukes were still in the anterior air sacs, and two were in the lungs.

On the basis of these data, it is suggested that *T. c. sisowi* and *T. c. cucumerinum* may travel from the abdominal cavity to the posterior air sacs and then enter the lungs and the trachea. The anterior air sacs are probably not a necessary step in the migration.

In both *T. c. sisowi* and *T. c. cucumerinum*, a low proportion of flukes was found in the lungs. The narrow ciliated passageways in the lungs (King, 1966) might expedite the migration of the flukes through the lungs, and might therefore account for the low recovery of specimens in the lungs.

b) SYNCHRONY OF MIGRATION

Given perfect synchrony of migration, one might expect that, at a certain day or period of days, all of the specimens would be located at one site and that, at a later date, they would all have moved to another site. If the migration of the flukes is asynchronous, at any one day specimens would be recovered from a variety of sites.

Although both subspecies apparently use the same route to reach the trachea, they do differ in the degree of synchrony of migration. It is possible to predict with reasonable accuracy where the majority of *T. c. cucumerinum* will be at any particular time. The flukes do not have an absolutely synchronous migration, but by 60 days post-infection, they have all reached the trachea. It is believed, therefore, that these flukes travel relatively synchronously.

In contrast, *T. c. sisowi* apparently migrate less synchronously. Flukes persist in the abdominal cavity and anterior air sacs for up to seven months post-infection. Whether or not these flukes eventually reach the trachea is not known.

c) TIMING OF MIGRATION

In addition to the differences in synchrony of migration between *T. c. sisowi* and *T. c. cucumerinum*, there is a substantial difference in the timing of the migration. *Typhlocoelum cucumerinum cucumerinum* were first recovered from the trachea at 12 days post-infection. Although two *T. c. sisowi* were found in the lungs of a mallard at 1 day post-infection, *T. c. sisowi* were not recovered from the trachea until 30 days post-infection. As the specimens can reach the lungs within 1 day post-infection, it is surprising that they require an additional 29 days to reach the trachea. It is possible that the juvenile flukes are not stimulated to enter the trachea, although Szidat (1933) recovered *T. c. sisowi* from the trachea at 6 days post-infection. He suggested that the deep indentations on the ventral surface of *T. c. sisowi* might aid in attachment to the trachea. As will be shown in Chapter VI, these are not present in the juvenile flukes. Perhaps, if *T. c. sisowi* do reach the trachea very early in the infection, they may be too small, or may not have developed sufficiently, to maintain their position in the trachea, and may be swept out by ciliary action. This would contribute to the high mortality of *T. c. sisowi* early in the infection.

d) FACTORS CONTRIBUTING TO THE DIFFERENCES IN SYNCHRONY AND TIMING OF MIGRATION

The differences observed in synchrony and timing of migration of the two subspecies of *T. cucumerinum* may be related to differences between the two types of experimental duck hosts, or they may be related to differences intrinsic to the parasites.

In Chapter IV, cross-infection experiments using *T. c. sisowi* revealed that at both 45 and 70 days post-infection, more flukes were located in the trachea of canvasbacks than mallards. This may reflect

differences between the two species of ducks. When the anatomy of the air sacs in mallards and canvasbacks were compared, the only apparent difference was the diameter of the ostia leading from the air sacs to the lungs. The major ostia between the abdominal air sacs and the lungs have a diameter of approximately 0.2 cm in mallards, and approximately 1.0 cm in canvasbacks. Similarly, the major ostia in the clavicular air sacs are larger in canvasbacks (0.8 cm) than in mallards (0.4 cm). The larger opening between the air sacs and the lungs in canvasbacks might expedite the fast, synchronous migration of *T. c. cucumerinum*. The differences between the diameter of the ostia in mallards and canvasbacks may therefore contribute to the differences observed in timing and synchrony of *T. c. sisowi* and *T. c. cucumerinum* migration. In addition, there are undoubtedly a number of other anatomical and physiological differences between mallards and canvasbacks that may be significant to the migration of the parasites.

In addition, from the cross-infection experiments, it has been shown that, at 70 days post-infection, two immature *T. c. sisowi* were located in the air sacs of canvasbacks. This persistence of immature flukes is a characteristic of *T. c. sisowi* infections in mallards. The presence of immature *T. c. sisowi* in the abdominal cavity and air sacs at late days post-infection, regardless of the host species, suggests that this phenomenon is a characteristic intrinsic to the parasite, and not a function of the host. This phenomenon does not occur in *T. c. cucumerinum* infections. It is therefore suggested that the differences in synchrony and timing of migration between the two subspecies may be related to differences intrinsic to the parasites as well as differences in the anatomy of the ducks.

3. PATHOLOGY OF THE RESPIRATORY TRACT

In addition to the differences in survivorship and migration of *T. c. sisowi* and *T. c. cucumerinum*, there are differences in the gross pathology associated with the presence of the flukes. The degree of tissue damage caused by respiratory tract parasites in general, is related to the activity of the worms. When they move along the mucosa, they cause irritation to the epithelium, resulting in an increased mucous production (LaPage, 1956). In addition, there may be mechanical damage to the epithelium

which may result in a sloughing of dead epithelial cells and hence an accumulation of mucus, epithelial cells, erythrocytes and various leucocytes in the lumen of the trachea (Clapham, 1935). This exudate may cause stenosis, obstruction of the air passages, coughing, gaping and ultimately asphyxia (Clapham, 1935; Stockdale *et al.*, 1972).

In three instances, *T. cucumerinum* has been cited as a mortality factor in ducks. Magalhães (1899) made reference to a case in Brazil where large numbers of *T. cucumerinum* in the trachea and bronchi of ducks were cited as a cause of suffocation promptly resulting in the death of the domestic ducks. Gower (1938b) described the symptoms of a *T. c. sisowi* infection in a mallard:

> "In July 1936, a half-grown mallard duck was noticed on the bank of a swale apparently dead. It suddenly jerked up its head, although unable to rise, and gasped spasmodically several times, and then settled back again as if in a coma. It was killed and autopsied immediately and 19 fully developed and mature *Typhlocoelum cucumerinum* were taken from the nasal passages, trachea, lungs, air sacs, and coelom. This duck was not more than two months of age ..."

Barry (1959) reported another case:

> "On 2/3/59, a Muscovy duckling aged about three months was presented for post mortem ... The young Muscovy ducklings, after growing at a normal rate over the early weeks of life, developed a noticeable cough and became stunted in growth ... Two of them died ... At autopsy, flukes were found actively moving in the trachea and air passages of the lung ... Twenty flukes were collected ... Two flukes were found ... in the caecum but these were apparently dead ... The parasites were identified as *Tracheophilus cymbius* ..."

Schafranski (1974) experimentally infected ducks with *T. c. cucumerinum* and found, in one case, dead specimens in nodules attached to the air sac walls and peritoneal cavity. She also found eggs within the nodules.

In this study, no experimentally infected ducks died, and there was no indication of coughing or gaping in any of the ducks. The only indication of pathology occurred in *T. c. cucumerinum*-infected canvasbacks. Undoubtedly the thickening of the air sac walls and the excessive mucous production

between 6 and 21 days post-infection were a result of irritation by the large number of juvenile *T. c. cucumerinum* in the air sacs (up to 24 in the abdominal air sacs of a single duck). Excessive mucus was also observed in the trachea in heavy infections and again this was probably a result of irritation by *T. c. cucumerinum*.

Those cases cited above demonstrate that *T. cucumerinum* can potentially kill ducks, if the ducks are young and if the infections are very heavy. The flukes are large (see Chapters III and VI) and they do move along the epithelium, presumably causing irritation and consequently mucous production. Large amounts of mucus could occlude the air passages and result in asphyxiation. Similarly, if large numbers of flukes are concentrated in the trachea, their sheer mass might be sufficient to cause respiratory difficulties. Due to their relatively small tracheas, ducklings would be most susceptible to these problems.

4. SUMMARY

Data presented in this chapter demonstrate that *T. c. sisowi* and *T. c. cucumerinum* reach the trachea of their hosts *via* the abdominal cavity, the posterior air sacs and the lungs. *Typhlocoelum cucumerinum cucumerinum* migrate synchronously and arrive at the trachea as early as 12 days post-infection whereas *T. c. sisowi* migrate asynchronously and can be found in the abdominal cavity and anterior air sacs up to seven months post-infection. *Typhlocoelum cucumerinum sisowi* were first found in the trachea at 30 days post-infection.

It has been suggested that the differences in size of the ostia connecting the air sacs and the lungs in mallards and canvasbacks may make it easier for *T. c. cucumerinum* to reach the upper respiratory tract of canvasbacks than for *T. c. sisowi* to do so in mallards. This may have contributed to the differences observed in synchrony and timing of migration of the flukes. In addition, factors intrinsic to the two subspecies of parasites may influence the patterns of migration.

Although *T. cucumerinum* has been reported as a cause of death of young ducks, this was not observed in this study. Thickened air sac walls and excessive mucus in the air sacs and trachea were evident in canvasbacks

with heavy infections, but no gross pathology was observed in mallards.

Survival of the two subspecies of parasites is in part related to their ability to enter the definitive host, reach the trachea and persist long enough to produce eggs. Evidence presented in this chapter has shown that the two subspecies differ in the way they accomplish this. *Typhlocoelum cucumerinum cucumerinum* migrates to the trachea quickly and can remain there for at least five months. *Typhlocoelum cucumerinum sisowi* migrates slowly and apparently only stays in the trachea until two months post-infection. Survival of the parasites is also dependent on their ability to grow and mature. In the next chapter, the growth and development patterns of *T. c. sisowi* and *T. c. cucumerinum* will be compared.

CHAPTER VI. COMPARATIVE GROWTH AND DEVELOPMENT OF *Typhlocoelum cucumerinum sisowi* IN MALLARDS AND *Typhlocoelum cucumerinum cucumerinum* IN CANVASBACKS

A. INTRODUCTION

Data presented in Chapter V have demonstrated that *T. c. sisowi* and *T. c. cucumerinum* differ in the synchrony and timing of their migration to the trachea, and also differ in longevity. These are two biologically important differences that underscore the morphological and ecological differences demonstrated in Chapter III. Subsequent to establishing in the definitive host, parasites must grow and develop to the egg-producing stage.

There have been very few experimental studies on the growth of *T. cucumerinum* in ducks. Schafranski (1974) and Schafranski *et al.* (1975) experimentally infected *Cairina moschata domestica* (Linnaeus) and *Anas boschas domestica* (Linnaeus) with *T. c. cucumerinum* and found specimens at necropsy 75 and 150 days after infection. With the exception of three specimens obtained from ectopic sites, all specimens were mature and were located in the trachea. Tang and Tang (1978) obtained adult *T. c. sisowi* from experimentally infected ducklings. However, the only work that has provided any information on the growth and development of either *T. c. sisowi* or *T. c. cucumerinum* was that of Szidat (1932, 1933) who used *Anas boschas domesticus* as the experimental host. At six days post-infection he noted that the specimens of *T. c. sisowi* were approximately 1.4 cm long and were morphologically similar to the cercariae. By day 12, they had doubled in size to a length of approximately 2.8 mm. Szidat (1933) reported that the sucking plate on *T. c. sisowi* could first be recognized at 14 days post-infection. He suggested that the ventral sucker was gradually replaced by the sucking plate which was adapted to attach to the bronchi and trachea. In specimens obtained at four weeks post-infection, the oral sucker was barely recognizable and the ventral sucker was still present. He reported that the flukes required six weeks to reach sexual maturity.

Krull (1940), in a detailed study on the oral and ventral suckers

in specimens of *T. c. sisowi* from a wild mallard, showed that both suckers have very weak musculature and that, in the case of the ventral sucker, the diameter was inversely proportional to the body length. He suggested that it, therefore, became less distinct in older specimens. When cyclocoelids were first classified, they were considered as monostomes, because the adult flukes did not have a ventral sucker (Dollfus, 1948). Life cycle work revealed, however, that the cercariae did have prominent ventral suckers, and that immature specimens in ducks also had ventral suckers (Stunkard, 1934b; Krull, 1940).

In this chapter the growth and development of *T. c. sisowi* in mallards and *T. c. cucumerinum* in canvasbacks are described and compared.

B. MATERIALS AND METHODS

All specimens obtained from mallards and canvasbacks in the migration study (see Table 5-1) were stained, mounted and measured, and the host species, age (days post-infection) and site of the specimens in the ducks were recorded. Measurements were also made on *T. c. sisowi* metacercariae from *Physa gyrina* that had been excysted *in vitro* in 0.5% trypsin and 0.5% duck bile in Tyrode's solution at 40°C, fixed in formalin, stained and mounted.

In addition to the numerical variables listed in Chapter III, the maximum lengths and widths of the oral and ventral suckers and the distance from the middle of the ventral sucker to the middle of the pharynx were determined. Character variables described in Chapter III were also assessed. In the case of the shape of the testes, the character states were altered to include: a) round testes; b) loosely-lobed testes; and c) deeply-lobed testes (Figure 6-1).

C. RESULTS

1. *Typhlocoelum cucumerinum sisowi* IN MALLARDS

a) GROWTH

The oral and ventral suckers, the pharynx and the circular intestine were the only prominent features in the excysted metacercariae. Measurements based on 14 excysted metacercariae are presented in Table 6-1.

Figure 6-1. Photographs of three character states for the character variables, anterior testis shape and posterior testis shape.

A, round;

B, loosely-lobed;

C, deeply-lobed;

at, anterior testis;

pt, posterior testis.

Bar represents 0.5 mm.

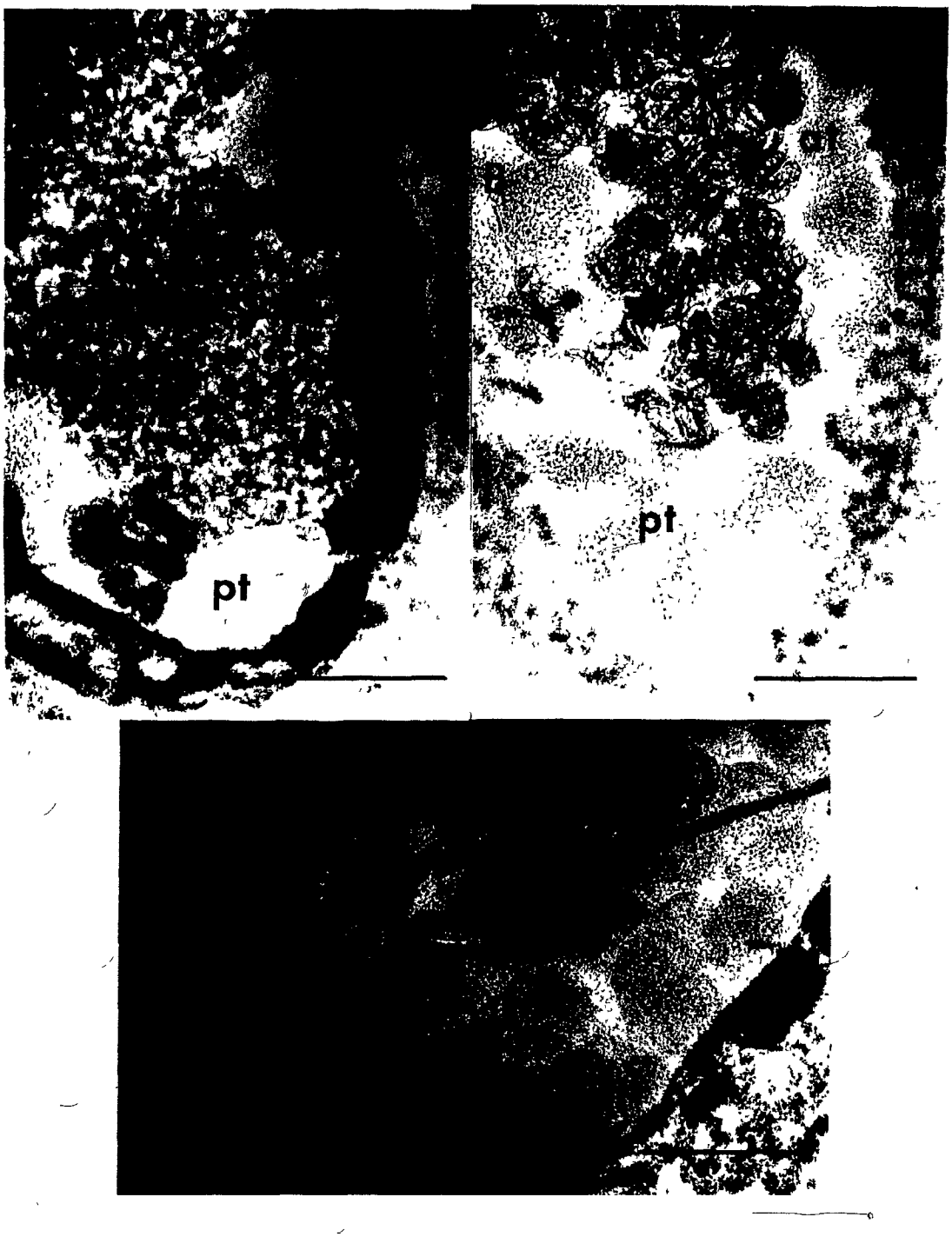

pt
pt

Table 6-1. Descriptive statistics of the numerical variables of excysted metacercariae of *Typhlocoelum cucumerinum sisowi* recovered from *Physa gyrina*.

NUMERICAL VARIABLE*	n	MEAN (mm) ± 95% C.L.
BL	14	0.194 ± 0.011
BW	13	0.075 ± 0.007
PL	11	0.018 ± 0.002
PW	11	0.015 ± 0.002
PA	11	0.055 ± 0.007
OES	10	0.013 ± 0.005
OSL	13	0.040 ± 0.002
OSW	13	0.046 ± 0.002
VSL	14	0.019 ± 0.001
VSW	14	0.022 ± 0.002
VSA	11	0.069 ± 0.009

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

They had a significantly shorter mean body length (0.194 mm) than the specimens recovered at 1 days post-infection (0.220 mm).

Figure 6-2 presents the data on mean body length of *T. c. sisowi* recovered from each site at different days post-infection. Between days 1 and 21 post-infection, no significant differences were detected in the body length of specimens recovered from different sites at any day. At day 30 post-infection, however, specimens recovered from the trachea were significantly longer than those recovered from the abdominal cavity or from the anterior air sacs. At 40 and 45 days post-infection, specimens recovered from the trachea were significantly longer than flukes recovered from any other sites. The decline in mean body length of flukes from the trachea between 45 and 60 days post-infection is the result of a small sample (only two specimens, 9.02 mm and 1.39 mm long) at day 60. After 60 days post-infection, all specimens recovered from all sites (including the trachea) were very small and did not differ significantly in body length from the flukes obtained at 12 days post-infection. Examination of Figure 6-2 clearly demonstrates that, throughout the course of the experimental infections, a proportion of the specimens remain at approximately the day 12 mean body length.

For all further analyses of growth and development, *T. c. sisowi* specimens obtained from the abdominal cavity and anterior air sacs on or after 30 days post-infection are deleted, and only specimens located in the trachea at 40 and 45 days post-infection are included. All specimens obtained after day 45 are deleted.

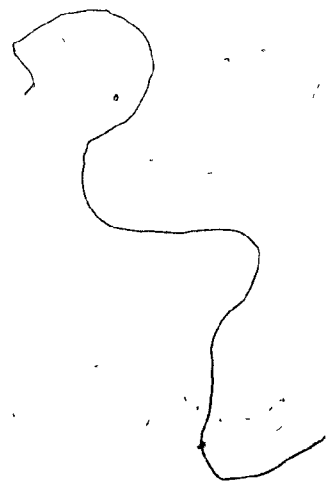
On the basis of a least-squares linear regression analysis, the growth of *T. c. sisowi* with respect to body length can be described by the following equation:

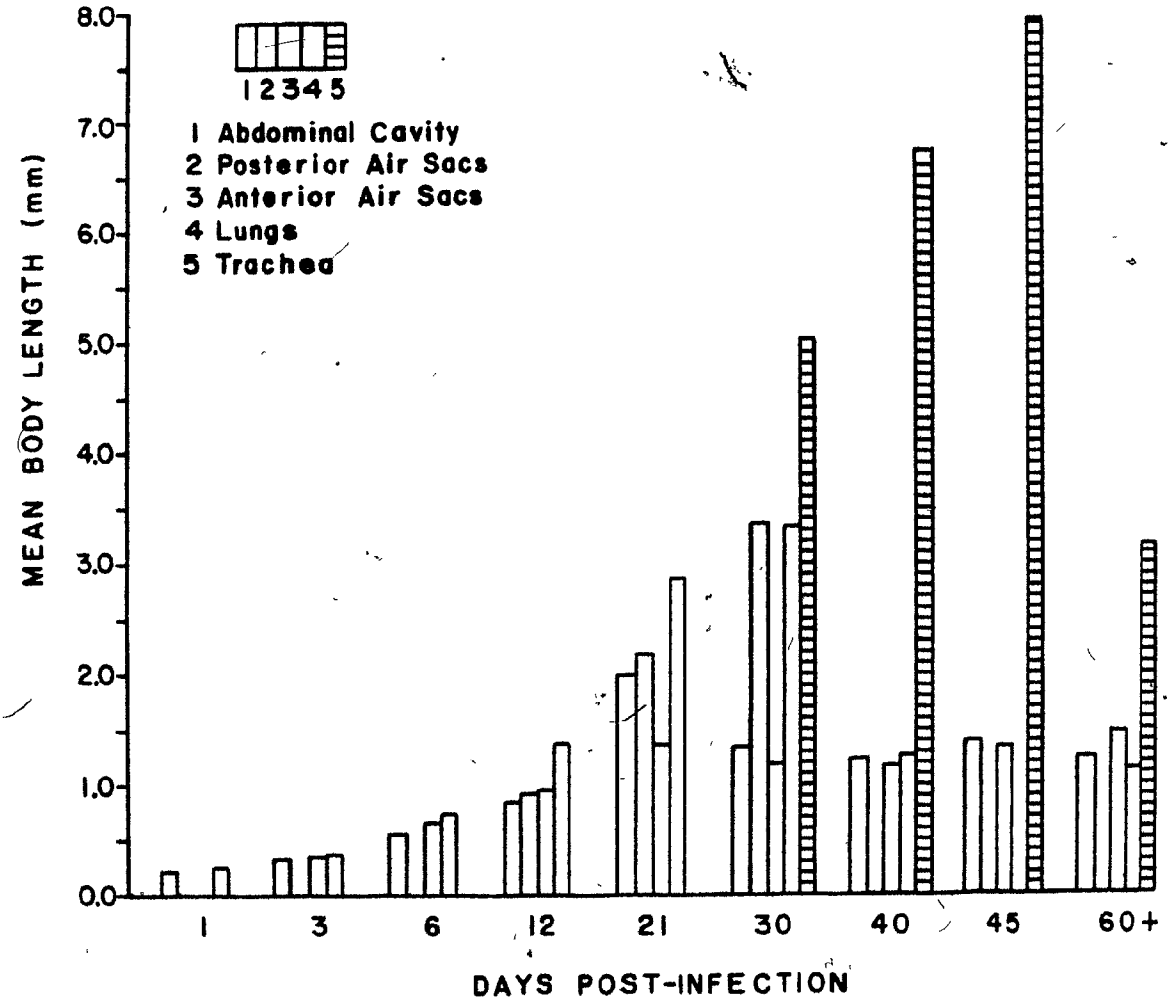
$$\ln(\text{body length}) = \ln(0.294) + 0.079\ (\text{days post-infection}).$$

The slope of the line (0.079) is significantly different from zero ($P < 0.001$); the Pearson correlation coefficient ($r = 0.931$, $P < 0.001$) demonstrates that the body length increases significantly with time.

The mean measurements for all other variables at each day post-infection are presented in Appendix 6-1. The relationship between the body length and each of these variables can be described by the generalized

Figure 6-2. Histograms of mean body length of *Typhlocoelum cucumerinum sisowi* recovered from various sites in mallards at various days post-infection.




8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
MEAN BODY LENGTH (mm)
1 2 3 4 5
1 Abdominal Cavity
2 Posterior Air Sacs
3 Anterior Air Sacs
4 Lungs
5 Trachea
1
3
6
12
21
30
40
45
60+
DAYS POST-INFECTION

allometric growth equation:

$$\ln(\text{variable}) = \ln(b) + \alpha\{\ln(\text{body length})\}, \text{ or}$$

$$\text{variable} = b(\text{body length})^{\alpha}$$

where b is the Y-intercept and α is the coefficient of allometry and indicates the slope of the regression line between body length and the variables. The parameters b and α for each numerical variable relative to body length are presented in Table 6-2. If the rates of growth are the same, α equals unity. If the variable increases at a faster or slower rate than the body length, α will be greater or smaller than unity. In three cases (anterior extent of eggs in the uterus, number of inner diverticula and cirrus sac length), the variables were not significantly correlated with body length. Nine of the variables increased at a significantly slower rate than the body length: body width, pharynx length and width, distance from the pharynx to the anterior, oesophagus length, oral sucker length and width and ventral sucker length and width. The dimensions of the ventral sucker did not continue to increase with increased body length (Figure 6-3) and the ventral sucker may actually get smaller (Figure 6-4). The remaining variables increased at approximately the same rate as the body length.

b) DEVELOPMENT

A summary of the character variable states for all *T. c. sisowi* specimens recovered at different days post-infection are presented in Appendix 6-2.

At 1 and 3 days post-infection, juvenile *T. c. sisowi* had a prominent oral and ventral sucker, pharynx and circular intestine. Inner diverticula on the intestinal caeca could be distinguished in 21% of the day 1 specimens and in 100% of the day 3 specimens. The uterus was represented by a chain of darkly staining cells that extended the length of the intercaecal region.

Specimens obtained at 6 days post-infection showed the first indication of indentations on the ventral surface. At this stage, the uterus had become tubular and coiled in the intercaecal region. At 12 days post-infection, a few specimens had the deep, obvious indentations on the ventral surface that are characteristic of mature *T. c. sisowi*.

Table 6-2. Parameters of the allometric growth equation* for numerical variables of *Typhlocoelum cucumerinum sisowi* recovered from mallards.

NUMERICAL VARIABLE**	n	ln b	α ± 95% C.L.	r	P > r
BW	185	-0.774	0.948 ± 0.026	0.982	0.0001
PL	163	-2.539	0.643 ± 0.025	0.971	0.0001
PW	163	-2.657	0.683 ± 0.027	0.968	0.0001
OL	50	-3.254	0.975 ± 0.188	0.830	0.0001
OW	50	-3.216	1.017 ± 0.233	0.794	0.0001
SRL	40	-3.589	1.045 ± 0.234	0.823	0.0001
SRW	40	-3.600	1.236 ± 0.275	0.824	0.0001
ATL	50	-3.348	1.015 ± 0.218	0.800	0.0001
ATW	50	-3.456	1.150 ± 0.219	0.833	0.0001
PTL	50	-3.536	1.140 ± 0.166	0.892	0.0001
PTW	50	-3.193	1.157 ± 0.231	0.822	0.0001
EGGA	18	0.179	-0.281 ± 0.550	0.253	0.2956
EYEA	23	-2.095	1.527 ± 0.723	0.683	0.0002
PA	144	-1.915	0.657 ± 0.024	0.976	0.0001
OA	48	-0.462	1.074 ± 0.100	0.953	0.0001
SRA	41	-0.240	0.983 ± 0.072	0.975	0.0001
ATA	49	-0.460	1.046 ± 0.099	0.951	0.0001
PTA	49	-0.373	1.069 ± 0.098	0.954	0.0001
NID	88	10.481	0.096 ± 0.256	0.080	0.4553
OES	109	-4.022	0.644 ± 0.135	0.672	0.0001
CL	5	-2.570	1.072 ± 1.211	0.776	0.0699
OSL	106	-2.310	0.450 ± 0.048	0.884	0.0001
OSW	110	-2.193	0.479 ± 0.046	0.883	0.0001
VSL	123	-2.658	0.473 ± 0.073	0.756	0.0001
VSW	123	-2.562	0.487 ± 0.070	0.779	0.0001
VSA	99	-1.072	0.960 ± 0.052	0.965	0.0001

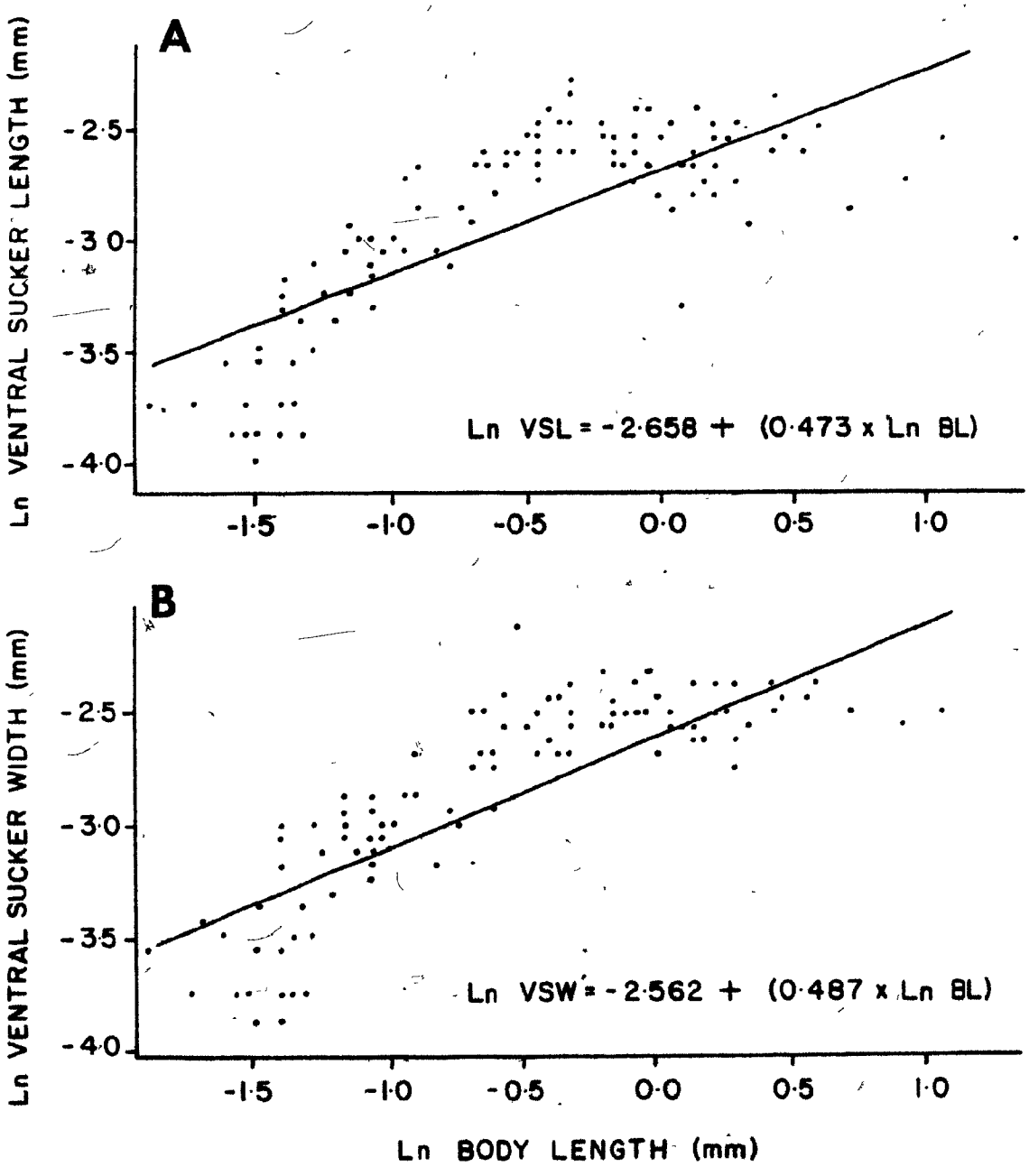
* $\ln Y = \ln b + \alpha \ln X$, where X is the body length and Y is the numerical variable listed in the table.

** The abbreviations for numerical variables are explained in the "List of Abbreviations".

Figure 6-3. Graphs showing the relationship between ventral sucker size and body length of *Typhlocoelum cucumerinum sisowi* from mallards.

A, ventral sucker length;

B, ventral sucker width.


A
Ln VENTRAL SUCKER LENGTH (mm)
-2·5
-3·0
-3·5
-4·0
Ln VSL = -2·658 + (0·473 x Ln BL)
-1·5
-1·0
-0·5
0·0
0·5
1·0
B
Ln VENTRAL SUCKER WIDTH (mm)
-2·5
-3·0
-3·5
-4·0
Ln VSW = -2·562 + (0·487 x Ln BL)
-1·5
-1·0
-0·5
0·0
0·5
1·0
Ln BODY LENGTH (mm)

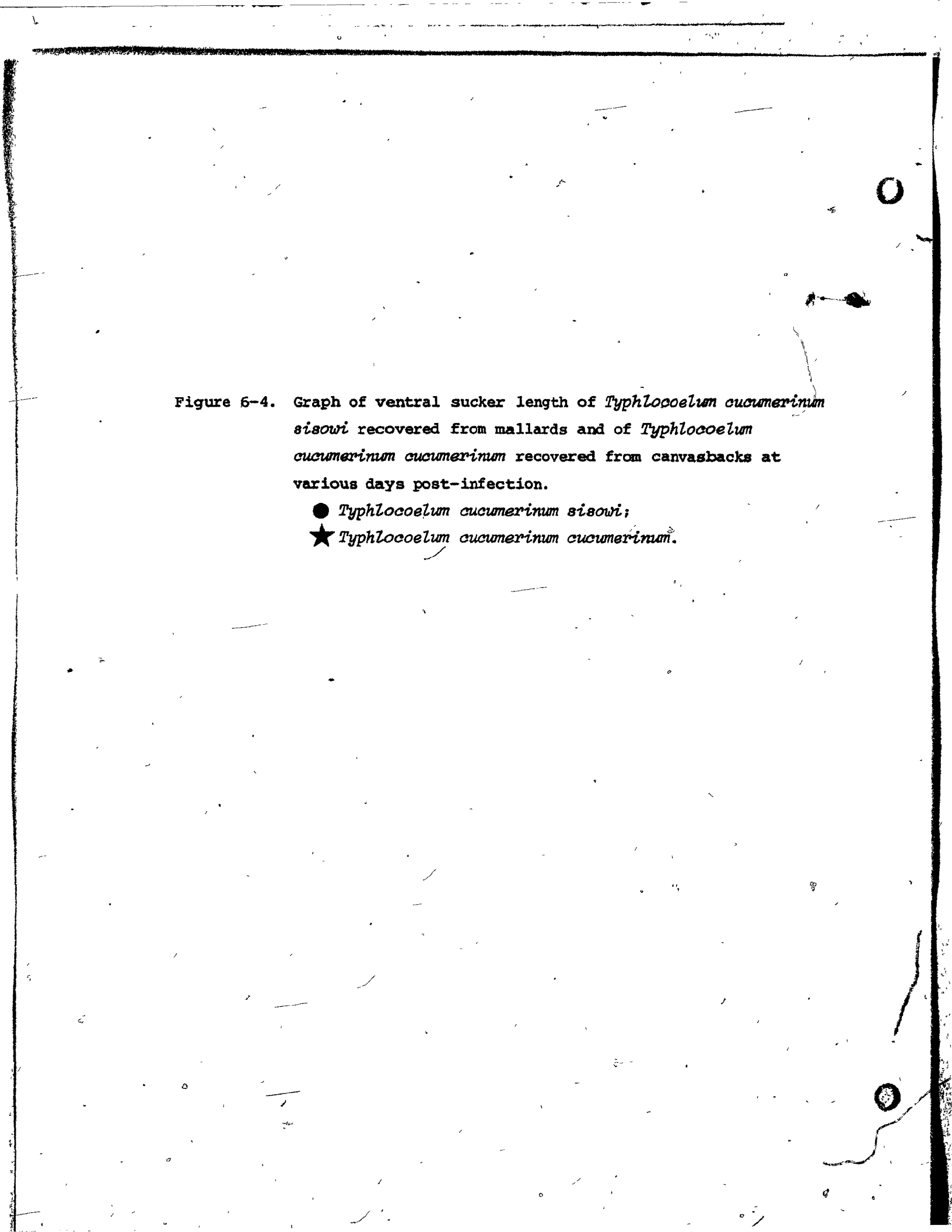
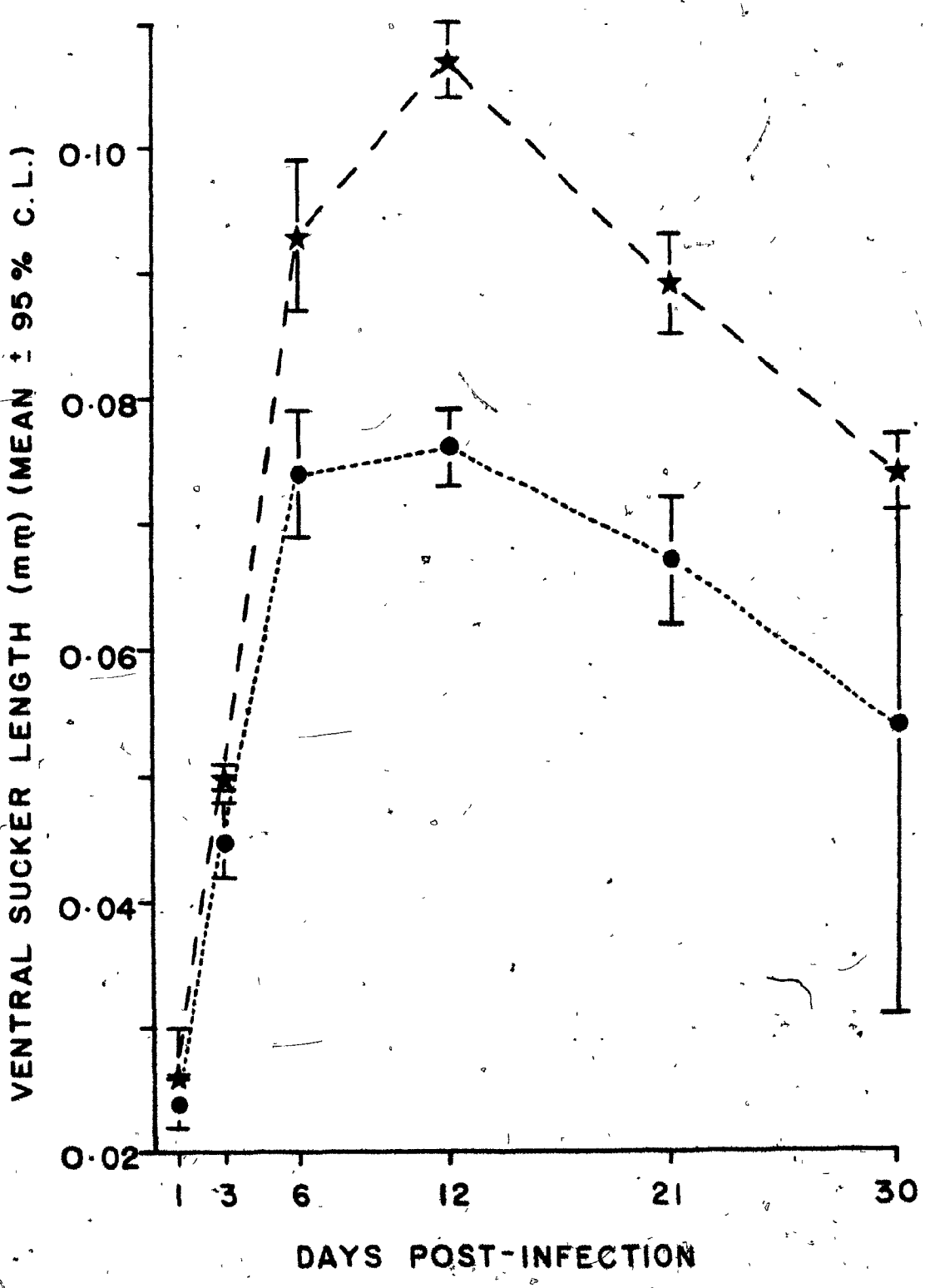


Figure 6-4. Graph of ventral sucker length of *Typhlocoelum cucumerinum sisowi* recovered from mallards and of *Typhlocoelum cucumerinum cucumerinum* recovered from canvasbacks at various days post-infection.

● *Typhlocoelum cucumerinum sisowi*;

★ *Typhlocoelum cucumerinum cucumerinum*.


VENTRAL SUCKER LENGTH (mm) (MEAN ± 95% C.L.)
0·10
0·08
0·06
0·04
0·02
1
3
6
12
21
30
DAYS POST-INFECTION

By 21 days post-infection, the ovary and testes were recognizable. The testes were round (Figure 6-5A). The coils of the uterus passed between the testes in all specimens. The cirrus sac, genital pore and vitellaria were first seen at 21 days post-infection. Eggs were present in the uterus of 47% of the specimens. The ventral sucker was distinguishable in only 46% of the day 21 specimens.

At 30 days post-infection, 50% of the *T. c. sisowi* recovered from mallards had deep, distinct indentations on the ventral surface. The vitellaria had developed fully and extended from the level of the pharynx posterior to the excretory bladder. Embryonated eggs with eyespots were observed for the first time at 30 days post-infection.

The rate of maturation of *T. c. sisowi* was determined by assessing the proportion of specimens with fully embryonated eggs in the uterus. Figure 6-6 shows that the first mature specimens were obtained at 30 days post-infection, and that, by 45 days post-infection, 85% of the specimens were mature.

Nine embryonated eggs were collected from five *T. c. sisowi* recovered from the trachea at 30 days post-infection. Three of these were tanned, and averaged 0.152 x 0.074 mm. In addition, two *T. c. sisowi* recovered from the lungs at 30 days post-infection contained 36 unembryonated eggs. At 40 days post-infection, 125, 150 and 775 fully embryonated eggs were contained within the uterus of three *T. c. sisowi*.

2. *Typhlocoelum cucumerinum cucumerinum* IN CANVASBACKS

a) GROWTH

Figure 6-7 presents the data on mean body length of *T. c. cucumerinum* from each site at different days post-infection. Significant differences in the body length of *T. c. cucumerinum* among sites were only detected at 52 days post-infection when those specimens recovered from the anterior air sacs were significantly shorter than those recovered from the trachea. For all further analyses, specimens recovered from the anterior air sacs and lungs at 52 days post-infection are deleted.

On the basis of a least-squares linear regression analysis, the growth of *T. c. cucumerinum* with respect to body length can be described by

Figure 6-5. Photographs of developmental stages of the testes of *Typhlocoelum cucumerinum sisowi* from mallards and of *Typhlocoelum cucumerinum cucumerinum* from canvasbacks.

A, *Typhlocoelum cucumerinum sisowi* at 21 days post-infection;

B, *Typhlocoelum cucumerinum cucumerinum* at 21 days post-infection;

C, *Typhlocoelum cucumerinum cucumerinum* at 30 days post-infection;

at, anterior testis;

pt, posterior testis.

Bar represents 0.5 mm.

at
at
at
pt
pt

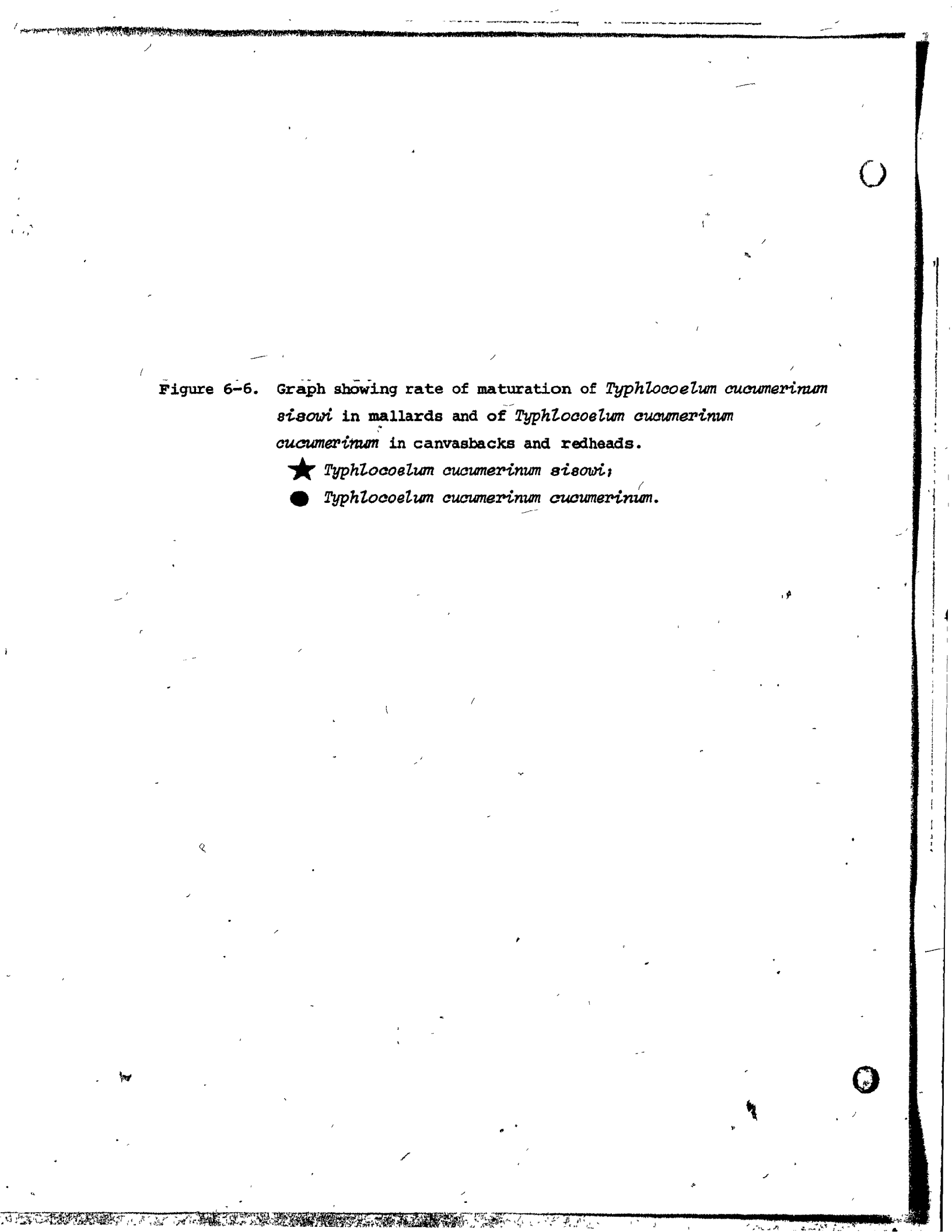


Figure 6-6. Graph showing rate of maturation of *Typhlocoelum cucumerinum sisowi* in mallards and of *Typhlocoelum cucumerinum cucumerinum* in canvasbacks and redheads.

★ *Typhlocoelum cucumerinum sisowi*;

● *Typhlocoelum cucumerinum cucumerinum*.

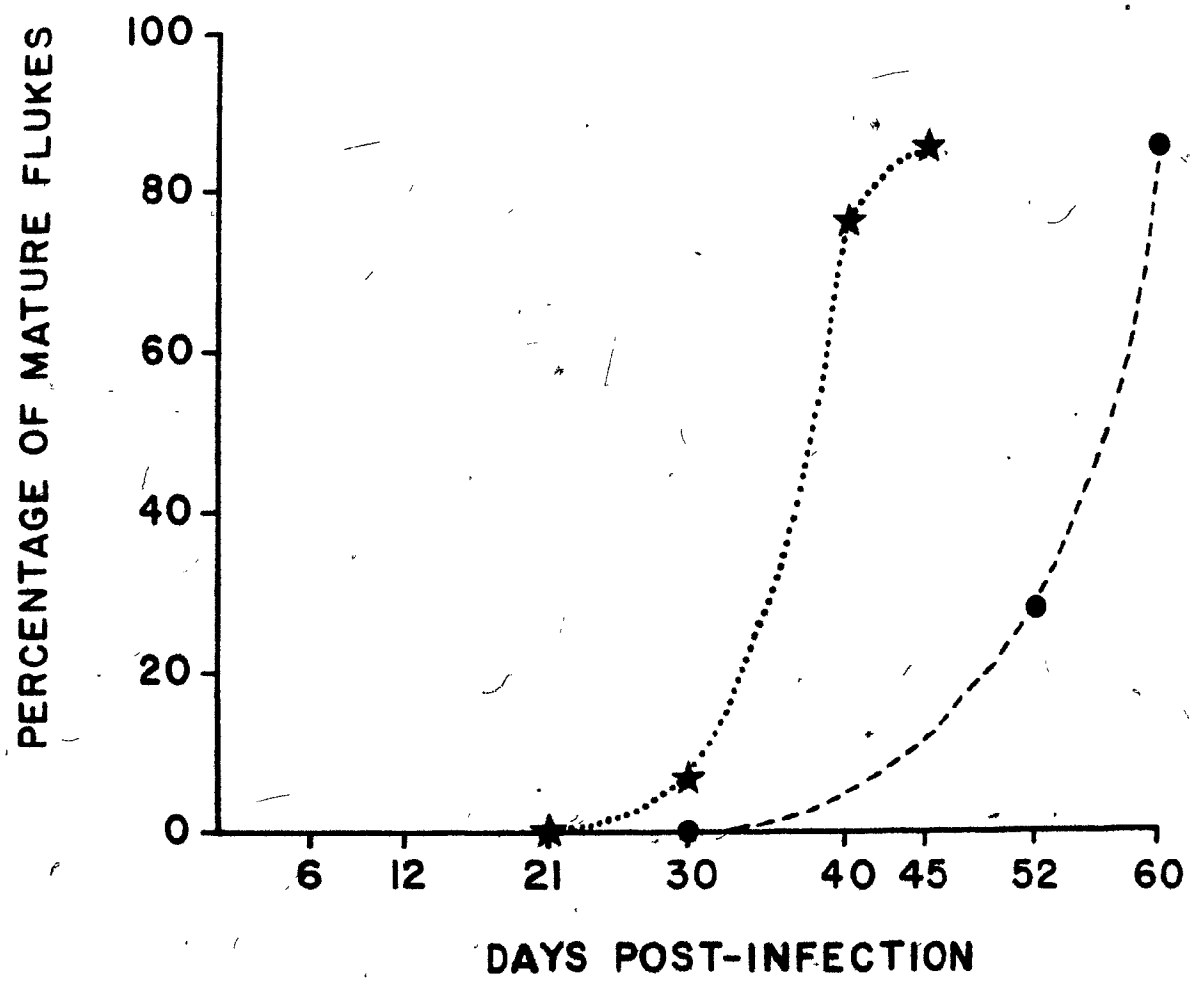
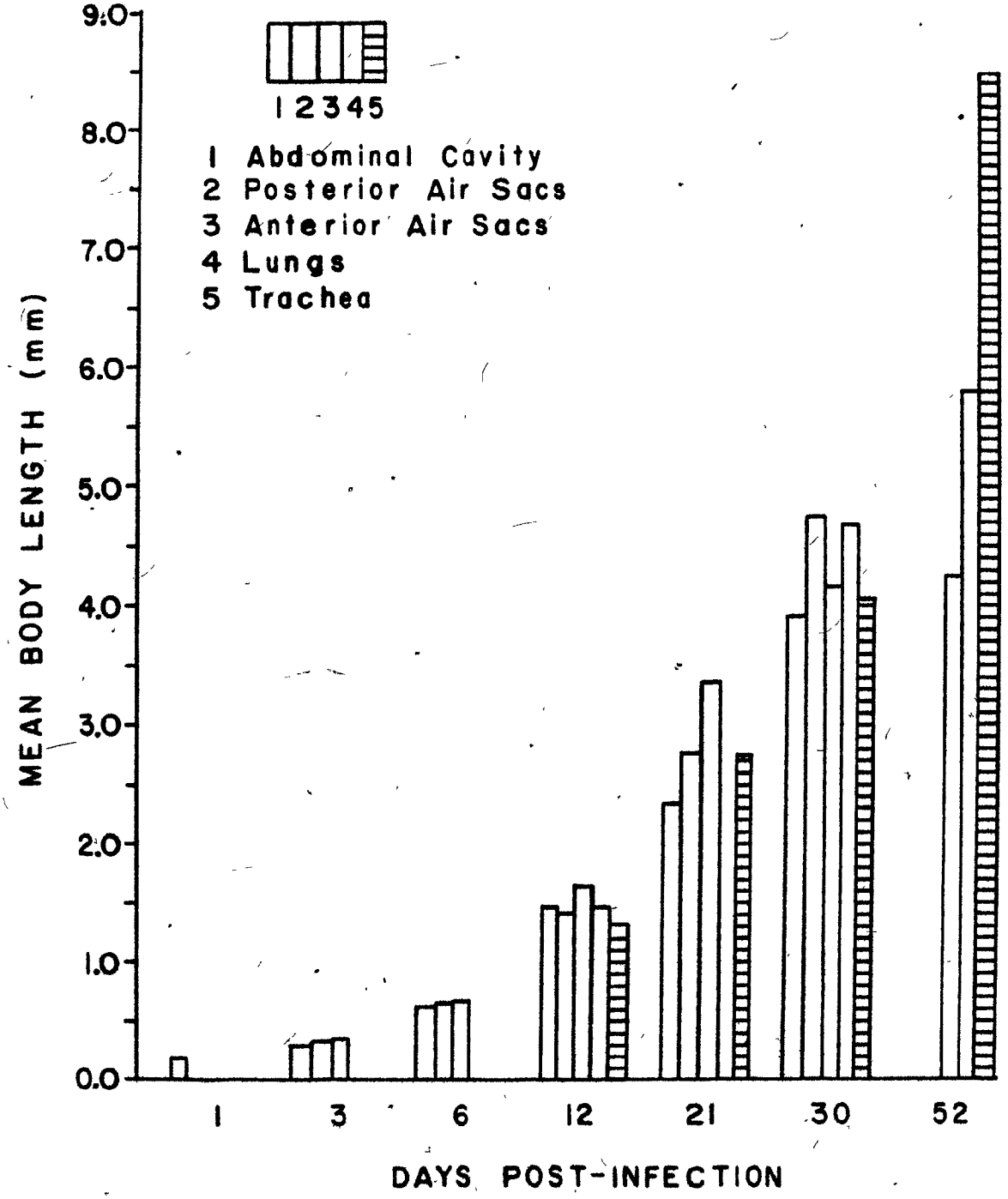

PERCENTAGE OF MATURE FLUKES
100
80
60
40
20
0
6
12
21
30
40
45
52
60
DAYS POST-INFECTION

Figure 6-7. Histograms of mean body length of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at various days post-infection.


9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
MEAN BODY LENGTH (mm)
1 2 3 4 5
1 Abdominal Cavity
2 Posterior Air Sacs
3 Anterior Air Sacs
4 Lungs
5 Trachea
1
3
6
12
21
30
52
DAYS POST-INFECTION

the following equation:

ln (body length) = ln (0.467) + 0.065 (days post-infection).

The slope of the line (0.065) is significantly greater than zero ($P < 0.0001$) and the Pearson correlation coefficient ($r = 0.903$, $P < 0.0001$) demonstrates that the body length increases significantly with time.

Mean measurements for all other numerical variables at each day post-infection are presented in Appendix 6-3. Allometric growth parameters were determined for each numerical variable relative to the body length (Table 6-3). The number of inner diverticula and the distance from the eggs to the anterior were not significantly correlated with the body length. Three variables increased at approximately the same rate as the body length: distance from the eyespots to the anterior, distance from the anterior testis to the pharynx and posterior testis width. The lengths and widths of the ovary, the testes and the seminal receptacle, and the distances from the ovary, seminal receptacle and posterior testis to the pharynx increased significantly faster than the body length. The remaining variables increased at a significantly slower rate relative to the body length. As was the case in *T. c. sisowi*, the dimensions of the ventral sucker were the most divergent from simple allometry (Figure 6-8) and the ventral sucker actually decreased in size after 12 days post-infection (Figure 6-4).

b) DEVELOPMENT

The data on character variables of *T. c. cucumerinum* are presented in Appendix 6-4. Inner diverticula were present in all specimens at 1 day post-infection. These flukes had a prominent pharynx together with oral and ventral suckers. The first indication of indentations on the ventral surface of *T. c. cucumerinum* occurred at 3 days post-infection. By day 12, the cirrus sac, genital pore and ovary were visible. The testes were first distinguished at 21 days post-infection, and in 96% of the specimens, they were loosely-lobed (Figure 6-5B). The remaining 4% of the flukes had deeply-lobed testes. At 30 days post-infection, the vitellaria had developed fully and eggs were first observed. All day 30 specimens had lobed testes, 95% of which were deeply-lobed (Figure 6-5C). Only 26% of the

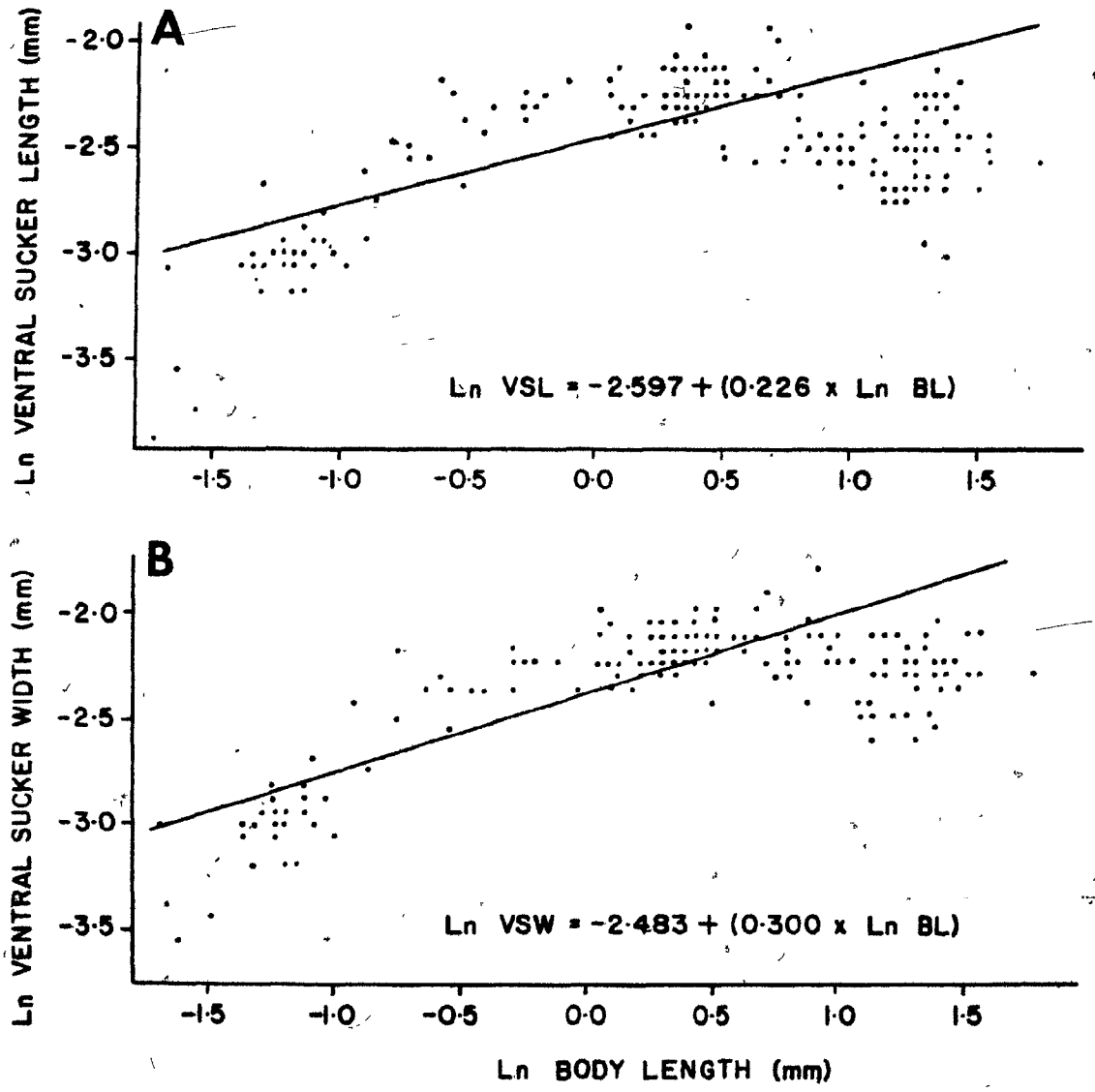
Table 6-3. Parameters of the allometric growth equation* for numerical variables of *Typhlocoelum cucumerinum cucumerinum* recovered from canvasbacks.

NUMERICAL VARIABLE**	n	ln b	α ± 95% C.L.	r	P > r
BW	382	-0.751	0.955 ± 0.017	0.985	0.0001
PL	356	-2.646	0.617 ± 0.013	0.981	0.0001
PW	357	-2.864	0.663 ± 0.013	0.982	0.0001
OL	278	-4.170	1.401 ± 0.057	0.946	0.0001
OW	278	-3.905	1.338 ± 0.057	0.940	0.0001
SRL	280	-4.047	1.186 ± 0.062	0.915	0.0001
SRW	280	-3.507	1.327 ± 0.061	0.931	0.0001
ATL	219	-2.554	1.223 ± 0.097	0.859	0.0001
ATW	218	-3.405	1.211 ± 0.113	0.819	0.0001
PTL	224	-3.117	1.230 ± 0.072	0.914	0.0001
PTW	224	-2.184	1.019 ± 0.071	0.882	0.0001
EGGA	27	0.017	0.157 ± 1.388	0.046	0.8173
EYEA	14	-3.152	1.913 ± 0.872	0.796	0.0004
PA	347	-1.885	0.638 ± 0.018	0.966	0.0001
OA	275	-0.409	1.029 ± 0.020	0.986	0.0001
SRA	280	-0.365	1.022 ± 0.021	0.985	0.0001
ATA	224	-0.428	1.020 ± 0.026	0.982	0.0001
PTA	227	-0.310	1.037 ± 0.024	0.984	0.0001
NID	290	11.557	-0.028 ± 0.165	0.020	0.7405
OES	321	-4.002	0.722 ± 0.054	0.826	0.0001
CL	176	-2.564	0.927 ± 0.064	0.906	0.0001
OSL	123	-2.327	0.437 ± 0.027	0.945	0.0001
OSW	122	-2.346	0.377 ± 0.029	0.919	0.0001
VSL	221	-2.597	0.226 ± 0.037	0.629	0.0001
VSW	221	-2.483	0.300 ± 0.032	0.777	0.0001
VSA	210	-1.141	0.929 ± 0.016	0.992	0.0001

* $\ln Y = \ln b + \alpha \ln X$, where X is the body length and Y is the numerical variable listed in the table.

** The abbreviations for numerical variables are explained in the "List of Abbreviations".

Figure 6-8. Graphs showing the relationship between ventral sucker size and body length of *Typhlocoelum cucumerinum cucumerinum* from canvasbacks.
A, ventral sucker length;
B, ventral sucker width.


A
Ln VENTRAL SUCKER LENGTH (mm)
-2·0
-2·5
-3·0
-3·5
Ln VSL = -2·597 + (0·226 x Ln BL)
-1·5
-1·0
-0·5
0·0
0·5
1·0
1·5
B
Ln VENTRAL SUCKER WIDTH (mm)
-2·0
-2·5
-3·0
-3·5
Ln VSW = -2·483 + (0·300 x Ln BL)
-1·5
-1·0
-0·5
0·0
0·5
1·0
1·5
Ln BODY LENGTH (mm)

day 30 flukes had a ventral sucker.

The rate of maturation is represented graphically in Figure 6-5. Mature flukes were not recovered until 52 days post-infection. One *T. c. cucumerinum* obtained at 52 days post-infection contained 15 fully embryonated eggs that measured 0.168 ± 0.002 x 0.086 ± 0.002 mm (mean ± 95% confidence limits). Three specimens obtained at 72 days post-infection (canvasbacks infected with metacercariae from *Lymnaea elodes*) contained 50, 75 and 825 fully embryonated eggs.

D. DISCUSSION

In comparing the growth and development of *T. c. sisowi* and *T. c. cucumerinum*, a number of similarities are apparent. Both parasites lose their ventral suckers, the testes are first recognizable at 21 days post-infection, and the vitellaria are fully developed at 30 days post-infection. However, when the patterns of growth and development are compared, a number of marked differences are found as well.

During the early period of growth, the location of *T. c. sisowi* and *T. c. cucumerinum* in the ducks did not significantly affect the body length. However, after 12 days post-infection, *T. c. sisowi* in mallards gradually split into two distinct groups (Figure 6-2): one continued to increase in body length and one stopped growing and remained at approximately the day 12 body length regardless of age or site. When the size and development of various organs in these small flukes were examined (Appendix 6-1, 6-2), it was obvious that they not only ceased to increase in body length but that they also ceased to develop. There were no indications that this residual group of day 12-sized worms commenced growing during the first seven months of the infection, and as a consequence, these specimens were eliminated from all growth and development comparisons with *T. c. cucumerinum*.

A similar separation into two distinct groups, observed in *T. c. sisowi* infections, did not occur in *T. c. cucumerinum* infections in canvasbacks (Figure 6-7) although at day 52, the two *T. c. cucumerinum* specimens recovered from the anterior air sacs were significantly shorter than the specimens recovered from the trachea.

1. GROWTH

a) TECHNIQUE USED TO STUDY GROWTH

Digenea are soft-bodied organisms and, as a consequence, all measurements of body and organ sizes are subject to extensive variability resulting from fixation and preparation techniques (Bakke, 1978b). It is very important to keep this in mind when discussing any size-related phenomena.

Growth of Digenea has been analyzed in the past primarily by determining the body length at various days post-infection. Because measurement of the parasites necessarily involves killing the host and usually also involves killing the parasite, actual growth of an individual can not be assessed. Therefore, it is essential to obtain samples at various days post-infection and it is imperative that the statistical parameters of each sample be determined. When growth is described, the mean, the standard error of the mean and the sample size, or the mean and the 95% confidence limits on the mean should be provided for each sample in order to compare the growth among the various days post-infection (Simpson *et al.*, 1960).

In the literature, examples can be found where growth is discussed in terms of a) a doubling of body length in a certain period of time (Szidat, 1933), b) average length and width at various days post-infection with no indication of the variability (Fried, 1973) and c) mean length and standard error at various days post-infection with no sample size (Colgan and Nollen, 1977). Although each of these does give some information about the growth of the organism, none of these methods of presenting growth data provide sufficient information for morphometric comparisons with other studies.

Recently, researchers have begun using variables other than body length to describe growth. Harris *et al.* (1972) described the growth of *Leucochloridiomorpha constantiae* (Mueller, 1935) on the basis of body area and testicular area with respect to time. Guy and Fried (1973) provided the mean as well as the individual values for body length, body width, oral and ventral sucker width, length and width of both testes and ovary of *Parorchis acanthus* (Nicoll, 1906).

The use of ratios in growth studies has also become common in the 1970's. For instance, Fried and Guy (1974) presented data for the ratio of total gonad area to body area at different days post-infection for *Leucochloridium variae* McIntosh, 1932. The use of ratios provides information on the relative size of two variables through time.

An analysis of allometry is the most recent technique that has been applied in digenean growth studies. Allometry measures the relative growth of a part of the organism in relation to the whole organism. Fischthal (1978a,b) has provided data on allometry for seven species of Digenea obtained from naturally infected marine fishes. In the present study, allometry was assessed in experimentally reared specimens. It has been suggested (Simpson *et al.*, 1960) that "the relative growth rate of two organs or dimensions is a fairly basic characteristic of a species" and hence the allometric parameters could become very useful in comparative and taxonomic studies.

b) COMPARISON OF GROWTH OF *Typhlocoelum cucumerinum sisowi* AND *Typhlocoelum cucumerinum cucumerinum*

When the values of α were compared between *T. c. sisowi* (Table 6-2) (Table 6-2) and *T. c. cucumerinum* (Table 6-3), no significant differences were detected in any of the numerical variables. This means that the growth rates of the various organs relative to body length are the same for both *T. c. sisowi* and *T. c. cucumerinum*. However, when the rates of increase of body length were compared, it was found that *T. c. sisowi* increase in length significantly faster than *T. c. cucumerinum* ($P < 0.05$). When these two observations are considered together, it can be suggested that, because the body lengths increase at different rates, and because the growth of the organs is a function of the body length, then the organs grow faster in *T. c. sisowi* than in *T. c. cucumerinum*.

The only other data on the growth of *T. cucumerinum* was provided by Szidat (1933). He reported that *T. c. sisowi* are 1.4 mm long at 6 days post-infection and 2.8 mm long at 12 days post-infection. These data do not agree with those obtained in the present study as, at 6 days post-infection the specimens were approximately 0.6 mm long, at 12 days post-

infection they were up to 1.4 mm long and it was not until 21 days post-infection that they reached 2.8 mm in length. Szidat (1933) used a different species of duck, *Anas boschas domesticus*, and this together with different techniques of preparation, may have contributed to the discrepancies in body length between the two studies.

2. COMPARISON OF DEVELOPMENT OF *Typhlocoelum cucumerinum sisowi* AND *Typhlocoelum cucumerinum cucumerinum*

Whereas size can be measured objectively, assessment of development is somewhat subjective. In some cases, the developmental stage can be very accurately categorized, for instance, in the case of presence or absence of a certain trait. However, very often development involves a gradual transition through a succession of stages. The point at which one stage ends and the next begins is often very difficult to define precisely. In this study, a number of character variables have been examined to determine how they change during the growth and development of the flukes.

Presence of inner diverticula on the intestinal caeca is a characteristic of the subfamily Typhlocoelinae and researchers have included the number of inner diverticula in species descriptions. This characteristic would obviously be of little value if the number of inner diverticula changed as the specimens grew. No significant changes in the number of inner diverticula with changes in the body length were detected in either *T. c. sisowi* or *T. c. cucumerinum*, thereby suggesting that the flukes do not acquire more inner diverticula as they grew. It was shown, however, that *T. c. cucumerinum* have, on the average, 11.5 inner diverticula whereas *T. c. sisowi* have approximately 10.6 inner diverticula. Although the difference was statistically significant, the character would only be useful, taxonomically, when large samples were available, as the actual number in any one specimen ranged from 8 to 14 in the case of *T. c. sisowi* and from 9 to 15 in the case of *T. c. cucumerinum*.

As has been noted in the Introduction to this chapter, there is a curious relationship between the ventral surface and the ventral sucker during the development of typhlocoelids. The ventral sucker grows during the first six days post-infection, but then remains at the same size until

approximately 21 days post-infection. It then decreases in size (Figure 6-4). When the size of the ventral sucker is related to the body length of the specimens (Figure 6-3 and Figure 6-8), the length and width of the ventral sucker increase initially and then reach a plateau at a body length of about 0.5 mm. The plateau persists until specimens reach approximately 1.4 mm in length, and then there is an indication that the ventral sucker may actually decrease in size with increased body length.

As the specimens grow, not only does the ventral sucker disappear, but also, indentations appear over the entire ventral surface. In the case of *T. c. sisowi*, there is an inverse relationship between the presence of the ventral sucker and the presence of indentations on the ventral surface (Figure 6-9). Szidat (1933) noted that indentations were present as early as 14 days post-infection but that the ventral sucker persisted until 28 days post-infection. The data from this study corroborates Szidat's observations and extends the range in both directions. Ventral indentations were first observed at 6 days post-infection and the ventral sucker persisted in a few specimens until 40 days post-infection.

In *T. c. cucumerinum*, the proportion of specimens with a ventral sucker followed the same pattern as that for *T. c. sisowi*, but the proportion with indentations on the ventral surface did not (Figure 6-10). The number of specimens with and without indentations at various days post-infection fluctuated and no trend could be detected.

When the ventral surfaces of *T. c. sisowi* and *T. c. cucumerinum* were compared, there were distinct differences in the size and depth of the indentations. Of all specimens obtained on or after 30 days post-infection, 52% of the *T. c. sisowi* had large, deep indentations, 27% had small indentations and 21% had no indentations. However, in the case of *T. c. cucumerinum*, no specimens had large, deep indentations, 39% had small indentations and 61% had no indentations. The function of the indentations on the ventral surface is not known, although Szidat (1933) suggested that they may function in attachment to the trachea.

The ovary, genital pore and cirrus sac could be distinguished at 12 days post-infection in *T. c. cucumerinum*, but not until 21 days post-infection in *T. c. sisowi*. In both subspecies the testes were recognizable

Figure 6-9. Graph showing relationship between proportion of *Typhlocoelum cucumerinum sisowi* with ventral suckers and proportion with indentations on the ventral surface at various days post-infection.

● proportion with ventral sucker;

✘ proportion with indentations on the ventral surface.

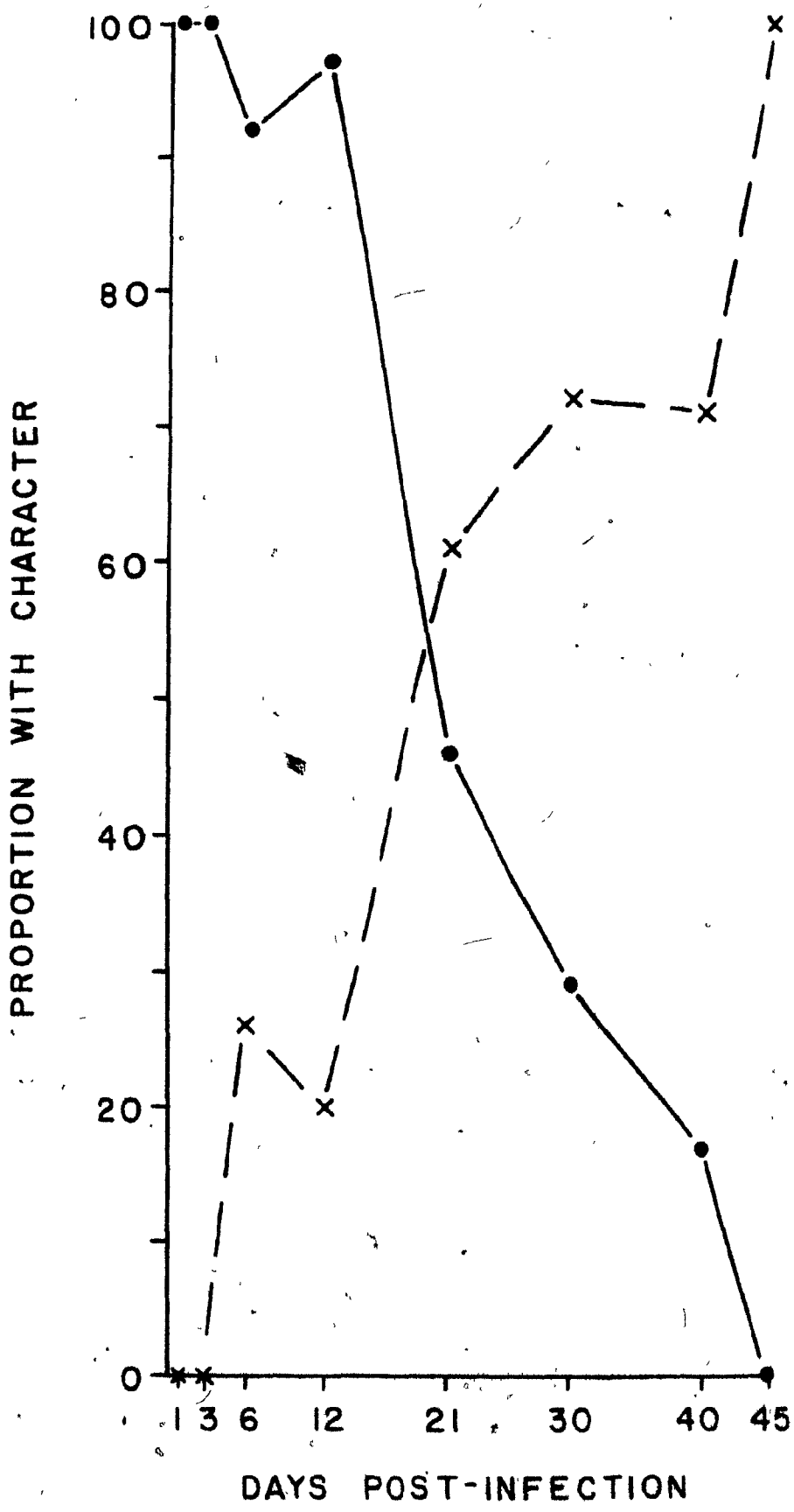
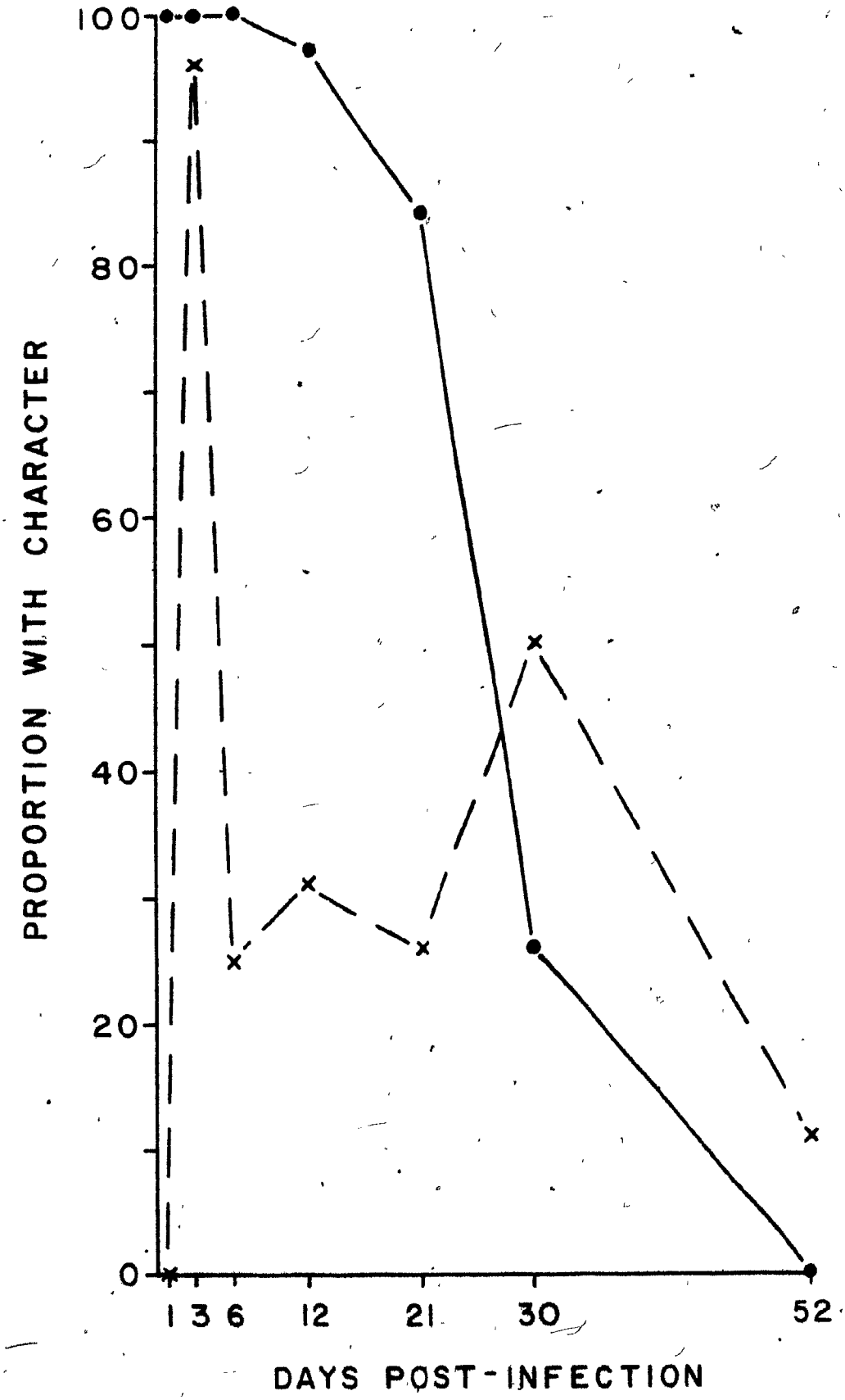

100
80
60
40
20
0
PROPORTION WITH CHARACTER
1 3 6 12 21 30 40 45
DAYS POST-INFECTION

Figure 6-10. Graph showing relationship between proportion of *Typhlocoelum cucumerinum cucumerinum* with ventral suckers and proportion with indentations on the ventral surface at various days post-infection.

● proportion with ventral sucker;

✕ proportion with indentations on the ventral surface.


100
80
60
40
20
0
PROPORTION WITH CHARACTER
1 3 6 12 21 30 52
DAYS POST-INFECTION

at 21 days post-infection.

The ovary had the same shape and was in the same position in specimens of *T. c. sisowi* and *T. c. cucumerinum*. There was a marked difference, however, in the size and shape of the testes between the two subspecies. All of the *T. c. sisowi* specimens had round or oval testes that increased in size with age, but did not change in shape. The testes of *T. c. cucumerinum* were loosely-lobed at 21 days post-infection and, as they continued to grow and develop, they expanded laterally and became deeply-lobed (see Figure 6-5B,C). Although one could postulate that either the lobed or the round testis shape might be a consequence of age, the data presented on the development of the testes through time demonstrate conclusively that the differences between the round and lobed testes are not a function of age, but that the loosely-lobed and deeply-lobed shapes are related to age.

The uterus passed between the testes only when there was a gap between the posterior margin of the anterior testis and the anterior margin of the posterior testis. Taxonomists have referred to the presence or absence of uterine loops between the testes as a diagnostic feature (see Chapter III). However, this appears to be solely a function of the size of the testes.

The vitellaria developed sooner in *T. c. sisowi* than in *T. c. cucumerinum*, and this is undoubtedly correlated with the earlier egg production in *T. c. sisowi*. In both *T. c. sisowi* and *T. c. cucumerinum*, the vitellaria extended anteriorly to the level of the pharynx. Most *T. c. sisowi* specimens had vitellaria extending as far posteriorly as the excretory bladder whereas this occurred in only about 50% of the *T. c. cucumerinum* specimens.

Eggs were present in *T. c. sisowi* nine days earlier than in *T. c. cucumerinum*, and *T. c. sisowi* reached maturity much sooner (Figure 6-6). There was no significant difference in the number of fully embryonated eggs in *T. c. sisowi* at 40 days post-infection and in *T. c. cucumerinum* at 72 days post-infection, although evidence from wild worms suggests that *T. c. cucumerinum* are capable of producing more eggs than *T. c. sisowi* (see Chapter III).

3. SUMMARY

In Chapter V, it was demonstrated that *T. c. sisowi* remain in the duck for a relatively short period of time in comparison with *T. c. cucumerinum*. Data presented in this chapter have shown that, in fact, *T. c. sisowi* grow and mature more quickly than *T. c. cucumerinum*. Obviously, the two subspecies have different survival strategies for development in the definitive host. In the next chapter, the larval development of the two subspecies will be compared.

CHAPTER VII. COMPARATIVE STUDY ON *Typhlocoelum cucumerinum sisowi* AND *Typhlocoelum cucumerinum cucumerinum* IN THREE DIFFERENT SPECIES OF SNAILS

A. INTRODUCTION

Considerably more experimental work has been done on the larval stages of *T. cucumerinum* than on the stages in the duck host. Szidat (1932, 1933) and Stunkard (1934a,b) presented detailed accounts of the morphology of the miracidia, rediae, cercariae and metacercariae of *T. c. sisowi* and, more recently, Schafranski (1974) and Schafranski *et al.* (1975) have described the morphology of these stages of *T. c. cucumerinum*.

Ciliated miracidia hatch from the eggs within 10 to 30 minutes of being released in water (Stunkard, 1934a,b). Each miracidium contains a prominent black eyespot, two flame cells and a single, fully formed redia occupies the posterior region of the miracidium (Stunkard, 1934a,b; Schafranski *et al.*, 1975). Miracidia attach to the snail and inject the rediae into the snail tissues. There is some confusion in the literature regarding the site in the snail where the parasites develop. Szidat (1932) reported that the rediae establish in a hidden portion of the gut of the snail, near the albuminous gland, but not in the liver. According to Stunkard (1934b) the rediae "lie in lymph spaces from which they readily dropped out into the salt solution when the snail was dissected". Schafranski *et al.* (1975) found that *T. c. cucumerinum* rediae migrate through the body and localize in the pericardial cavity.

The redia has two pairs of ventrolateral appendages. The digestive system consists of an oral opening, a pharynx and a simple intestine (Stunkard, 1934b; Schafranski *et al.*, 1975). Stunkard (1934b) reported that maximum growth of the rediae occurs during the first two weeks, but that growth continues for at least eight weeks.

There is a single asexual generation in the snail; germinal masses in the rediae develop directly into cercariae. Stunkard (1934b) noted that the germinal masses were present at least until eight weeks post-infection in *T. c. sisowi* rediae, and Schafranski *et al.* (1975) found

germinal masses in 41 day-old *T. c. cucumerinum* rediae. Cercarial development is sequential with only a few fully formed cercariae in a redia at a given time. Szidat (1932) found no more than two mature *T. c. sisowi* cercariae within a redia whereas Schafranski *et al.* (1975) found three to four completely developed *T. c. cucumerinum* cercariae within a single redia.

Tailless cercariae are released from the birth pore of the rediae as early as 8 days post-infection and remain in the vicinity of the rediae, never emerging from the snail (Szidat, 1932, 1933). A conspicuous organ is present at the anterior end of the cercariae. Although Szidat (1932) reported that this organ was separate from the subterminal oral opening on the ventral surface, Krull (1940) reported that this organ was on the ventral surface. The circular intestine, characteristic of adult flukes, is present in the cercariae as well. Two lateral groups of gland cells including cystogenous glands, as well as a ventral sucker, can be seen on the ventral surface (Szidat, 1932; Schafranski *et al.*, 1975).

According to Sreekumaran and Peter (1973a,b) metacercariae encyst in the albuminous gland. Stunkard (1934b) reported that *T. c. sisowi* metacercariae were "near the body wall ... in front of the digestive gland, about midway between the anterior and posterior ends of the snail. After the shell had been removed the cysts were visible at the surface of the body." The cyst wall has two layers, an outer delicate layer and an inner tough, fibrous layer (Stunkard, 1934b; Schafranski *et al.*, 1975). Based on Stunkard's (1934b) observations on metacercariae, he suggested that the metacercariae grow very little. Sreekumaran and Peter (1973a) noted that metacercariae are infective as early as 15 days post-infection.

A number of different species of snails have been exposed to *T. cucumerinum*. Szidat (1932) used *Planorbis corneus* (Linnaeus) and *Planorbis planorbis* (Linnaeus) as experimental hosts for *T. c. sisowi*, and noted that lymnaeids would also act as intermediate hosts. Stunkard (1934a) exposed three species, *Lymnaea stagnalis* (Say), *Physella heterostropha* (Say) and *Helisoma trivolvis* (Say) to *T. c. sisowi*, but only obtained infections in *H. trivolvis*. Sreekumaran and Peter (1973a,b) used *Indoplanorbis exustus* Larambergue as the intermediate host for *T. c. sisowi*. Schafranski (1974) and Schafranski *et al.* (1975) used *Biomphalaria glabrata* (Say) and

Biomphalaria tenagophila (d'Orbigny) as experimental hosts for *T. c. cucumerinum*.

In this study, a series of experiments was undertaken to compare the infectivity, the migration, growth and development, and the host response to larval stages of *T. c. sisowi* and *T. c. cucumerinum* in three different species of snails.

B. MATERIALS AND METHODS

Typhlocoelum cucumerinum sisowi obtained from wild mallards and a wild black duck, and *T. c. cucumerinum* obtained from wild canvasbacks and a wild redhead were used to infect three species of laboratory-reared snails: *Physa gyrina*, *Helisoma trivolvis* and *Lymnaea elodes*. Snails were exposed to five active miracidia for two hours in petti-cups and maintained at 20°C. The experimental protocol is summarized in Table 7-1. An additional 20 *P. gyrina* were each exposed to 25 *T. c. sisowi* miracidia in a petti-cup, and fixed for sectioning at 2, 4, 6 and 12 hours post-infection, in order to follow the migration of the rediae.

The snails were measured (length from apex to aperature) prior to dissection. The shell was gently crushed in 0.75% saline and the tissues were removed and teased apart under a stereomicroscope. The location of the rediae and metacercariae was noted, and the numbers of larval stages were recorded. Before the snail tissues were discarded, they were crushed between two glass slides and scanned using a compound microscope to ensure that all larval stages had been recovered. All rediae and metacercariae were transferred to fresh saline for approximately six hours, and then measured using a compound microscope. The length and width of all rediae, and the maximum diameter of all metacercariae were recorded. Samples of the rediae were also fixed in formalin, stained, mounted and measured.

Snails fixed for sectioning were embedded in Paraffin, serially sectioned at 7 µm and stained in Ehrlich's Haematoxylin and Eosin (Humason, 1972). The detailed synopsis of snail histology by Pan (1958) was used as a guideline in examining snail sections.

As each miracidium gives rise to a single redia, and as there is only one redial generation, the infectivity of the miracidia can be defined

Table 7-1. Experimental protocol for snail experiments.

PARASITE	SNAIL	NUMBER OF SNAILS DISSECTED AT DAYS POST-INFECTION: 1	6	12	21	30	45	60
T. c. sisowi	*P. gyrina*	10	10	10	10	10	0	10
T. c. sisowi	*H. trivolvis*	10	10	10	4	10	0	10
T. c. sisowi	*L. elodes*	10	10	10	10	10	0	10
T. c. cucumerinum	*P. gyrina*	10	10	10	10	10	0	1
T. c. cucumerinum	*H. trivolvis*	10	10	10	10	6	0	7
T. c. cucumerinum	*L. elodes*	10	10	10	10	10	10	4

PARASITE	SNAIL	NUMBER OF SNAILS SECTIONED AT DAYS POST-INFECTION: 1	6	12	21	30	45	60
T. c. sisowi	*P. gyrina*	4	5	3	5	4	0	5
T. c. sisowi	*H. trivolvis*	5	4	2	5	5	0	3
T. c. sisowi	*L. elodes*	5	5	5	2	6	0	5
T. c. cucumerinum	*P. gyrina*	5	4	5	4	4	0	0
T. c. cucumerinum	*H. trivolvis*	5	5	5	5	2	0	2
T. c. cucumerinum	*L. elodes*	5	1	5	5	4	5	0

as the number of rediae present in the snail divided by the total number of miracidia used to infect the snail. Using this definition, the overall percent infectivity of miracidia was determined for each sample of dissected snails.

C. RESULTS

1. *Typhlocoelum cucumerinum sisowi* IN THREE SPECIES OF SNAILS

a) INFECTIVITY OF MIRACIDIA

Although there was variability in the percentage of rediae recovered within each species of snail at different days post-infection, Pearson product-moment correlations between infectivity and days post-infection were not significant. Therefore, the data from all days post-infection were pooled for each snail species, and the overall percent infectivity of *T. c. sisowi* miracidia in each species of snail were calculated (Figure 7-1A). *Typhlocoelum cucumerinum sisowi* miracidia had a significantly higher infectivity in *P. gyrina* and *H. trivolvis* than in *L. elodes*. The size of the snail did not significantly influence the recovery of rediae in *P. gyrina*, *H. trivolvis* or *L. elodes*.

b) MIGRATION AND LOCALIZATION OF THE REDIAE

Physa gyrina exposed to *T. c. sisowi* were sectioned to determine the route of migration of the rediae. At two hours post-infection, *T. c. sisowi* miracidia were attached to the epidermis, and rediae were found in the pericardial cavity (Figure 7-2). At four hours post-infection, some miracidia were attached to the epidermis, and rediae were found in the connective tissue, the sinuses, the pericardial cavity and within the heart (Figure 7-3). By six hours post-infection, all of the rediae in four of five snails were located in the pericardial cavity. The fifth snail sectioned at six hours post-infection was very small, and rediae were dispersed throughout the tissues.

In all snails sectioned and dissected after six hours post-infection, rediae and metacercariae were only found in the pericardial cavity regardless of the snail species or the time post-infection (Figure 7-4).

Figure 7-1. Graphs of miracidial infectivity, production of metacercariae and infectivity of metacercariae in *Typhlocoelum cucumerinum eiseni* infections in three species of snails.

A, miracidial infectivity;

B, production of metacercariae;

C, infectivity of metacercariae to mallards;

P, *Physa gyrina*;

H, *Helisoma trivolvis*;

L, *Lymnaea elodes*.

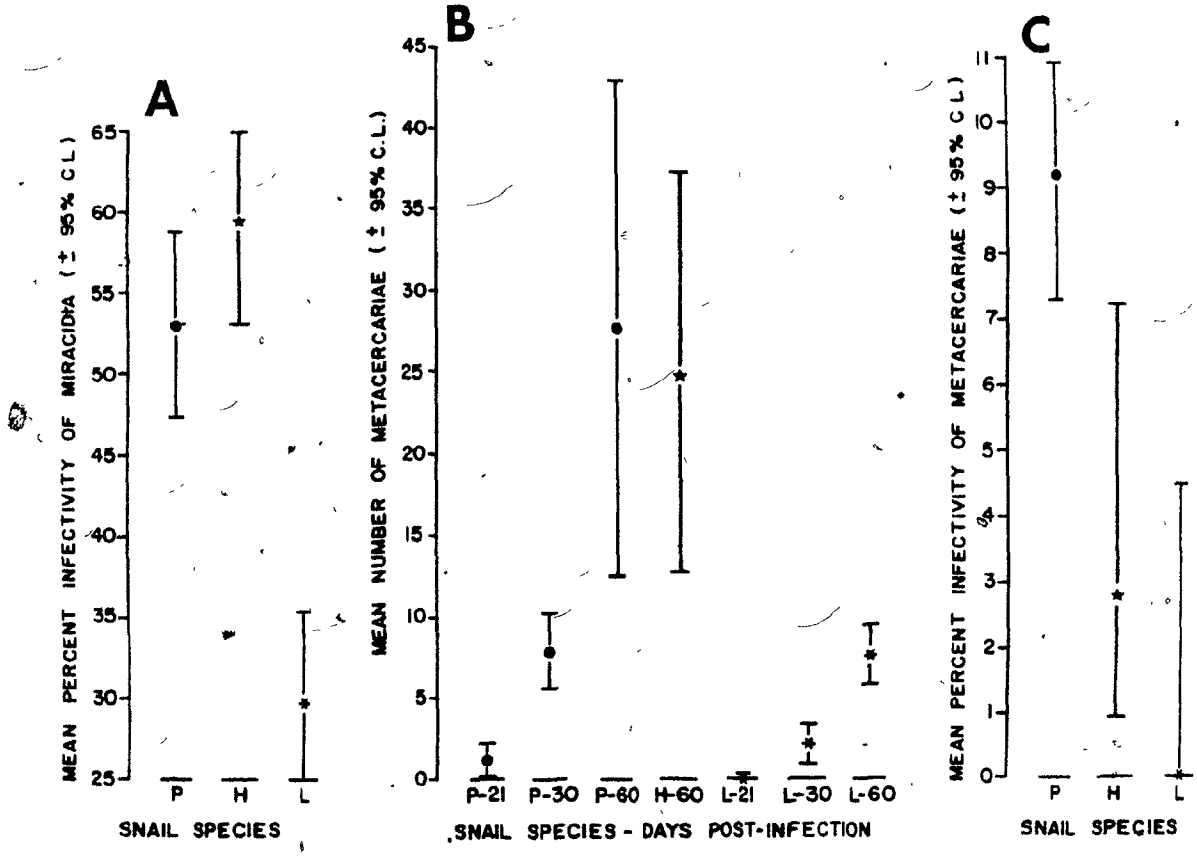

A
MEAN PERCENT INFECTIVITY OF MIRACIDIA (± 95% C.L.)
65
60
55
50
45
40
35
30
25
P
H
L
SNAIL SPECIES
B
MEAN NUMBER OF METACERCARIAE (± 95% C.L.)
45
40
35
30
25
20
15
10
5
0
P-21
P-30
P-60
H-60
L-21
L-30
L-60
SNAIL SPECIES - DAYS POST-INFECTION
C
MEAN PERCENT INFECTIVITY OF METACERCARIAE (± 95% C.L.)
11
10
9
8
7
6
5
4
3
2
1
0
P
H
L
SNAIL SPECIES

Figure 7-2. Photographs of *Typhlocoelum cucumerinum sisowi* miracidia and rediae in *Physa gyrina* at two hours post-infection.
A, miracidium attached to epidermis;
B, rediae in pericardial cavity;
m, miracidium;
r, redia;
h, heart;
p, pericardial cavity.

Bar represents 0.1 mm.

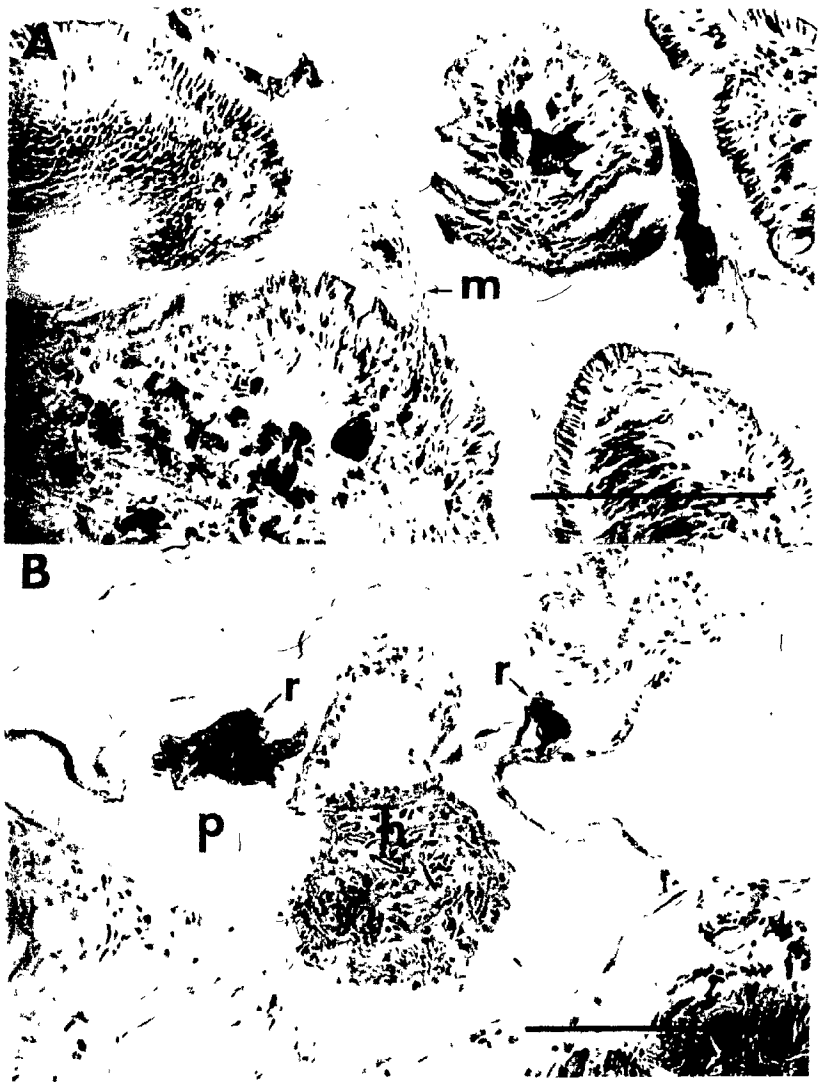

A
m
B
r
r
P
h

Figure 7-3. Photographs of *Typhlocoelum cucumerinum sisowi* rediae in *Physa gyrina* at four hours post-infection.

A, redia in connective tissue;

B, redia in sinus;

C, rediae in pericardial cavity and in heart;

r, redia;

s, sinus;

h, heart;

p, pericardial cavity.

Bar represents 0.1 mm.

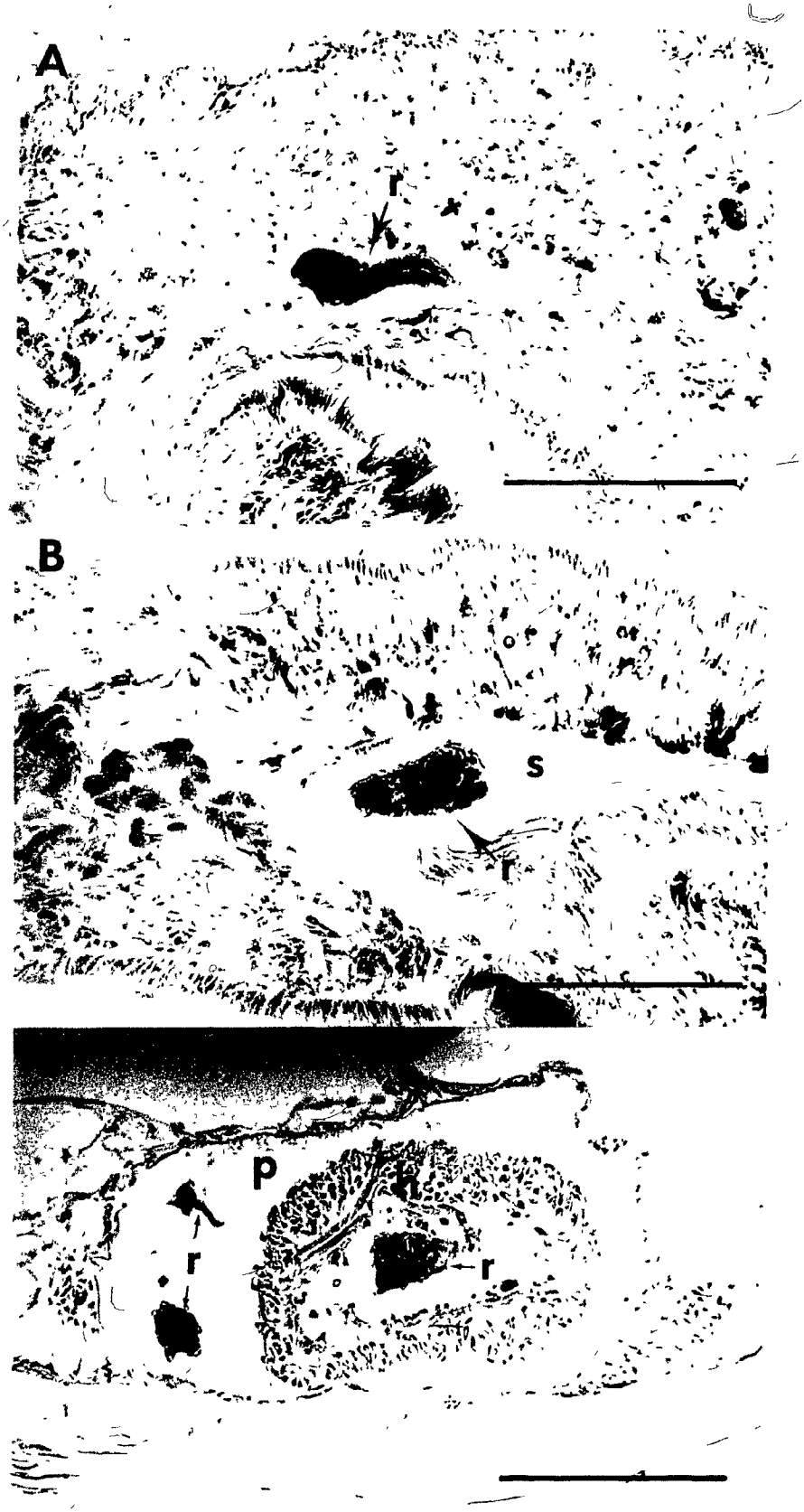

A
r
B
s
r
p
r
r

Figure 7-4. Photograph of *Helisoma trivolvis* (shell removed) showing rediae and metacercariae of *Typhlocoelum cucumerinum sisowi* in the pericardial cavity.
m, metacercaria;
r, redia.

Bar represents 3.0 mm.

COLOURED PICTURE

c) GROWTH AND DEVELOPMENT OF REDIAE

Measurements of rediae contained within the miracidia are presented in Table 7-2. Three rediae were also measured shortly after escaping from the miracidia; the body lengths and widths were: 0.228 x 0.060, 0.200 x 0.042 and 0.172 x 0.049 mm.

Data on the mean length and width of live *T. c. sisowi* rediae at different days post-infection in each of the three snail species are presented in Figure 7-5. Whereas rediae from *L. elodes* increased in length only during the first 12 days of the infection, rediae from *P. gyrina* and *H. trivolvis* continued to increase in length until 30 days post-infection. As a consequence, the rediae from *L. elodes* were significantly smaller than those from both *P. gyrina* and *H. trivolvis* at both 30 and 60 days post-infection. Preserved rediae from both *P. gyrina* and *H. trivolvis* were significantly smaller than live rediae (Table 7-3).

Cercariae could first be distinguished within the rediae at 12 days post-infection. The length and width of the larger cercariae contained within a representative redia from each snail species at each day post-infection are presented in Table 7-4. A maximum of five large cercariae were contained within a single redia, but the usual number was one or two. Each redia also contained smaller developing cercariae and germinal masses at least until 60 days post-infection.

d) PRODUCTION AND DESCRIPTION OF METACERCARIAE

Metacercariae were first found in *P. gyrina* and *L. elodes* at 21 days post-infection, but metacercariae were not recovered from *H. trivolvis* until 60 days post-infection (Figure 7-1B). At 60 days post-infection, both *P. gyrina* and *H. trivolvis* had significantly more metacercariae than *L. elodes*.

In an attempt to discover factors that potentially influence the rate of metacercarial production, a series of Pearson product-moment correlations were calculated for each snail species, using the number of metacercariae in each snail, *vs.* the number of rediae in the infection, and the snail length. The results are presented in Tables 7-5 and 7-6. At 21 days post-infection in *P. gyrina* and at 30 days post-infection in *L. elodes*, large

Table 7-2. Measurements of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* miracidia.

VARIABLE	*T. c. sisowi*		*T. c. cucumerinum*		
MIRACIDIAL LENGTH	0.228	0.217	0.282	0.252	0.296
MIRACIDIAL WIDTH	0.066	0.074	0.070	0.105	0.049
REDIAL LENGTH*	0.140	-	0.212	0.231	0.210
REDIAL WIDTH*	0.052	-	0.056	0.032	0.035
PHARYNX LENGTH*	0.021	0.018	-	-	-
PHARYNX WIDTH*	0.018	0.018	-	-	-

* Measurements made on rediae contained within miracidia.

Figure 7-5. Graph showing mean length and width of live *Typhlocoelum cucumerinum sisowi* rediae at different days post-infection in three species of snails.

✱ *Physa gyrina;*

★ *Helisoma trivolvis;*

✱ *Lymnaea elodes.*

Large symbols represent length and small symbols represent width.

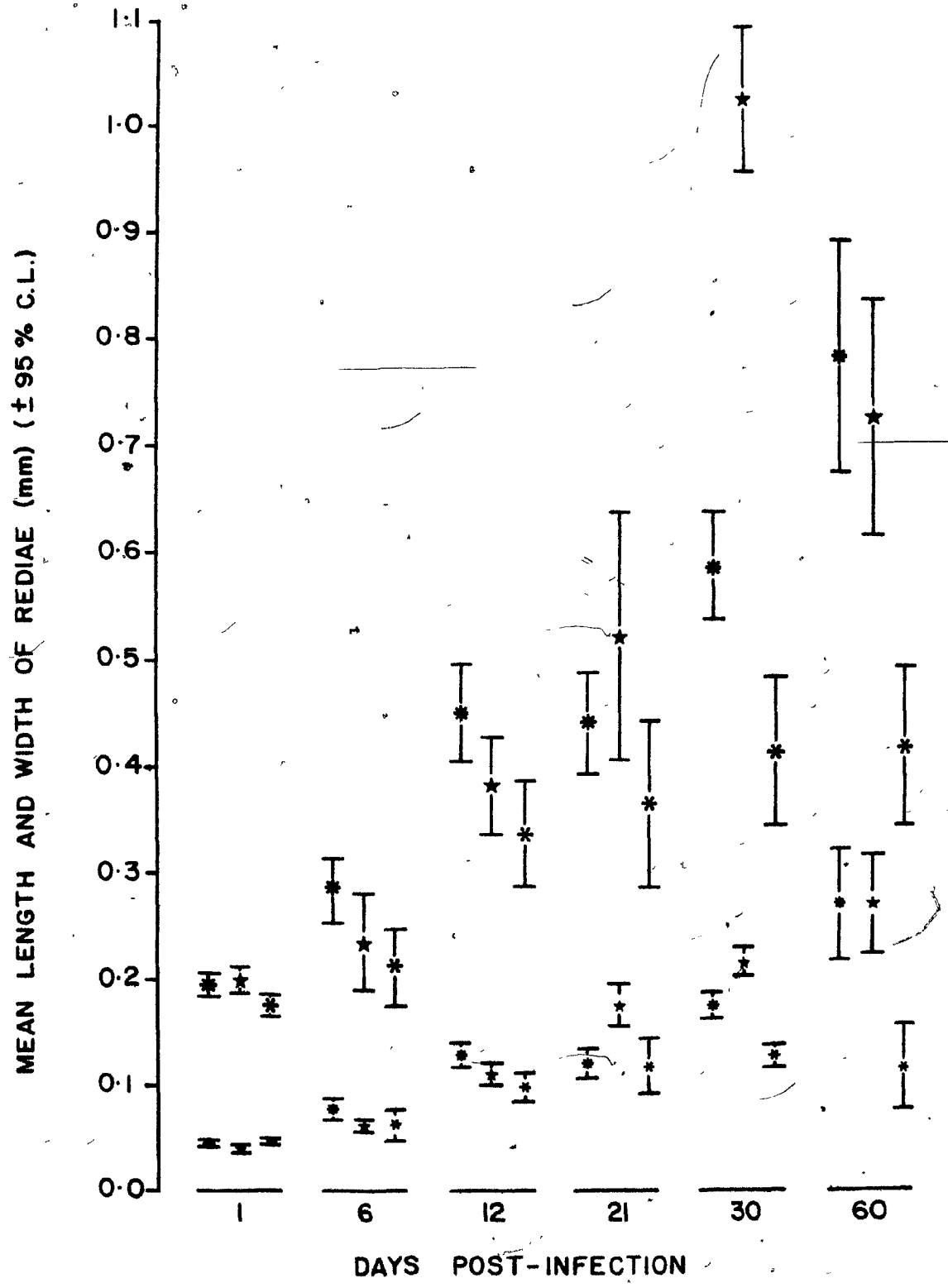

MEAN LENGTH AND WIDTH OF REDIAE (mm) (± 95 % C.L.)
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0·0
1
6
12
21
30
60
DAYS POST-INFECTION

Table 7-3. Measurements of preserved *Typhlocoelum cucumerinum sisowi* rediae recovered at various days post-infection from various species of snails.

DAY	SNAIL	MEAN LENGTH (mm) ± 95% C.L.	MEAN WIDTH (mm) ± 95% C.L.
6	*P. gyrina*	0.192 ± 0.025	0.064 ± 0.011
6	*L. elodes*	0.192 -	0.046 -
12	*P. gyrina*	0.323 ± 0.026	0.096 ± 0.008
12	*H. trivolvis*	0.300 ± 0.028	0.084 ± 0.006
12	*L. elodes*	0.289 ± 0.056	0.080 ± 0.017
21	*P. gyrina*	0.353 ± 0.041	0.097 ± 0.008
21	*H. trivolvis*	0.391 ± 0.077	0.135 ± 0.017
21	*L. elodes*	0.248 -	0.080 -
30	*P. gyrina*	0.426 ± 0.053	0.111 ± 0.011
30	*L. elodes*	0.327 ± 0.106	0.088 ± 0.031
60	*P. gyrina*	0.498 ± 0.062	0.149 ± 0.026
60	*H. trivolvis*	0.395 ± 0.154	0.115 ± 0.044
60	*L. elodes*	0.268 -	0.080 -

Table 7-4. Measurements of the larger cercariae contained within a representative *Typhlocoelum cucumerinum sisowi* redia, recovered from various species of snails at various days post-infection.

DAY	SNAIL	CERCARIA LENGTH (mm)	CERCARIA WIDTH (mm)
12	*P. gyrina*	0.175	0.066
		0.102	0.054
12	*H. trivolvis*	0.088	0.035
		0.070	0.032
12	*L. elodes*	0.105	0.049
		0.102	0.046
21	*P. gyrina*	0.175	0.084
21	*H. trivolvis*	0.280	0.084
		0.294	0.060
21	*L. elodes*	0.270	0.080
30	*P. gyrina*	0.296	0.119
30	*H. trivolvis*	0.522	0.108
60	*P. gyrina*	0.423	0.056
		0.310	0.070
		0.296	0.070
		0.212	0.070
60	*H. trivolvis*	0.212	0.113
60	*L. elodes*	0.226	0.056

Table 7-5. Parameters of linear regression analyses between the number of *Typhlocoelum cucumerinum sisowi* metacercariae and the number of *Typhlocoelum cucumerinum sisowi* rediae.

DAY	SNAIL	n	r	Y INT	SLOPE	SIGNIFICANCE
21	*P. gyrina*	9	-0.706	3.07	-0.42	* (-)
30	*P. gyrina*	9	0.314	4.93	1.11	
30	*L. elodes*	8	-0.769	2.40	-0.44	* (-)
60	*P. gyrina*	9	-0.158	33.86	-2.41	
60	*H. trivolvis*	7	0.099	30.06	-1.64	
60	*L. elodes*	4	0.977	5.22	1.44	* (+)

* P > r, 0.05

Table 7-6. Parameters of linear regression analyses between the number of *Typhlocoelum cucumerinum sisowi* metacercariae and the length of the snails.

DAY	SNAIL	n	r	Y INT	SLOPE	SIGNIFICANCE
21	*P. gyrina*	9	0.019	0.96	0.07	
30	*P. gyrina*	9	0.674	-2.93	4.57	* (+)
30	*L. elodes*	9	0.506	-1.06	1.26	
60	*P. gyrina*	10	0.729	-5.94	9.98	* (+)
60	*H. trivolvis*	9	0.887	-9.18	8.89	** (+)
60	*L. elodes*	6	0.316	1.03	2.57	

* P > r, 0.05

** P > r, 0.01

numbers of rediae were significantly correlated with low numbers of metacercariae, whereas, at 60 days post-infection, high numbers of rediae in *L. elodes* were correlated with high numbers of metacercariae. In *P. gyrina*, at both 30 and 60 days post-infection, and in *H. trivolvis* at 60 days post-infection, large snails had significantly more metacercariae than small snails.

Although significant correlations were obtained for both the number of rediae and the snail length, the results were confusing. Therefore, the total data set for each host species was subjected to a Stepwise Multiple Regression Anlaysis. Parameters included in the analysis were time post-infection, snail length, number of rediae, and mean redial length. The results are summarized in Table 7-7. In all three snail species, the most important parameter in determining the number of metacercariae was the time post-infection. In *P. gyrina* and *H. trivolvis*, the size of the snail was found to be important as well.

The mean diameter of metacercariae recovered from *P. gyrina* increased significantly between 30 and 60 days post-infection but did not change in *L. elodes* (Figure 7-6). At 60 days post-infection, metacercariae from *P. gyrina* and *H. trivolvis* were significantly larger than those from *L. elodes*. Metacercariae from *P. gyrina* and *L. elodes* were almost always seen moving within the cyst while being measured whereas similar movements were seen in less than 20% of the metacercariae from *H. trivolvis*.

e) HOST RESPONSE

At two months post-infection, a slight host response around *T. c. sisowi* metacercariae in *H. trivolvis* was observed in sectioned material. This was evidenced by amoebocytes in the pericardial cavity (Figure 7-7) and a thickened pericardium. These responses were not seen in *P. gyrina* or *L. elodes* at two months post-infection.

Additional *H. trivolvis* were sectioned at four months post-infection to monitor the host response. No rediae were seen in these snails, but a number of degenerated metacercariae were surrounded by amoebocytes which filled the pericardial cavity and also pervaded the heart muscle (Figure 7-8).

Table 7-7. Parameters of Stepwise Multiple Regression Analyses on number of *Typhlocoelum cucumerinum sisowi* metacercariae and days post-infection (DPI), snail length (SL), redial length (RL) and number of rediae (NR) in *Physa gyrina* (A), *Helisoma trivolvis* (B) and *Lymnaea elodes* (C).*

A.	B VALUE	F	P > F	r^2
INTERCEPT	-12.824			0.8080
DPI	0.473	161.90	0.0001	
SL	3.260	15.54	0.0002	

B.	B VALUE	F	P > F	r^2
INTERCEPT	-10.351			0.5870
DPI	0.395	41.26	0.0001	
SL	1.998	5.35	0.0263	

C.	B VALUE	F	P > F	r^2
INTERCEPT	-1.153			0.6003
DPI	0.111	52.57	0.0001	

* The parameters presented represent the analysis that incorporated the greatest number of variables with significant F values.

Figure 7-6. Graph showing mean diameter of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* metacercariae in three species of snails at different days post-infection.
A, *Typhlocoelum cucumerinum sisowi*;
B, *Typhlocoelum cucumerinum cucumerinum*;
P, *Physa gyrina*;
H, *Helisoma trivolvis*;
L, *Lymnaea elodes*.

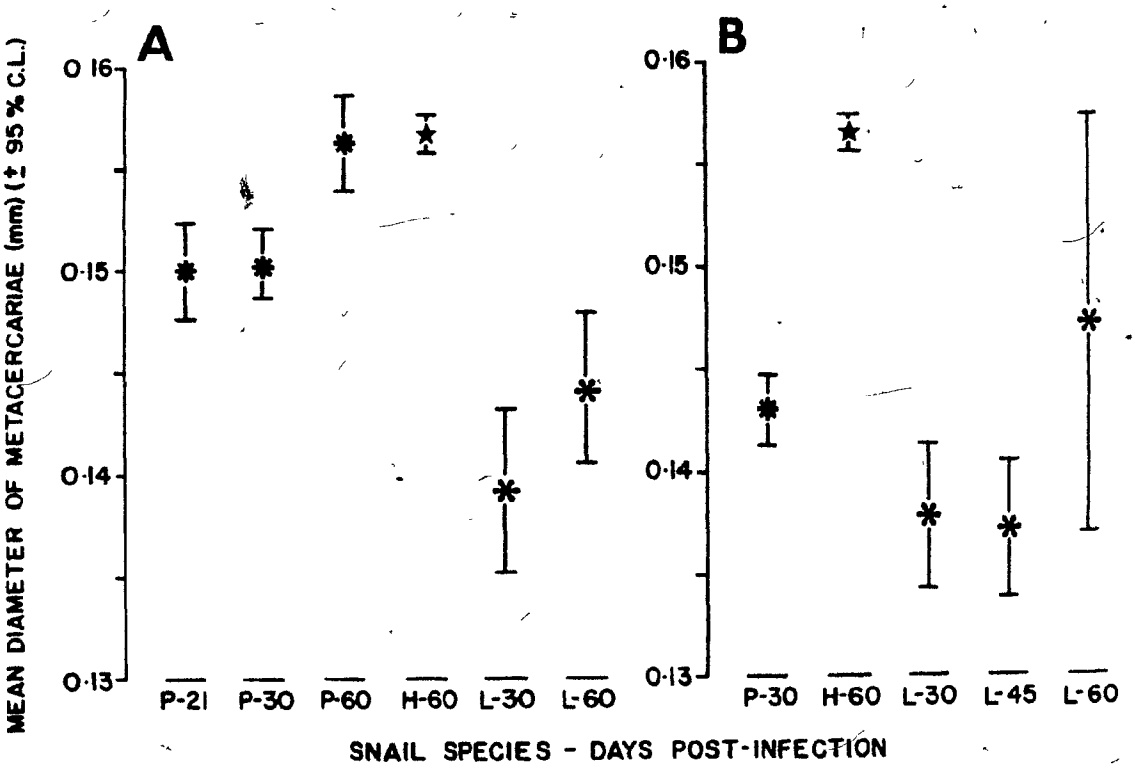

A
B
0·16
0·15
0·14
0·13
MEAN DIAMETER OF METACERCARIAE (mm) (± 95 % C.L.)
P-21
P-30
P-60
H-60
L-30
L-60
P-30
H-60
L-30
L-45
L-60
SNAIL SPECIES - DAYS POST-INFECTION

Figure 7-7. Photographs of host response of *Helisoma trivolvis* to *Typhlocoelum cucumerinum sisowi* at two months post-infection.

A, uninfected *Helisoma trivolvis*;

B, metacercaria in pericardial cavity surrounded by few amoebocytes;

a, amoebocytes;

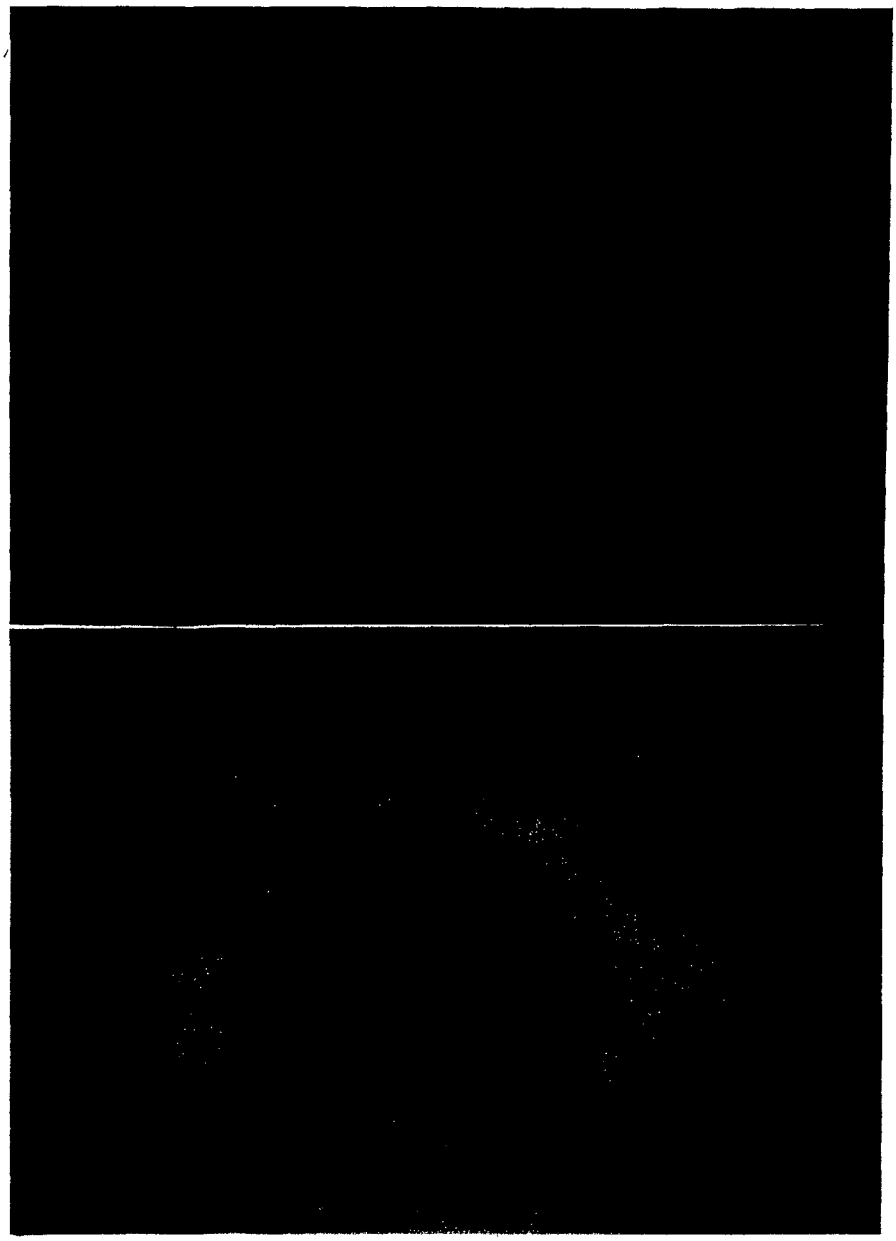
h, heart;

m, metacercaria;

p, pericardial cavity;

pe, pericardium.

Scale is in mm.



COLOURED PICTURE

Figure 7-8. Photographs of host response of *Helisoma trivolvis* to *Typhlocoelum cucumerinum sisowi* at four months post-infection.

A, metacercariae concentrated around heart in pericardial cavity;

B, concentration of amoebocytes around metacercariae;

C, amoebocytes in close association with cyst wall;

a, amoebocytes;

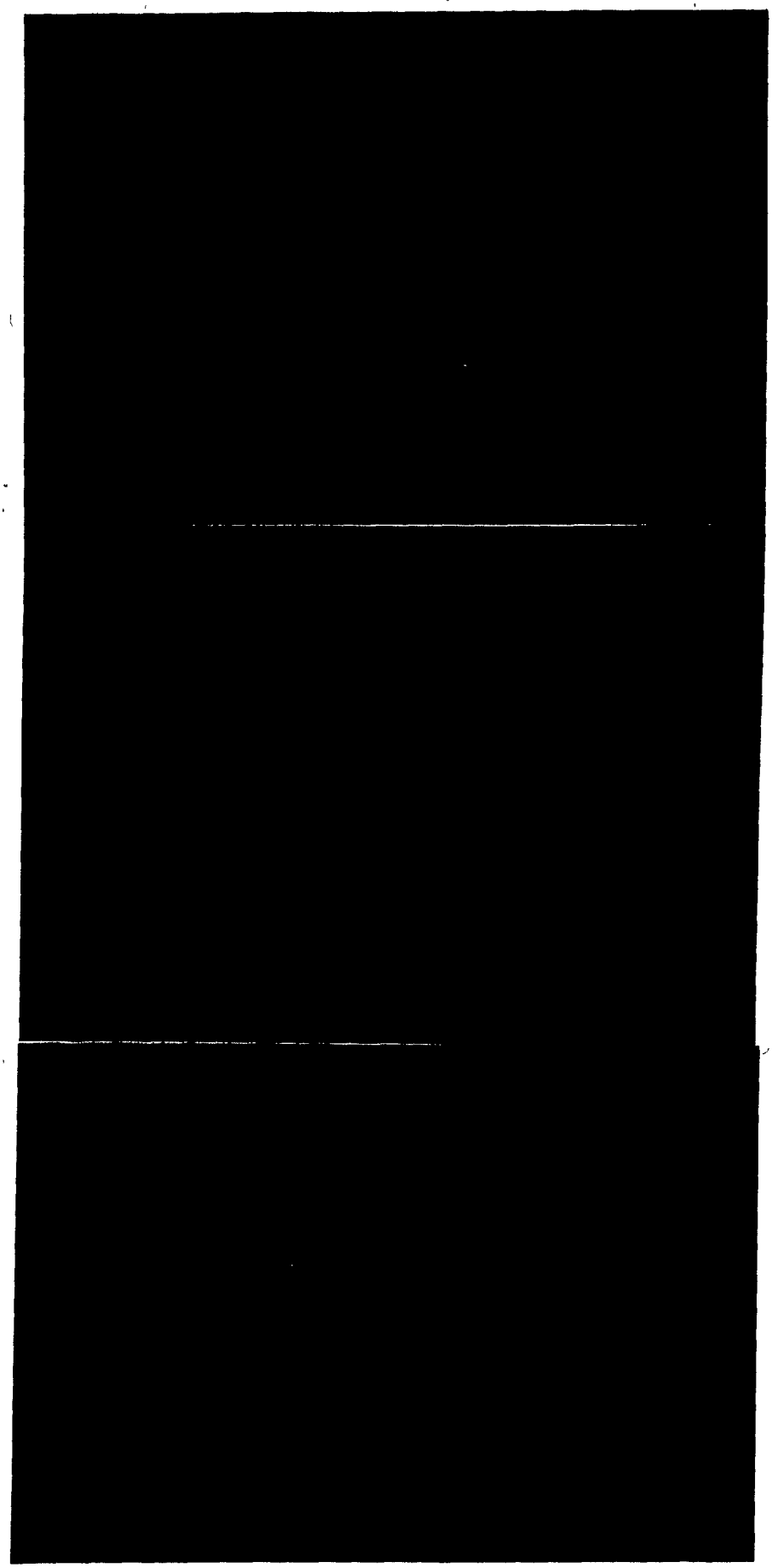
c, cyst wall;

h, heart;

m, metacercaria;

p, pericardial cavity.

Scale is in mm.



COLOURED PICTURE

f) INFECTIVITY OF METACERCARIAE

The mean percent recovery of *T. c. sisowi* in ducks fed whole snails and necropsied between 1 and 60 days post-infection are presented in Figure 7-1C. Metacercariae from *P. gyrina* were significantly more infective to mallards than those from *L. elodes* and *H. trivolvis*.

2. *Typhlocoelum cucumerinum cucumerinum* IN THREE SPECIES OF SNAILS

a) INFECTIVITY OF MIRACIDIA

Although the percent recovery of rediae in *P. gyrina*, *H. trivolvis* and *L. elodes* was variable at different days post-infection, it was not significantly correlated with the time post-infection. Based on the pooled data, *T. c. cucumerinum* miracidia have a significantly higher infectivity in *P. gyrina* than in *H. trivolvis* or *L. elodes* (Figure 7-9A). A significant negative correlation between the infectivity of *T. c. cucumerinum* miracidia and the size of the snail was detected in *L. elodes* at 45 days post-infection (Table 7-8).

b) LOCALIZATION, GROWTH AND DEVELOPMENT OF REDIAE

Rediae and metacercariae were located in the pericardial cavity in all snail species regardless of the time post-infection.

Measurements on *T. c. cucumerinum* rediae contained within the miracidia are presented in Table 7-2, and mean lengths and widths of live *T. c. cucumerinum* rediae at different days post-infection in the three species of snails are presented in Figure 7-10. Rediae from all three species of snails increased in size between 1 and 6 days post-infection. Rediae from *P. gyrina* and *H. trivolvis* continued to grow whereas rediae from *L. elodes* did not. At no time, however, did the rediae from the three species of snails differ significantly in body length or width. Measurements on preserved rediae from all three species of snails (Table 7-9) were significantly smaller than those of live rediae.

Recognizable cercariae were first observed within the rediae at 6 days post-infection and germinal masses were seen in the rediae as late as 60 days post-infection. A maximum of five well developed cercariae were contained within a single redia. Measurements of cercariae contained

Figure 7-9. Graphs of miracidial infectivity, production of metacercariae and infectivity of metacercariae in *Typhlocoelum cucumerinum cucumerinum* infections in three species of snails.

A, miracidial infectivity;

B, production of metacercariae;

C, infectivity of metacercariae to canvasbacks and redheads;

P, *Physa gyrina*;

H, *Helisoma trivolvis*;

L, *Lymnaea elodes*.

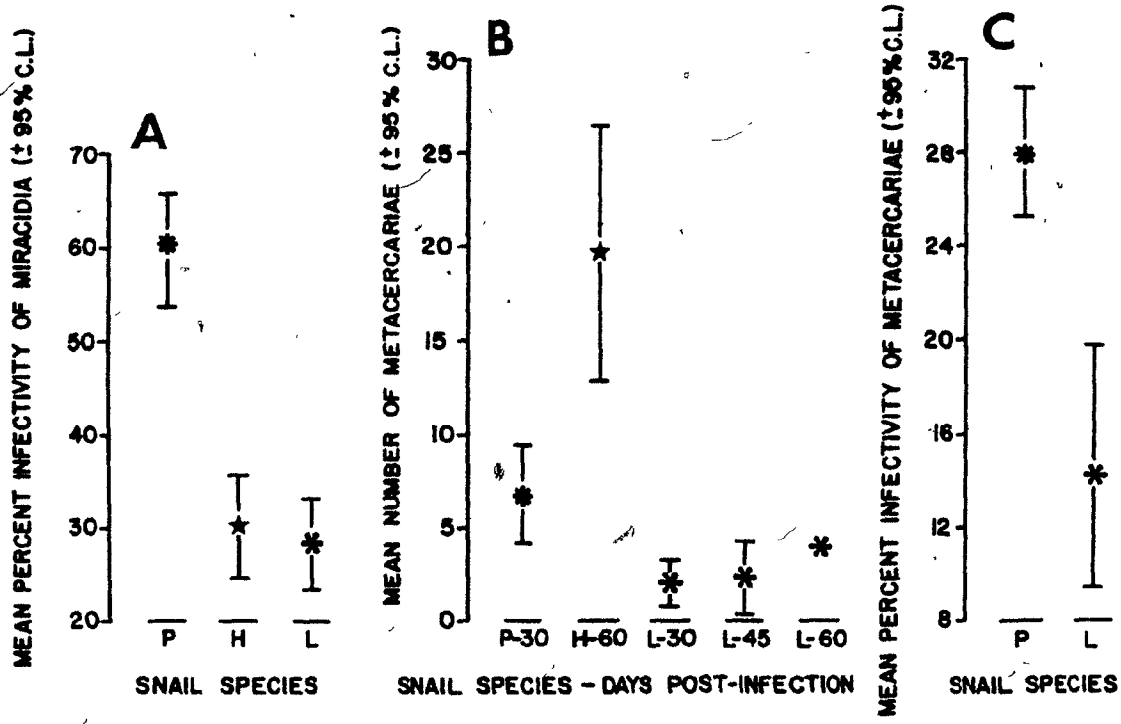

A
MEAN PERCENT INFECTIVITY OF MIRACIDIA (± 95% C.L.)
70
60
50
40
30
20
P
H
L
SNAIL SPECIES
B
MEAN NUMBER OF METACERCARIAE (± 95% C.L.)
30
25
20
15
10
5
0
P-30
H-60
L-30
L-45
L-60
SNAIL SPECIES - DAYS POST-INFECTION
C
MEAN PERCENT INFECTIVITY OF METACERCARIAE (± 95% C.L.)
32
28
24
20
16
12
8
P
L
SNAIL SPECIES

Table 7-8. Parameters of linear regression analyses between the number of *Typhlocoelum cucumerinum cucumerinum* rediae recovered and the length of the snails.

DAY	SNAIL	n	r	Y INT	SLOPE	SIGNIFICANCE
1	*P. gyrina*	10	0.158	1.79	1.07	
1	*H. trivolvis*	10	-0.069	2.25	-0.45	
1	*L. elodes*	9	0.535	-3.51	2.60	
6	*P. gyrina*	10	-0.434	6.23	-1.35	
6	*H. trivolvis*	9	-0.260	3.93	-1.76	
6	*L. elodes*	10	0.483	-4.06	2.58	
12	*P. gyrina*	10	-0.005	2.92	-0.01	
12	*H. trivolvis*	10	-0.206	4.16	-1.32	
12	*L. elodes*	10	0.262	0.75	0.62	
21	*P. gyrina*	10	0.458	-2.55	1.89	
21	*H. trivolvis*	10	-0.607	7.26	-2.63	
21	*L. elodes*	10	-0.427	5.64	-1.06	
30	*P. gyrina*	9	-0.269	4.36	-0.27	
30	*H. trivolvis*	6	-	0.00	0.00	
30	*L. elodes*	10	-0.080	1.20	-0.09	
45	*L. elodes*	10	-0.670	1.98	-0.25	* (-)
60	*H. trivolvis*	7	-0.210	2.88	-0.14	
60	*L. elodes*	4	-0.053	0.65	-0.02	

* $p > r$, 0.05

Figure 7-10. Graph showing mean length and width of live *Typhlocoelum cucumerinum cucumerinum* rediae at different days post-infection in three species of snails.

* ✱ *Physa gyrina;*
* ★ *Helisoma trivolvis;*
* ✱ *Lymnaea elodes.*

Large symbols represent length and small symbols represent width.

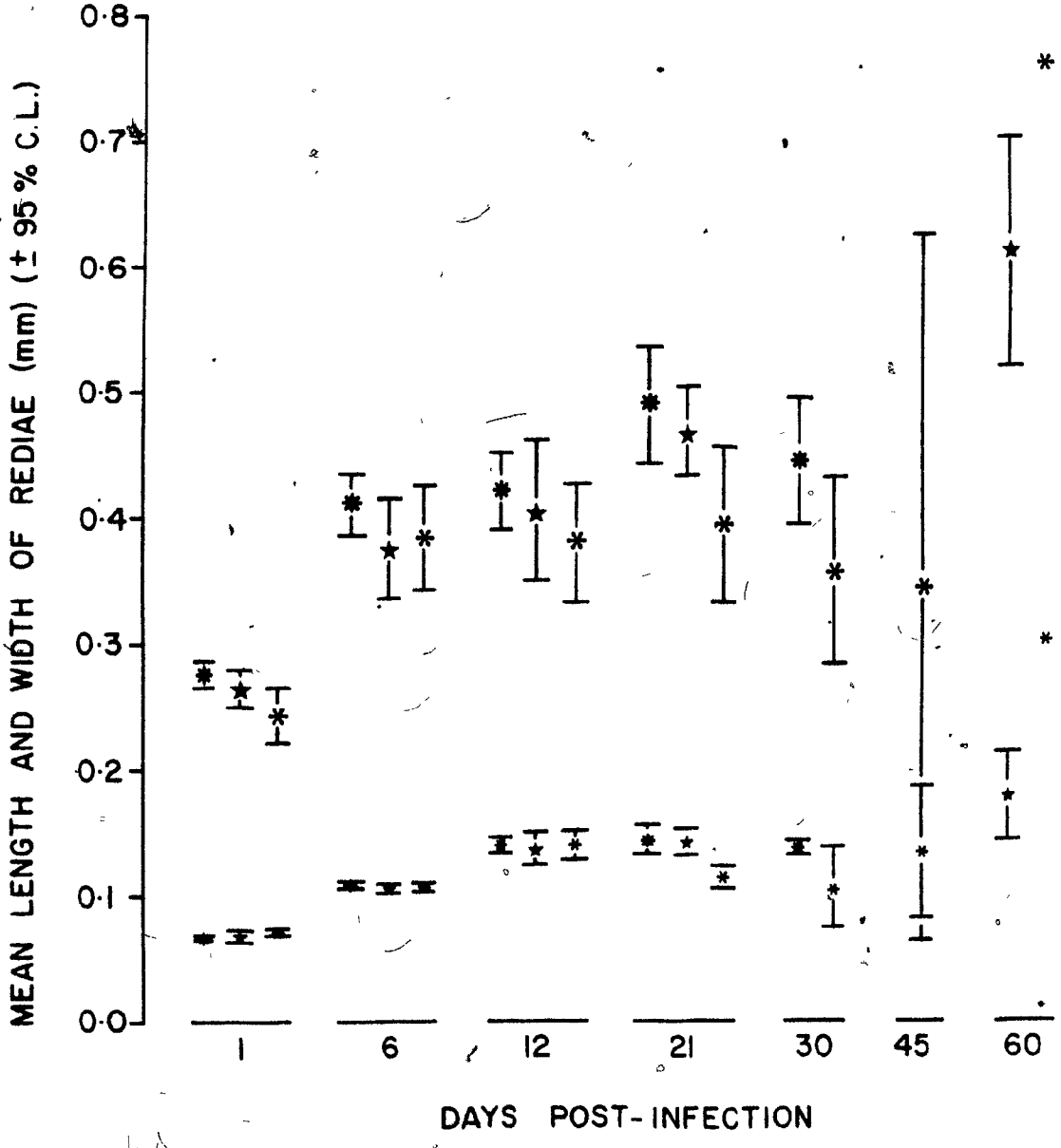

MEAN LENGTH AND WIDTH OF REDIAE (mm) (± 95 % C.L.)
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0·0
1
6
12
21
30
45
60
DAYS POST-INFECTION

Table 7-9. Measurements of preserved *Typhlocoelum cucumerinum cucumerinum* rediae recovered at various days post-infection from various species of snails.

DAY	SNAIL	MEAN LENGTH (mm) ± 95% C.L.	MEAN WIDTH (mm) ± 95% C.L.
1	*L. elodes*	0.145 ± 0.015	0.056 ± 0.013
6	*P. gyrina*	0.233 ± 0.015	0.081 ± 0.005
6	*H. trivolvis*	0.195 ± 0.050	0.065 ± 0.010
6	*L. elodes*	0.211 ± 0.030	0.074 ± 0.008
12	*P. gyrina*	0.226 ± 0.080	0.104 ± 0.016
12	*H. trivolvis*	0.221 ± 0.024	0.106 ± 0.013
12	*L. elodes*	0.213 ± 0.075	0.110 ± 0.032
21	*P. gyrina*	0.343 ± 0.058	0.102 ± 0.008
30	*P. gyrina*	0.355 ± 0.061	0.127 ± 0.023
30	*H. trivolvis*	0.613 ± 0.042	0.122 ± 0.010
30	*L. elodes*	0.352 -	0.080 -
60	*H. trivolvis*	0.443 ± 0.077	0.152 ± 0.026
60	*L. elodes*	0.552 -	0.177 -

within a representative redia from each snail species at each day post-infection are presented in Table 7-10. No free cercariae were found in the snails.

c) PRODUCTION AND DESCRIPTION OF METACERCARIAE

Metacercariae were first found in *P. gyrina* and *L. elodes* at 30 days post-infection, and were first found in *H. trivolvis* at 60 days post-infection (Figure 7-9B). When the number of metacercariae in each snail species at each day post-infection were correlated with the number of rediae, the correlation coefficient was not significant. Similarly, no significant correlations were detected between the number of metacercariae and the length of the snails (Appendix 7-1). However, when Stepwise Multiple Regression Analyses were done on the pooled data for each snail species, the number of metacercariae was shown to be significantly correlated with the time post-infection in *P. gyrina* and *H. trivolvis*, with the snail length in all three species of snails, and with the redial length in *P. gyrina* (Table 7-11).

The mean maximum diameter of *T. c. cucumerinum* metacercariae from the three species of snails at different days post-infection are presented in Figure 7-6. Significant differences were not detected in the size of metacercariae from *L. elodes* between 30 and 60 days post-infection. Metacercariae from *H. trivolvis* and *P. gyrina* were available at only one time post-infection, and therefore their change in diameter with time could not be assessed.

d) HOST RESPONSE

Sufficient *L. elodes* and *P. gyrina* were not available for sectioning at 60 days post-infection; consequently, only *H. trivolvis* were examined for the presence of a host response. At 60 days post-infection, a few amoebocytes were observed in the pericardial cavity of infected *H. trivolvis*.

e) INFECTIVITY OF METACERCARIAE

A comparison of data on infectivity of *T. c. cucumerinum*

Table 7-10. Measurements of the larger cercariae contained within a representative *Typhlocoelum cucumerinum cucumerinum* redia, recovered from various species of snails at various days post-infection.

DAY	SNAIL	CERCARIA LENGTH (mm)	CERCARIA WIDTH (mm)
6	*P. gyrina*	0.133	-
6	*H. trivolvis*	0.108	-
		0.108	-
6	*L. elodes*	0.091	-
12	*P. gyrina*	0.168	-
		0.116	-
12	*H. trivolvis*	0.262	-
		0.234	-
12	*L. elodes*	0.112	-
		0.102	-
21	*P. gyrina*	0.210	0.080
		0.192	0.084
		0.119	-
		0.112	-
21	*H. trivolvis*	0.200	0.088
		0.175	0.060
21	*L. elodes*	0.259	0.066
		0.144	-
30	*P. gyrina*	0.332	0.150
		0.266	0.130
		0.228	0.098
		0.214	0.119
		0.210	0.105
30	*L. elodes*	0.200	0.070
45	*L. elodes*	0.338	0.108

Table 7-11. Parameters of Stepwise Multiple Regression Analyses on number of *Typhlocoelum cucumerinum cucumerinum* metacercariae and days post-infection (DPI), snail length (SL), redial length (RL) and number of rediae (NR) in *Physa gyrina* (A), *Helisoma trivolvis* (B) and *Lymnaea elodes* (C).*

A.	B VALUE	F	P > F	r^2
INTERCEPT	-1.454			0.6388
DPI	0.204	27.77	0.0001	
SL	2.228	16.08	0.0002	
RL	-0.012	8.81	0.0049	

B.	B VALUE	F	P > F	r^2
INTERCEPT	-9.313			0.8858
DPI	0.140	7.83	0.0090	
SL	4.756	24.90	0.0001	

C.	B VALUE	F	P > F	r^2
INTERCEPT	-1.242			0.6167
SL	0.615	46.67	0.0001	

* The parameters presented represent the analysis that incorporated the greatest number of variables with significant F values.

metacercariae obtained between 1 and 60 days post-infection of the ducks, revealed that metacercariae from *P. gyrina* have a significantly higher infectivity to redhead and canvasback ducklings than metacercariae from *L. elodes* (Figure 7-9C). A shortage of infected *H. trivolvis* made it impossible to test the infectivity of *T. c. cucumerinum* metacercariae from *H. trivolvis* in ducks.

D. DISCUSSION

The combination of three features make the larval development of cyclocoelids different from the typical digenean life cycles in snails. The typical digenean miracidium penetrates the tissues of the snail and the sporocyst or redia develops subsequently. In cyclocoelids, a precocial redia is fully formed within the miracidium and it is injected directly into the snail tissues. Precocial rediae do occur in a few other digeneans, e.g. *Parorchis acanthus* and *Philophthalmus gralli* (Mathis and Leger, 1910), and are also injected into the snail (Rees, 1940; Alicata, 1962).

The second difference in cyclocoelid larval development is the reduction in intramolluscan stages. There are no sporocyst generations and a single exposure produces only one redia that produces cercariae asexually. Cyclocoelids produce comparatively few cercariae; this is, to some extent, a function of the reduction in asexual generations.

A final difference in the larval development of cyclocoelids is that the cercariae encyst within the first intermediate host. The typical digenean cercariae leave the snail, and have a free-swimming dispersal phase.

The above-mentioned aspects of cyclocoelid life cycles apply to both *T. c. sisowi* and *T. c. cucumerinum*. The larval development of both subspecies has been investigated and will now be compared.

1. THE MIRACIDIAL PHASE OF THE INFECTION

Typhlocoelum cucumerinum cucumerinum miracidia are larger than *T. c. sisowi* miracidia (0.277 and 0.220 mm long, respectively); this is not surprising, as *T. c. cucumerinum* eggs are also significantly longer than *T. c. sisowi* eggs (0.165 and 0.137 mm long, respectively). Stunkard

(1934b) reported that *T. c. sisowi* miracidia are 0.015 mm long. Although *T. c. sisowi* miracidia were much larger in this study (approximately 0.22 mm long), they were similar in all other respects to Stunkard's material. Measurements made by Schafranksi *et al.* (1975) on *T. c. cucumerinum* miracidia (0.238 x 0.108 mm) agree with the measurements of *T. c. cucumerinum* miracidia in this study (0.277 x 0.075 mm).

The attraction of miracidia to snails has been subject to considerable investigation recently, and a number of factors have been shown to influence miracidial behaviour. These have been reviewed by Ulmer (1971), Erasmus (1972) and Etges *et al.* (1975). It is generally agreed that miracidia move at random until they detect a snail. In some species, the miracidia apparently respond to metabolic products and secretions by an increased rate of turning (Etges *et al.*, 1975) that presumably keeps them in the vicinity of the snail. Inorganic ions have been shown to influence miracidial activity (Mason, 1977) as have amino acids (Ulmer, 1971) and neurotransmitters (Roberts *et al.*, 1978). Researchers have proposed that the miracidia of some digeneans distinguish between the chemicals released from different snail species and "choose" the appropriate intermediate host (Nansen *et al.*, 1976).

In this study, significantly more *T. c. sisowi* rediae were recovered from *P. gyrina* and *H. trivolvis* than from *L. elodes* whereas more *T. c. cucumerinum* rediae were obtained from *P. gyrina* than from *L. elodes* or *H. trivolvis*. While it is possible that the differential recovery of rediae from the three species of snails is related to a differential attractiveness of the miracidia to the three snail species, this is not a likely factor in these experiments as the miracidia and snails were in close proximity during exposure (approximately 2 ml of water). Alternatively, the ability of the miracidia to attach to the snail and inject the rediae may differ among the three snail species, or once inside the snail tissues, the rediae may have differential success in establishing an infection in the different snail species.

Loker (1978) has shown that very young *Lymnaea catascopium* Say are less susceptible to *Schistosomatium douthitti* (Cort, 1915) than 12 to 55 day-old snails, and suggested that the miracidia may not be attracted to

the neonatal snails. He also demonstrated a decreased success of miracidia in older snails and related this to an entanglement of the miracidia in the mucous secretions. In this study, a significant negative relationship between the size of the snail and the number of rediae was detected only in *L. elodes* infected with *T. c. cucumerinum* and examined at 45 days post-infection. It is therefore suggested that, within the size range of snails used in this study, the snail size is not an important factor influencing the infectivity of *T. c. sisowi* or *T. c. cucumerinum* miracidia.

The mean recovery of rediae in the snails did not decrease as the infections progressed. If rediae were dying, or if the heavily infected snails were dying, the overall mean recovery of rediae would be expected to gradually decrease. As this did not happen, it can be assumed that an exposure to five miracidia does not result in rediae or snail mortality at least during the first two months of the infection.

2. PENETRATION, MIGRATION AND LOCALIZATION OF REDIAE

Coil (1977) suggested that miracidia of *Fasciola hepatica* Linnaeus, 1758 penetrate *Lymnaea truncatula* (Muller) in 15 minutes. Although the time required for *T. c. sisowi* miracidia to inject the rediae was not determined, it is less than two hours, because, in this time, rediae have penetrated the snail and reached the pericardial cavity.

Specimens were found in the connective tissues of the head-foot and in the sinuses at four hours post-infection. Robinson (1977) followed the migration of *Cyclocoelum mutabile* in *Promenetus exacuous* (Say) and found that the rediae travel through the epithelium and connective tissues to the sinuses where they remain for at least four months. In *T. c. sisowi* and presumably in *T. c. cucumerinum*, the rediae probably travel through the connective tissues into the sinuses and then to the pericardial cavity.

The rediae and the metacercariae of both *T. c. sisowi* and *T. c. cucumerinum* develop in the pericardial cavity of *P. gyrina H. trivolvis* and *L. elodes*. In dissections, the rediae often fall out as soon as the snail is opened, and this may explain why Stunkard (1934b) associated the rediae with the lymph spaces, and why Szidat (1932) and Sreekumaran and Peter (1973a,b) associated them with the nearby albuminous gland.

Schafranski *et al.* (1975) reported that *T. c. cucumerinum* rediae and metacercariae are found in the pericardial cavity. This was substantiated in the present study. As the pericardial cavity becomes filled with rediae and metacercariae, it resembles a "cyst-like swelling" (see Figure 7-4) as described by Sreekumaran and Peter (1973b).

One small *P. gyrina* exposed to 25 *T. c. sisowi* miracidia for sectioning shortly after exposure had rediae in the digestive gland and ovotestis at six hours post-infection. Perhaps, in heavy infections of small snails, the rediae "overload" the system and travel throughout the body of the snail. No rediae or metacercariae were ever found outside the pericardial cavity after one day post-infection; therefore, any rediae which settle in sites other than the pericardial cavity are presumably unsuccessful, either because the host sequesters and subsequently kills these rediae, or because these rediae cause the death of the snail.

3. THE REDIAL PHASE OF THE INFECTION

At 1 and 6 days post-infection, *T. c. cucumerinum* rediae were larger than *T. c. sisowi* rediae, but no significant differences in size between the two subspecies were detected at 12 or 21 days post-infection. Growth of both *T. c. cucumerinum* and *T. c. sisowi* rediae was evident in *P. gyrina* and *H. trivolvis* after 6 days post-infection, but not in *L. elodes*. At 30 days post-infection, *T. c. sisowi* rediae were significantly longer than *T. c. cucumerinum* rediae in both *P. gyrina* and *H. trivolvis*.

The length of the rediae was found to be highly variable. For example, at 21 days post-infection, one *T. c. sisowi* redia from *L. elodes* measured 0.423 x 0.212 mm when contracted and 1.198 x 0.155 mm when extended. In an attempt to reduce this source of variability, all rediae were left for six hours in 0.75% saline before they were measured, thereby decreasing their activity. For the most part, measurements made on preserved rediae were significantly smaller than those made on live rediae.

The size of the rediae is dependent, to a large extent, on the size of the developing cercariae. At 30 days post-infection, for instance, *T. c. sisowi* rediae from *H. trivolvis* contained the largest cercariae (Table 7-4, Table 7-10), and were significantly longer than any other

rediae from any other host species at any time post-infection (Figure 7-5, Figure 7-10).

Two previous researchers have measured *T. cucumerinum* rediae. Stunkard (1934b) reported that preserved rediae of *T. c. sisowi* were 0.65 - 0.70 x 0.18 - 0.19 mm at 2 weeks post-infection and 0.86 x 0.027 mm at 8 weeks post-infection. These measurements are considerably larger than those made on preserved *T. c. sisowi* in this study (Table 7-3). Schafranski *et al.* (1975) reported that live *T. c. cucumerinum* rediae measured 0.344 ± 0.041 x 0.121 ± 0.019 mm at 5 days post-infection, 0.444 ± 0.063 x 0.156 ± 0.13 mm at 8 days post-infection, 0.669 ± 0.047 x 0.221 ± 0.019 mm at 11 days post-infection and 0.934 ± 0.055 x 0.269 ± 0.044 mm at 14 days post-infection. These rediae attained a much larger size than *T. c. cucumerinum* rediae in this study (Figure 7-10). The differences demonstrated between this study and other studies are not surprising in view of the variability in size of the rediae demonstrated within and among different species of snails.

Free cercariae were never observed in the pericardial cavity. However, a single cercaria was observed on a slide as it emerged through the birth pore of a redia, and it had encysted within one minute. This would explain the absence of free cercariae in the snails examined in this study.

4. PRODUCTION OF METACERCARIAE

In these experiments, *T. c. sisowi* metacercariae were first obtained at 21 days post-infection in *P. gyrina* and *L. elodes* whereas *T. c. cucumerinum* metacercariae were first obtained at 30 days post-infection in *P. gyrina* and *L. elodes*. Although neither *T. c. sisowi* nor *T. c. cucumerinum* metacercariae were obtained in *H. trivolvis* dissected at 30 days post-infection, *T. c. cucumerinum* metacercariae were seen in the *H. trivolvis* sectioned at 30 days post-infection, and the *T. c. sisowi* rediae contained large cercariae that were undoubtedly released shortly after day 30. By 60 days post-infection both *T. c. sisowi* and *T. c. cucumerinum* had produced large numbers of metacercariae in *H. trivolvis*.

Although *T. c. sisowi* infections produced more metacercariae than *T. c. cucumerinum* infections, the differences were not statistically

significant. In both *T. c. sisowi* and *T. c. cucumerinum* infections, the rate of metacercarial production was significantly higher in *P. gyrina* and *H. trivolvis* than in *L. elodes*.

The results from multiple regression analyses suggest that metacercarial production occurs at a higher rate in large snails than in small snails. This suggests that some sort of regulatory mechanism may control the development of the cercariae within the rediae. The functional significance of this type of regulation is obvious as a high rate of cercarial production in small snails, and the resultant accumulation of metacercariae, could potentially kill the snail.

Observations made during this research indicate that two other factors influence the rate of metacercarial production: temperature and season. The snails used for experimental infections in ducks were maintained at ambient laboratory temperatures that varied between approximately 24 and 28°C. Under these conditions, production of metacercariae occurred earlier (as soon as 12 days post-infection) and at a faster rate than metacercarial production in the snails maintained at 20°C for the snail experiments. Temperature has frequently been reported to influence the rate of digenean development in the snail (Nice and Wilson, 1974; Anderson *et al.*, 1977), and it had an effect on both *T. c. sisowi* and *T. c. cucumerinum* in this study as well. Szidat (1932, 1933) reported that *T. c. sisowi* metacercariae were present in *Planorbis* spp. as early as 8 days post-infection. This is comparable with results obtained from snails maintained at higher temperatures in this study.

During this research, an attempt was also made to obtain metacercariae from snails experimentally infected in October through December. Although the snails were maintained at relatively high temperatures parasite development was very slow. This suggests that an endogenous rhythm within the snails may exist, and that this may influence the parasite's growth and development.

5. METACERCARIAE

The mean diameter of *T. c. sisowi* metacercariae increased significantly between 30 and 60 days post-infection (0.150 and 0.156 mm,

respectively). This may be related to an increased thickness of the cyst wall (0.007 mm thick at 21 days post-infection and 0.014 mm thick at 60 days post-infection). Stunkard (1934b) noted that *T. c. sisowi* metacercarial cysts are "all about the same size and ... that the metacercariae do not grow or develop in the cyst". This observation is supported in the present study, although, at least in *P. gyrina*, the diameter of the cyst does increase.

Comparison of the mean diameter of *T. c. sisowi* and *T. c. cucumerinum* metacercariae revealed that, in *P. gyrina* at 30 days post-infection, *T. c. sisowi* metacercariae were significantly larger than *T. c. cucumerinum* metacercariae (0.150 and 0.143 mm, respectively). No other significant differences were detected between the metacercariae of the two subspecies. Stunkard (1934b) found that *T. c. sisowi* metacercariae from *H. trivolvis* at 8 weeks post-infection had a diameter of approximately 0.175 mm. These metacercariae were considerably larger than those obtained from *H. trivolvis* at 60 days post-infection in the present study (0.157 ± 0.001 mm). According to Schafranski *et al.* (1975), *T. c. cucumerinum* metacercariae from *Biomphalaria* spp. measure 0.151 ± 0.006 x 0.141 ± 0.010 mm. This corresponds closely with the diameter of *T. c. cucumerinum* metacercariae from *H. trivolvis* at 60 days post-infection (0.156 mm).

Both Stunkard (1934b) and Schafranski *et al.* (1975) described two layers, a fragile outer layer and a fibrous inner layer, in the metacercarial cysts. In this study, the fragile outer layer was associated with host cells and was easily removed under coverslip pressure. A thick translucent layer, resistant to mechanical damage, was found, however, between the fragile outer layer and the inner fibrous layer (Figure 7-11).

6. HOST RESPONSE

Helisoma trivolvis were found to mount a severe host response to *T. c. sisowi* metacercariae at four months post-infection as evidenced by dense accumulations of amoebocytes in the pericardial cavity and heart muscle, and the presence of apparently degenerated metacercariae. This response was not as severe at two months post-infection, although a few amoebocytes were found in the pericardial cavity, and the metacercariae were often covered

Figure 7-11. Photographs of metacercariae of *Typhlocoelum cucumerinum sisowi*.

A, metacercaria with fragile outer layer still attached, showing association of host cells;

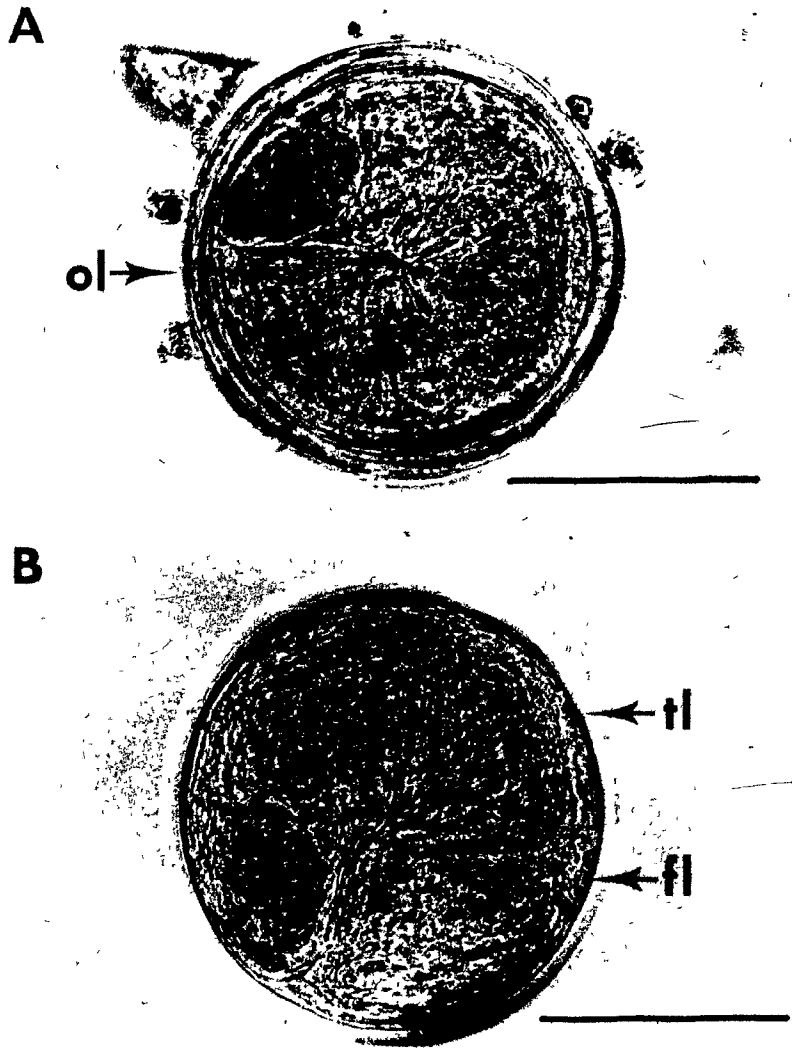
B, metacercaria with fragile outer layer removed, showing thick translucent layer and inner fibrous layer.

fl, fibrous layer;

ol, outer layer;

tl, translucent layer.

Bar represents 0.1 mm.


A
ol
B
tl
fl

by a layer of host cells. A similar response was seen in *T. c. cucumerinum*-infected *H. trivolvis* at 60 days post-infection, but no response was found in either *P. gyrina* or *L. elodes*.

Research on the cellular response of snails to larval Digenea has been reviewed by Malek and Cheng (1974) and by Michelson (1975). When parasites elicit a host response, they are characteristically surrounded by amoebocytes in a foreign body response. They become encapsulated and may eventually be destroyed.

It is difficult to determine, in the case of *T. c. sisowi* in *H. trivolvis*, whether the host actually induced the degeneration of the metacercariae, or whether the metacercarial degeneration resulted in the subsequent invasion of amoebocytes to the site of the infection. A few amoebocytes do surround the cyst wall of live metacercariae, and this may suggest that the host response begins prior to parasite degeneration. Presumably, the degenerated metacercariae obtained in *H. trivolvis* at four months post-infection were incapable of producing an infection in ducks.

7. INFECTIVITY OF METACERCARIAE

Throughout the course of this research, a large number of ducks have been exposed to metacercariae. The data on the recovery of flukes from all of the ducks necropsied between 1 and 60 days post-infection were used to assess the infectivity of the metacercariae.

A comparison of metacercarial infectivity from different snail hosts reveals that, for both *T. c. sisowi* and *T. c. cucumerinum*, metacercariae from *P. gyrina* have the highest infectivity. *Typhlocoelum cucumerinum sisowi* metacercariae from *H. trivolvis* have a low infectivity and no infections were established using *T. c. sisowi* metacercariae from *L. elodes*. However, *T. c. cucumerinum* metacercariae from *L. elodes* were a good source for infective material. The shortage of *T. c. cucumerinum* metacercariae from *H. trivolvis* prevented an assessment of their infectivity to ducks.

A comparison of the infectivity of *T. c. sisowi* and *T. c. cucumerinum* from *P. gyrina* and *L. elodes* reveals that the recovery of *T. c. cucumerinum* in canvasbacks and redheads is higher than the recovery

of *T. c. sisowi* in mallards. It has been shown in Chapter V that the death rate of *T. c. sisowi* is greater than that of *T. c. cucumerinum* and this has undoubtedly contributed to the apparently lower infectivity of *T. c. sisowi* metacercariae.

8. THEORETICAL COMPARISON OF *Typhlocoelum cucumerinum sisowi* AND *Typhlocoelum cucumerinum cucumerinum* IN THREE SPECIES OF SNAILS

Transmission of *T. c. sisowi* and *T. c. cucumerinum* through the snail is dependent on the infectivity of the miracidia, the rate of production of metacercariae and the infectivity of the metacercariae to ducks. On the basis of the data presented in this chapter, it is possible to theoretically predict the outcome of 300 *T. c. sisowi* eggs if 100 eggs were used to infect each of *P. gyrina*, *H. trivolvis* and *L. elodes*. In *P. gyrina*, approximately 53 rediae would develop and these would produce approximately 760 metacercariae by 60 days post-infection. If all of these metacercariae were then fed to mallards, approximately 70 flukes would be recovered at 60 days post-infection. In *H. trivolvis*, 59 rediae would produce 426 metacercariae which would result in approximately 12 flukes in the mallards. Approximately 30 rediae would develop in *L. elodes*, and these would yield 152 metacercariae by 60 days post-infection. However, none of these metacercariae would develop in mallards. From this, it can be suggested that *P. gyrina* may be the most important intermediate host for transmission of *T. c. sisowi*.

Unfortunately, a value for production of *T. c. cucumerinum* metacercariae in *P. gyrina* at 60 days post-infection is not available, and the infectivity of *T. c. cucumerinum* from *H. trivolvis* is unknown. However, based on the high infectivity of miracidia in *P. gyrina*, the early production of metacercariae in *P. gyrina* and the high infectivity of metacercariae from *P. gyrina*, it could be suggested that, at least under experimental conditions, *P. gyrina* is a better host for *T. c. cucumerinum* than *H. trivolvis* or *L. elodes*. It seems reasonable to suppose that *P. gyrina* might also be an important host in the field situation.

In this chapter, additional differences between the two subspecies of *T. cucumerinum* in the snail phase of the life cycle have been demonstrated,

particularly with respect to infectivity of the miracidia, growth of the rediae, and production and infectivity of the metacercariae.

CHAPTER VIII. DISTRIBUTION OF *Typhlocoelum cucumerinum sisowi* AND *Typhlocoelum cucumerinum cucumerinum* IN WILD DUCKS OF SOUTHERN QUEBEC AND SOUTHERN MANITOBA

A. INTRODUCTION

At the outset of this research, a survey of wild ducks was undertaken and two morphological forms of *Typhlocoelum cucumerinum* were obtained from the trachea of ducks. The presence of these two forms initiated a series of investigations that have clarified their taxonomic and biological relationships. It is now possible to examine the ecological relationships between the two subspecies as indicated in the field survey data.

Typhlocoelum cucumerinum has a cosmopolitan distribution (McDonald, 1969). In North America, records have come from Florida (Kinsella and Forrester, 1972), Texas (Broderson *et al.*, 1977), Iowa (Taft, 1971), Michigan (Gower, 1938a,b; Town, 1960), Manitoba (Cornwell and Cowan, 1963; Buscher, 1966) and eastern Canada (Mahoney and Threlfall, 1978).

This chapter presents the results of surveys of wild ducks in southern Québec and southern Manitoba undertaken to determine the distribution of *T. c. sisowi* and *T. c. cucumerinum* in naturally occurring duck populations.

B. MATERIALS AND METHODS

The 536 ducks representing 20 species examined from southern Québec were shot by duck hunters on Lac Saint-Louis (75°25'N, 73°45'W) during September and October, 1976, 1977 and 1978, and on Lac Saint-François (45°00'N, 74°38'W) and at Thurso (45°35'N, 75°13'W) during September, 1978.

Material from southern Manitoba was collected during 1977, 1978 and 1979. The majority of the 3442 ducks examined were shot by hunters on the Delta Marsh during the first two weeks of October (1977 and 1978). When direct contact with the hunters was established, ducks were identified to species, aged (juvenile or adult) and sexed. When duck cleaners were contacted, only the neck and head of ducks were available; therefore, age and (or) sex data were frequently unobtainable.

Ducks examined during the summers of 1977, 1978 and 1979 were shot as part of this and four other research programs sponsored by the Delta Waterfowl Research Station: Mr. Rod Sayler provided redhead tracheas and Mr. Paul DuBowy provided blue-winged teal and shoveler tracheas from the Delta Marsh; Mr. Michael Tome provided four ruddy duck tracheas from the Minnedosa area (50°15'N, 99°50'W); and Mr. Bill Hohman provided 14 ring-necked duck tracheas from the Roseau Marshes (49°N, 96°W). Additional tracheas from six canvasbacks shot in the Minnedosa region were provided by Dr. Jerome Serie (Northern Prairie Wildlife Research Center, Jamestown, North Dakota) and tracheas from ten white-winged scoter shot at Gordon Lake, Saskatchewan (55°50'N, 106°26'W) were provided by Mr. Patrick Brown (University of Missouri).

The prevalence (% infected), abundance (total number of worms recovered divided by the total number of ducks examined), and intensity (total number of worms recovered divided by the total number of infected ducks) of both *T. c. sisowi* and *T. c. cucumerinum* were calculated for each duck species from both the southern Québec and the southern Manitoba data. These three terms will be referred to collectively, as the levels of infection.

C. RESULTS

1. THE SOUTHERN QUEBEC DATA

The results of the wild duck survey in Québec are presented in Table 8-1. Five of eight species of dabbling ducks (black duck, blue-winged teal, green-winged teal, mallard and pintail) and two of five species of diving ducks (canvasback and greater scaup) were infected with *T. cucumerinum*. Seven other species of ducks were examined (Table 8-1), but none were infected. In all uninfected species of ducks the sample was small (less than 25 ducks).

Two subspecies of *T. cucumerinum* were identified: *Typhlocoelum cucumerinum sisowi* occurred exclusively in dabbling ducks and *Typhlocoelum cucumerinum cucumerinum* occurred exclusively in diving ducks. This separation was highly significant (Fisher's exact probability test, $P = 0.0014$).

Table 8-1. Prevalence and intensity of *Typhlocoelum cucumerinum sisowi*, *Typhlocoelum cucumerinum cucumerinum* and total *Typhlocoelum cucumerinum* in wild ducks shot in southern Québec during hunting seasons (1976, 1977, 1978).

		T. c. sisowi		*T. c. cucumerinum*		TOTAL *T. cucumerinum***	
HOST SPECIES*	n	PREVALENCE # INFECTED (%)	MEAN INTENSITY (RANGE)	PREVALENCE # INFECTED (%)	MEAN INTENSITY (RANGE)	PREVALENCE # INFECTED (%)	MEAN INTENSITY (RANGE)
DABBLING DUCKS							
BLA	52	6 (11.5)	1.33 (1-2)	0 (0.0)	-	8 (15.4)	1.38 (1-3)
BWT	170	13 (7.6)	1.23 (1-2)	0 (0.0)	-	16 (9.4)	1.25 (1-3)
GAD	14	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
GWT	21	1 (4.8)	1.00 (1)	0 (0.0)	-	1 (4.8)	1.00 (1)
MAL	90	14 (15.6)	1.43 (1-3)	0 (0.0)	-	15 (16.7)	1.53 (1-3)
PIN	16	2 (12.5)	1.50 (1-2)	0 (0.0)	-	2 (12.5)	1.50 (1-2)
SHO	4	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
WIG	12	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
MAL - BLA	2	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
TOTAL	381	36 (9.4)	1.33 (1-3)	0 (0.0)	-	42 (11.0)	1.43 (1-3)

... CONTINUED ...

Table 8-1. ... CONTINUED ...

HOST SPECIES*	n	*T. c. sisowi* PREVALENCE # INFECTED (%)	*T. c. sisowi* MEAN INTENSITY (RANGE)	*T. c. cucumerinum* PREVALENCE # INFECTED (%)	*T. c. cucumerinum* MEAN INTENSITY (RANGE)	TOTAL *T. cucumerinum*** PREVALENCE # INFECTED (%)	TOTAL *T. cucumerinum*** MEAN INTENSITY (RANGE)
DIVING DUCKS							
CAN	7	0 (0.0)	-	1 (14.3)	7.00 (7)	1 (14.3)	8.00 (8)
GSC	53	0 (0.0)	-	1 (1.9)	3.00 (3)	2 (3.8)	2.00 (1-3)
LSC	18	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
RED	20	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
RIN	7	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
TOTAL	105	0 (0.0)	-	2 (1.9)	5.00 (3-7)	3 (2.9)	4.00 (1-8)
OTHERS							
BUF	3	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
GOL	15	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
OLD	1	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
RUD	1	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
SSC	2	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
WOO	23	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
WSC	5	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-

* The abbreviations for host species are explained in the "List of Abbreviations".

** Total *T. cucumerinum* includes *T. c. sisowi*, *T. c. cucumerinum* and worms identified only to the species.

Although mallards had the highest levels of *T. c. sisowi* infection among the dabbling ducks, there were no significant differences in the prevalence (Figure 8-1), abundance (Figure 8-2) or intensity (Figure 8-3) among the various species of infected dabbling ducks. Among the diving ducks, canvasbacks had a higher prevalence and intensity of *T. c. cucumerinum* than greater scaup (Table 8-1), but the differences were not significant.

Sufficient data from mallards, black ducks and blue-winged teal were available to test for age and sex influences on the prevalence of *T. c. sisowi* infections but no significant effects were detected. There was insufficient data to make seasonal comparisons of *T. cucumerinum* infections within duck species.

2. SOUTHERN MANITOBA DATA

a) *Typhlocoelum cucumerinum sisowi* AND *Typhlocoelum cucumerinum cucumerinum* IN VARIOUS DUCK SPECIES

The survey data from southern Manitoba are summarized in Table 8-2. Seven of eight species of dabbling ducks (black duck, blue-winged teal, green-winged teal, mallard, pintail, shoveler and wigeon) and three of five species of diving ducks (canvasback, lesser scaup and redhead) were infected with *T. c. sisowi*. All five species of diving ducks (canvasback, greater scaup, lesser scaup, redhead and ring-necked duck) and three of eight species of dabbling ducks (blue-winged teal, green-winged teal and mallard) were infected with *T. c. cucumerinum*.

Of the 220 infected dabbling ducks, 91.8% were hosts for *T. c. sisowi* and only 1.4% were hosts for *T. c. cucumerinum*. The remaining 6.8% harboured flukes that were identified to the species level only. Of the 152 infected diving ducks, 92.1% were hosts for *T. c. cucumerinum* and only 7.9% were hosts for *T. c. sisowi*. Only 1.6% of all infected ducks (five canvasbacks and one blue-winged teal) harboured mixed infections of *T. c. sisowi* and *T. c. cucumerinum*.

Of 294 *T. c. sisowi* obtained, 95.6% came from dabbling ducks and 4.4% came from diving ducks. Of 427 *T. c. cucumerinum* obtained, 99.3% came from diving ducks and only 0.7% came from dabbling ducks. This almost complete segregation of the two subspecies into dabbling and diving ducks

Figure 8-1. Graphs of 95% confidence limits for prevalence of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* in wild ducks shot in southern Québec during hunting seasons (1976, 1977, 1978).

A *Typhlocoelum cucumerinum sisowi*;

B *Typhlocoelum cucumerinum cucumerinum*.

The abbreviations for host species are explained in the "List of Abbreviations".

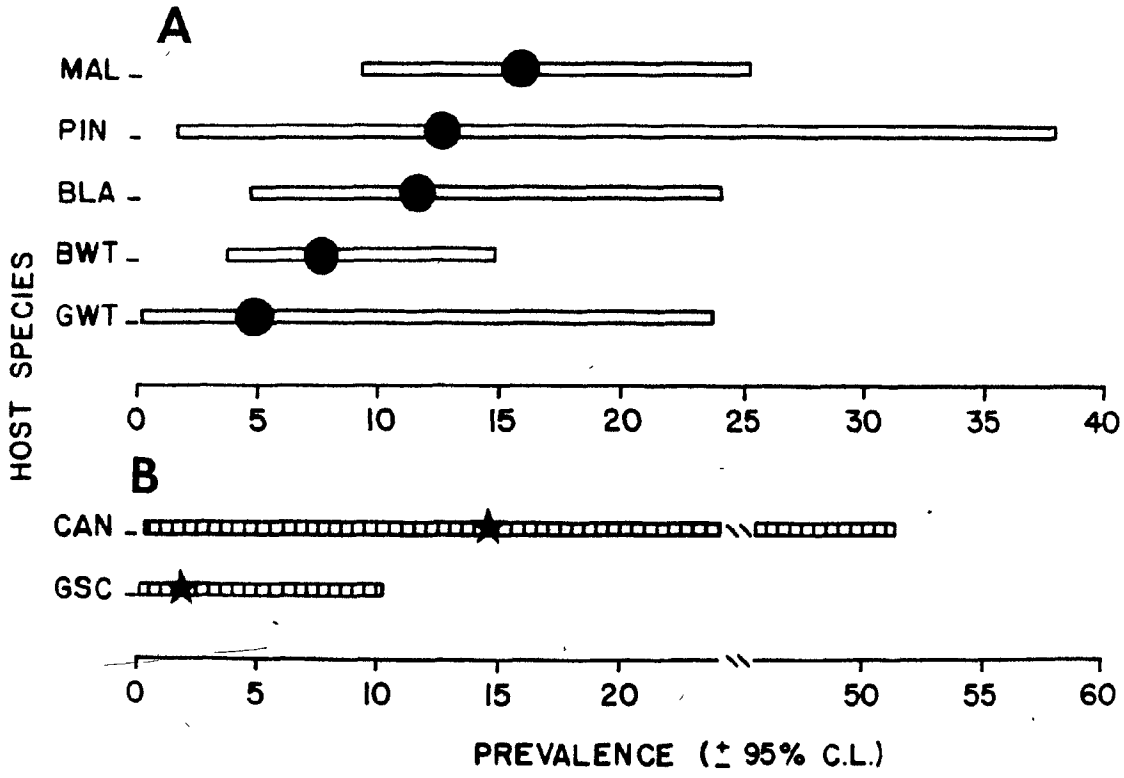

A
MAL
PIN
BLA
BWT
GWT
HOST SPECIES
0
5
10
15
20
25
30
35
40
B
CAN
GSC
0
5
10
15
20
50
55
60
PREVALENCE (± 95% C.L.)

Figure 8-2. Graphs of 95% confidence limits for abundance of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* in wild ducks shot in southern Québec during hunting seasons (1976, 1977, 1978).

A *Typhlocoelum cucumerinum sisowi*;

B *Typhlocoelum cucumerinum cucumerinum*.

The abbreviations for host species are explained in the "List of Abbreviations".

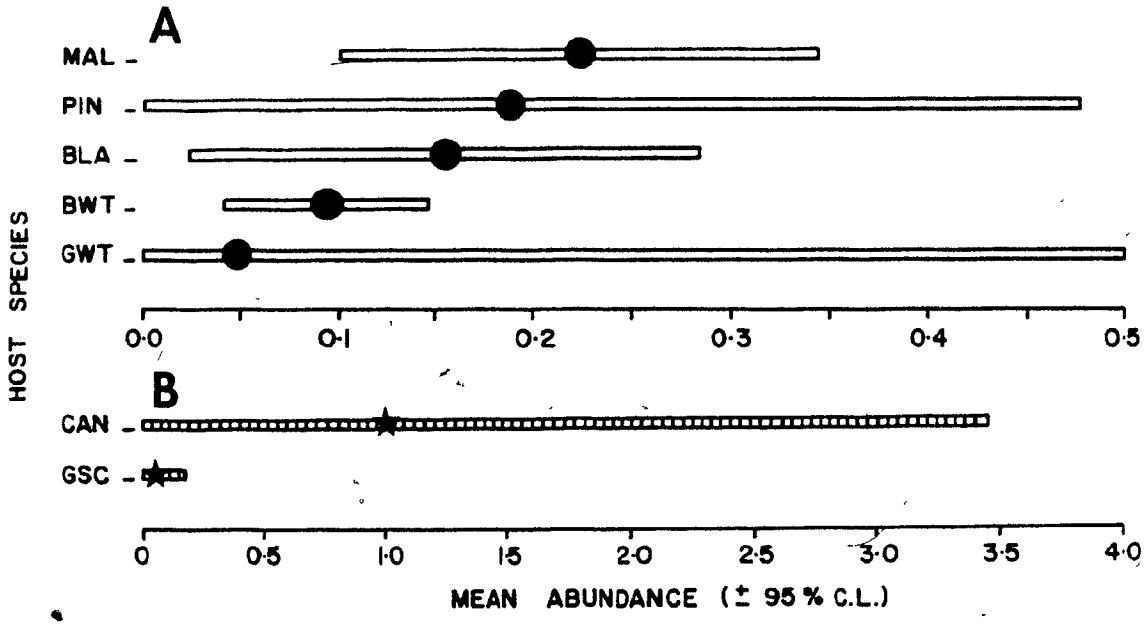

A
MAL
PIN
BLA
BWT
GWT
0·0
0·1
0·2
0·3
0·4
0·5
B
CAN
GSC
0
0·5
1·0
1·5
2·0
2·5
3·0
3·5
4·0
HOST SPECIES
MEAN ABUNDANCE (± 95 % C.L.)

Figure 8-3. Graph of 95% confidence limits for intensity of *Typhlocoelum cucumerinum sisowi* in wild ducks shot in southern Québec during hunting seasons (1976, 1977, 1978).

The abbreviations for host species are explained in the "List of Abbreviations".

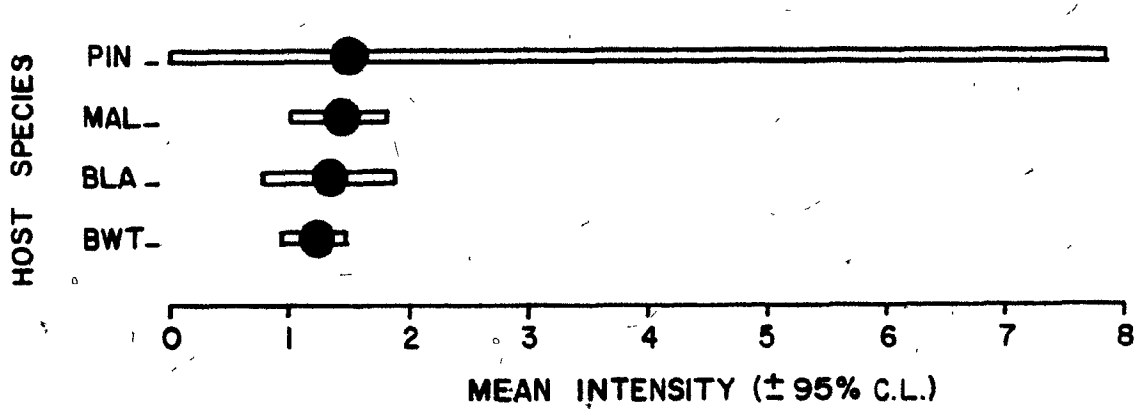

HOST SPECIES
PIN
MAL
BLA
BWT
0
1
2
3
4
5
6
7
8
MEAN INTENSITY (± 95% C.L.)

Table 8-2. Prevalence and intensity of *Typhlocoelum cucumerinum sisowi*, *Typhlocoelum cucumerinum cucumerinum* and total *Typhlocoelum cucumerinum* in wild ducks shot in southern Manitoba (1977, 1978, 1979).

HOST SPECIES*	n	*T. c. sisowi* # INFECTED (%)	*T. c. sisowi* MEAN INTENSITY (RANGE)	*T. c. cucumerinum* # INFECTED (%)	*T. c. cucumerinum* MEAN INTENSITY (RANGE)	TOTAL *T. cucumerinum*** # INFECTED (%)	TOTAL *T. cucumerinum*** MEAN INTENSITY (RANGE)
DABBLING DUCKS							
BLA	3	1 (33.3)	4.00 (4)	0 (0.0)	-	1 (33.3)	4.00 (4)
BWT	201	20 (10.0)	1.10 (1-2)	1 (0.5)	1.00 (1)	20 (10.0)	1.25 (1-2)
GAD	282	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
GWT	117	4 (3.4)	1.00 (1)	1 (0.8)	1.00 (1)	5 (4.3)	1.20 (1-2)
MAL	875	128 (14.6)	1.46 (1-7)	1 (0.1)	1.00 (1)	140 (16.0)	1.48 (1-7)
PIN	137	26 (19.0)	1.54 (1-3)	0 (0.0)	-	28 (20.4)	1.61 (1-3)
SHO	325	22 (6.8)	1.05 (1-2)	0 (0.0)	-	25 (7.7)	1.04 (1-2)
WIG	49	1 (2.0)	1.00 (1)	0 (0.0)	-	1 (2.0)	1.00 (1)
TOTAL	1989	202 (10.2)	1.39 (1-7)	3 (0.2)	1.00 (1)	220 (11.1)	1.43 (1-7)

... CONTINUED ...

Table 8-2. ... CONTINUED ...

HOST SPECIES*	n	*T. c. sisowi* PREVALENCE # INFECTED (%)	*T. c. sisowi* MEAN INTENSITY (RANGE)	*T. c. cucumerinum* PREVALENCE # INFECTED (%)	*T. c. cucumerinum* MEAN INTENSITY (RANGE)	TOTAL *T. cucumerinum*** PREVALENCE # INFECTED (%)	TOTAL *T. cucumerinum*** MEAN INTENSITY (RANGE)
DIVING DUCKS							
CAN	272	7 (2.6)	1.14 (1-2)	81 (29.8)	3.65 (1-17)	86 (31.6)	3.81 (1-18)
GSC	7	0 (0.0)	-	3 (42.9)	3.67 (3-4)	3 (42.9)	3.67 (3-4)
LSC	750	2 (0.3)	1.00 (1)	35 (4.7)	1.74 (1-8)	39 (5.2)	1.74 (1-9)
RED	274	3 (1.1)	1.00 (1)	18 (6.6)	2.83 (1-10)	21 (7.7)	2.71 (1-12)
RIN	97	0 (0.0)	-	3 (3.1)	1.67 (1-3)	3 (3.1)	1.67 (1-3)
TOTAL	1400	12 (0.9)	1.08 (1-2)	140 (10.0)	3.03 (1-17)	152 (10.9)	3.09 (1-18)
OTHERS							
BUF	21	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
GOL	11	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
RUD	9	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
WOO	2	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
WSC	10	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-

* The abbreviations for host species are explained in the "List of Abbreviations".

** Total *T. cucumerinum* includes *T. c. sisowi*, *T. c. cucumerinum* and flukes identified to the species level only.

is highly significant ($\chi^2 = 656.48$, df = 1, $P < 0.001$). Because of the small number of *T. c. sisowi* obtained from diving ducks (twelve) and the small number of *T. c. cucumerinum* obtained from dabbling ducks (three), these flukes have been disregarded in all further analyses.

Six species of ducks examined were not infected. In five of these (bufflehead, goldeneye, wood duck, ruddy duck and white-winged scoter), the sample was small (less than 25) (Table 8-2). However, 282 gadwall were examined and none were infected.

b) COMPARISON OF LEVELS OF INFECTION AMONG DIFFERENT DUCK SPECIES

The prevalence, abundance and intensity of *T. c. sisowi* *T. c. cucumerinum* were compared among the various duck species and the results are presented in Figures 8-4, 8-5 and 8-6, respectively. Non-overlapping bars represent significant differences. Because of the small numbers of greater scaup (seven) and black ducks (three) examined, these species were omitted from this analysis. In addition, with the exception of gadwall, all uninfected species were omitted.

In all cases, canvasbacks had the highest level of *T. c. cucumerinum* infection. Pintails and mallards had consistently higher levels of *T. c. sisowi* infections than blue-winged teal, green-winged teal and shoveler, and wigeon had the lowest levels of *T. c. sisowi* infections. All diving duck species had a higher intensity of infection with *T. c. cucumerinum* than the dabbling ducks had with *T. c. sisowi* (Figure 8-6); such distinct segregations between diving ducks and dabbling ducks were not observed for prevalence (Figure 8-4) or abundance (Figure 8-5).

c) COMPARISON OF AGE AND SEX CLASSES WITHIN DUCK SPECIES

Where age data were available, comparison of prevalence, abundance and intensity were made between juvenile and adult ducks shot in the fall. Only two host species revealed significant age effects (Table 8-3). Juvenile pintails had a significantly higher prevalence and abundance of *T. c. sisowi* than did adults (Table 8-3A). Similarly, juvenile mallards had a significantly higher prevalence and abundance of *T. c. sisowi* than did adults (Table 8-3B).

Figure 8-4. Graphs of 95% confidence limits for prevalence of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* in wild ducks shot in southern Manitoba (1977, 1978, 1979).

A *Typhlocoelum cucumerinum sisowi*;

B *Typhlocoelum cucumerinum cucumerinum*.

The abbreviations for host species are explained in the "List of Abbreviations".

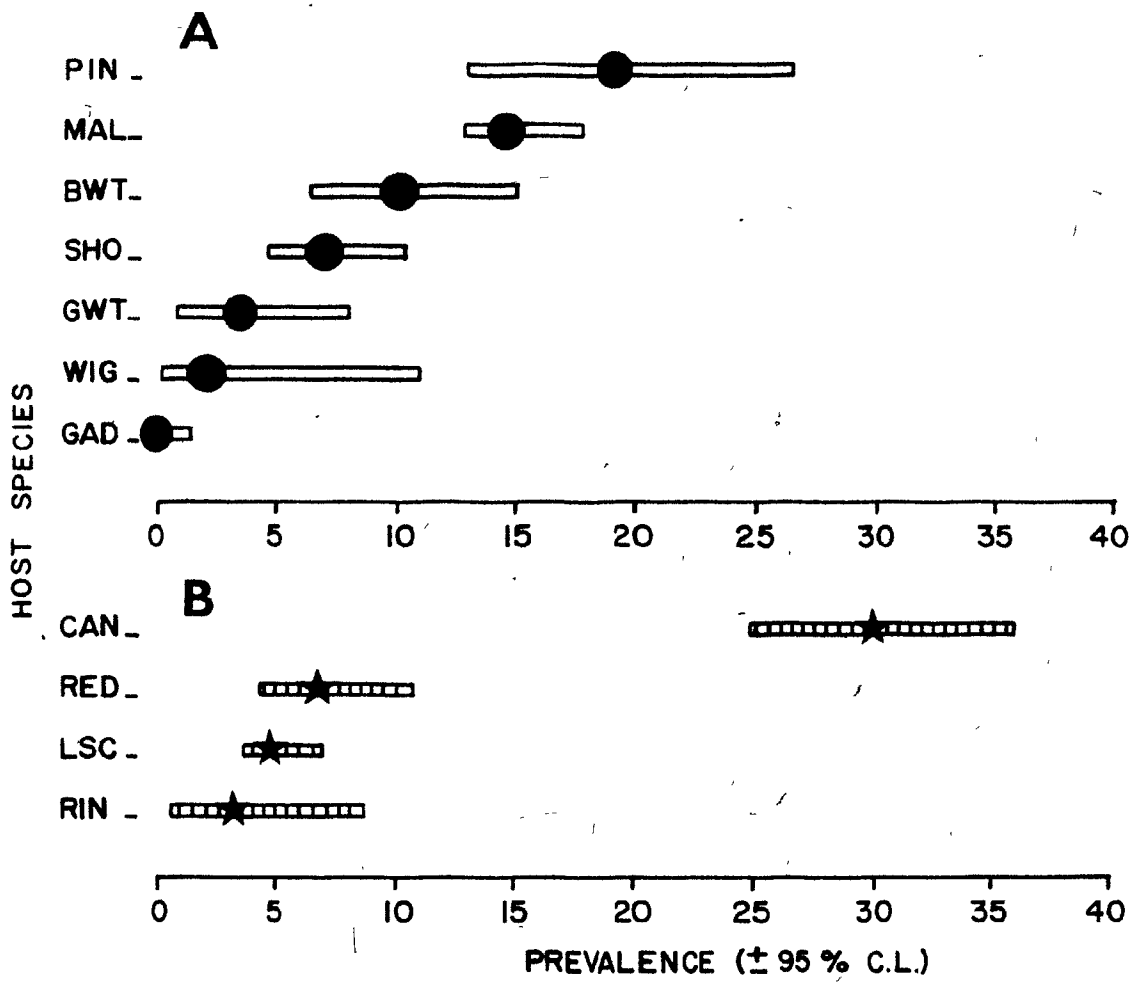

A
PIN
MAL
BWT
SHO
GWT
WIG
GAD
HOST SPECIES
0 5 10 15 20 25 30 35 40
B
CAN
RED
LSC
RIN
0 5 10 15 20 25 30 35 40
PREVALENCE (± 95 % C.L.)

Figure 8-5. Graphs of 95% confidence limits for abundance of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* in wild ducks shot in southern Manitoba (1977, 1978, 1979).

A *Typhlocoelum cucumerinum sisowi*;

B *Typhlocoelum cucumerinum cucumerinum*.

The abbreviations for host species are explained in the "List of Abbreviations".

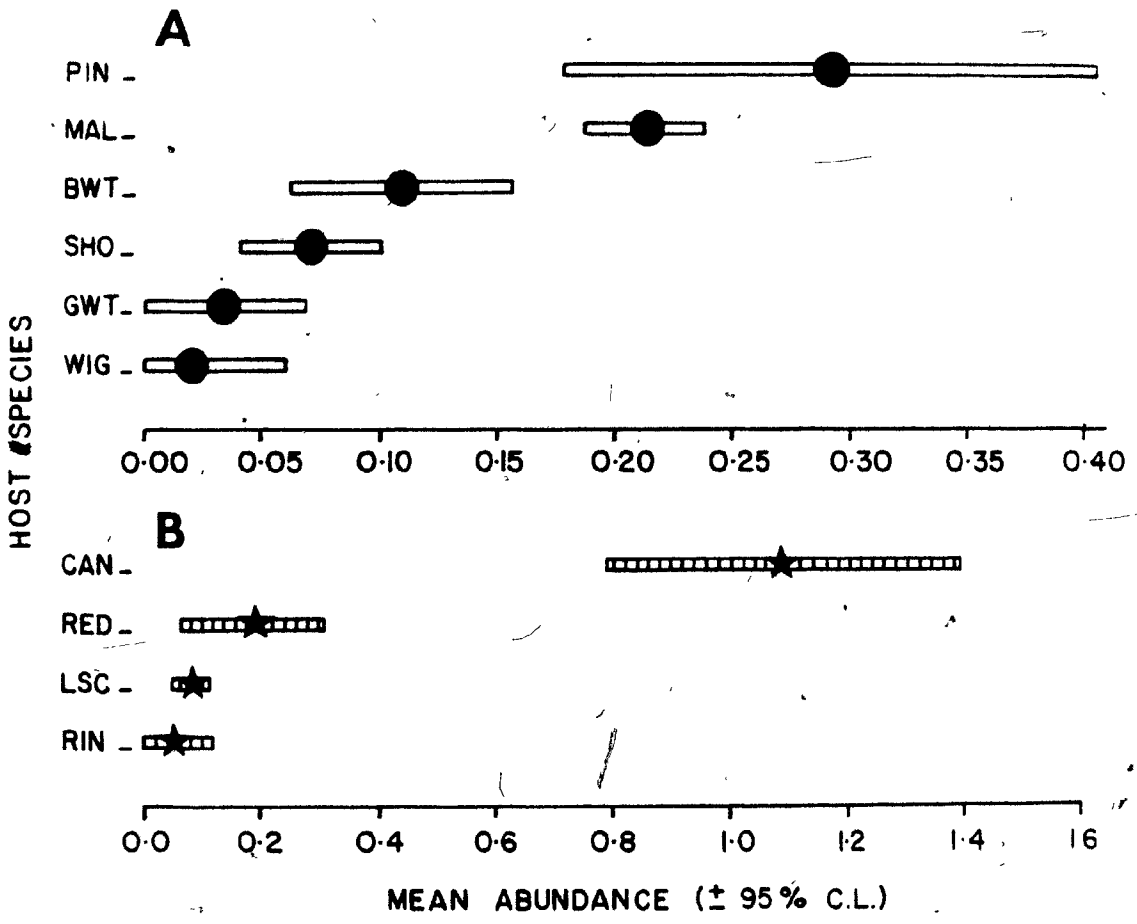

A
PIN
MAL
BWT
SHO
GWT
WIG
0·00
0·05
0·10
0·15
0·20
0·25
0·30
0·35
0·40
B
CAN
RED
LSC
RIN
0·0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
HOST SPECIES
MEAN ABUNDANCE (± 95% C.L.)

Figure 8-6. Graphs of 95% confidence limits for intensity of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* in wild ducks shot in southern Manitoba (1977, 1978, 1979).

A *Typhlocoelum cucumerinum sisowi*;

B *Typhlocoelum cucumerinum cucumerinum*.

The abbreviations for host species are explained in the "List of Abbreviations".

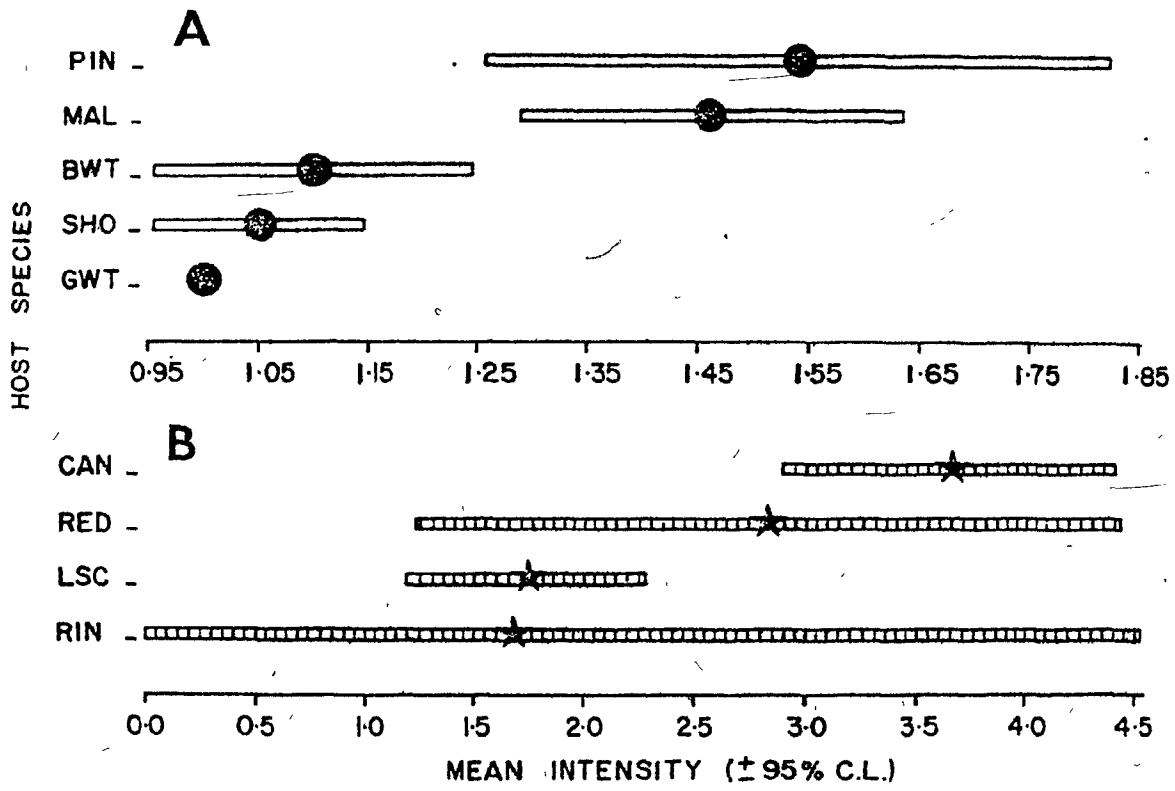

A
PIN
MAL
BWT
SHO
GWT
HOST SPECIES
0·95
1·05
1·15
1·25
1·35
1·45
1·55
1·65
1·75
1·85
B
CAN
RED
LSC
RIN
0·0
0·5
1·0
1·5
2·0
2·5
3·0
3·5
4·0
4·5
MEAN INTENSITY (± 95% C.L.)

Table 8-3. Comparison of prevalence, abundance and intensity of *Typhlocoelum cucumerinum sisowi* between juvenile and adult pintails (A) and mallards (B) shot during hunting seasons (1977, 1978).†

	DUCK	AGE CLASS	n	PREVALENCE	ABUNDANCE	INTENSITY
A.	PIN	JUVENILE	16	50.0% **	0.875 **	1.75
		ADULT	29	6.9%	0.069	1.00
B.	MAL	JUVENILE	37	29.7% **	0.432 **	1.45
		ADULT	215	11.6%	0.153	1.32

† Values joined by the same bar are not significantly different.

** $p < 0.01$

No significant differences in prevalence, abundance or intensity of infection were detected between male and female ducks in any species.

d) COMPARISON OF SEASONS AND YEARS WITHIN DUCK SPECIES

Comparison within years in adult mallards revealed significantly higher prevalences and abundances of *T. c. sisowi* in the resident summer mallard population than in the population of mallards that migrate through the Delta Marsh in the fall, in both 1977 and 1978, but there were no significant differences in the intensity of infection in either year (Table 8-4A,B).

In only three cases did comparisons within host species yield significant differences between years. Although no significant differences in prevalence, abundance or intensity of *T. c. sisowi* in mallards shot in the summers of 1977, 1978 and 1979 were seen (Table 8-4C), mallards and shovelers shot in the fall of 1978 had a significantly higher prevalence and abundance of *T. c. sisowi* than those shot in the fall of 1977 (Table 8-4D,E). Canvasbacks had a significantly higher abundance of *T. c. cucumerinum* in the fall of 1978 than in the fall of 1977 (Table 8-4F).

e) THEORETICAL FREQUENCY DISTRIBUTIONS FOR *Typhlocoelum cucumerinum sisowi* AND *Typhlocoelum cucumerinum cucumerinum* IN VARIOUS DUCK SPECIES

Theoretical frequency distributions for both *T. c. sisowi* and *T. c. cucumerinum* in the ten duck hosts were determined (Table 8-5; Figure 8-7). In eight of these (blue-winged teal, green-winged teal, mallard, pintail, canvasback, lesser scaup, redhead and ring-necked duck) the distribution was overdispersed, and was described by the negative binomial distribution. In the remaining host species (shoveler and wigeon) the distribution was random and was described by a Poisson distribution.

D. DISCUSSION

1. UNINFECTED DUCK SPECIES AND NEW HOST RECORDS

Four of the duck species reported as uninfected in this study have previously been recorded as hosts for *T. cucumerinum*: gadwall (Quortrup and Shillinger, 1941; Bezubik, 1956); goldeneyes (Town, 1960;

Table 8-4. Comparison between seasons and among years of prevalence, abundance and intensity of *Typhlocoelum cucumerinum sisowi* in mallards (A,B,C,D) and shovelers (E) and of *Typhlocoelum cucumerinum cucumerinum* in canvasbacks (F) shot in southern Manitoba.†

	DUCK	SEASON	YEAR	n	PREVALENCE	ABUNDANCE	INTENSITY
A.	MAL[1]	SUMMER	1977	29	27.6%	0.345	1.25
					**	*	
		FALL	1977	101	8.9%	0.148	1.67
B.	MAL[1]	SUMMER	1978	78	28.2%	0.474	1.68
					*	*	
		FALL	1978	114	14.0%	0.158	1.12
C.	MAL[1]	SUMMER	1977	29	27.6%	0.345	1.25
		SUMMER	1978	78	28.2%	0.474	1.68
		SUMMER	1979	32	21.9%	0.531	2.43
D.	MAL[2]	FALL	1977	291	7.9%	0.117	1.48
					**	**	
		FALL	1978	445	17.8%	0.245	1.36
E.	SHO[2]	FALL	1977	66	1.5%	0.015	1.00
					*	*	
		FALL	1978	191	11.0%	0.115	1.05
F.	CAN[2]	FALL	1977	108	30.6%	1.139	3.73
						**	
		FALL	1978	156	32.1%	1.256	3.96

† Values joined by the same bar are not significantly different.

1 Only adult ducks included.

2 All ducks included.

* $P < 0.05$

** $P < 0.01$

Table 8-5. Parameters of observed and expected theoretical frequency distributions of *Typhlocoelum cucumerinum sisowi* (A) and *Typhlocoelum cucumerinum cucumerinum* (B) in various duck species shot in southern Manitoba.

A. DUCK HOST[1]	n	MEAN[2]	$s^2/\bar{x}$ [3]	EMPIRICAL DISTRIBUTION	k	$P > \chi^2$	FIGURE
DABBLING DUCKS							
BWT	201	0.124	1.2839	-VE BINOMIAL	0.334	0.171	8-7 E
GWT	117	0.051	1.2931	-VE BINOMIAL	0.142	0.578	8-7 F
MAL	875	0.237	1.8828	-VE BINOMIAL	0.297	0.433	8-7 B
PIN	137	0.328	1.6202	-VE BINOMIAL	0.365	0.149	8-7 C
SHO	325	0.080	1.0011	POISSON	-	0.970	8-7 D
WIG	49	0.020	1.0000	POISSON	-	0.966	8-7 G

B. DUCK HOST[1]	n	MEAN[2]	$s^2/\bar{x}$ [3]	EMPIRICAL DISTRIBUTION	k	$P > \chi^2$	FIGURE
DIVING DUCKS							
CAN	272	1.206	6.2434	-VE BINOMIAL	0.194	0.574	8-7 H
LSC	750	0.091	3.1689	-VE BINOMIAL	0.054	0.216	8-7 A
RED	274	0.208	6.2920	-VE BINOMIAL	0.047	0.355	8-7 I
RIN	97	0.052	2.1582	-VE BINOMIAL	0.034	0.200	8-7 J

[1] The abbreviations for host species are explained in the "List of Abbreviations"

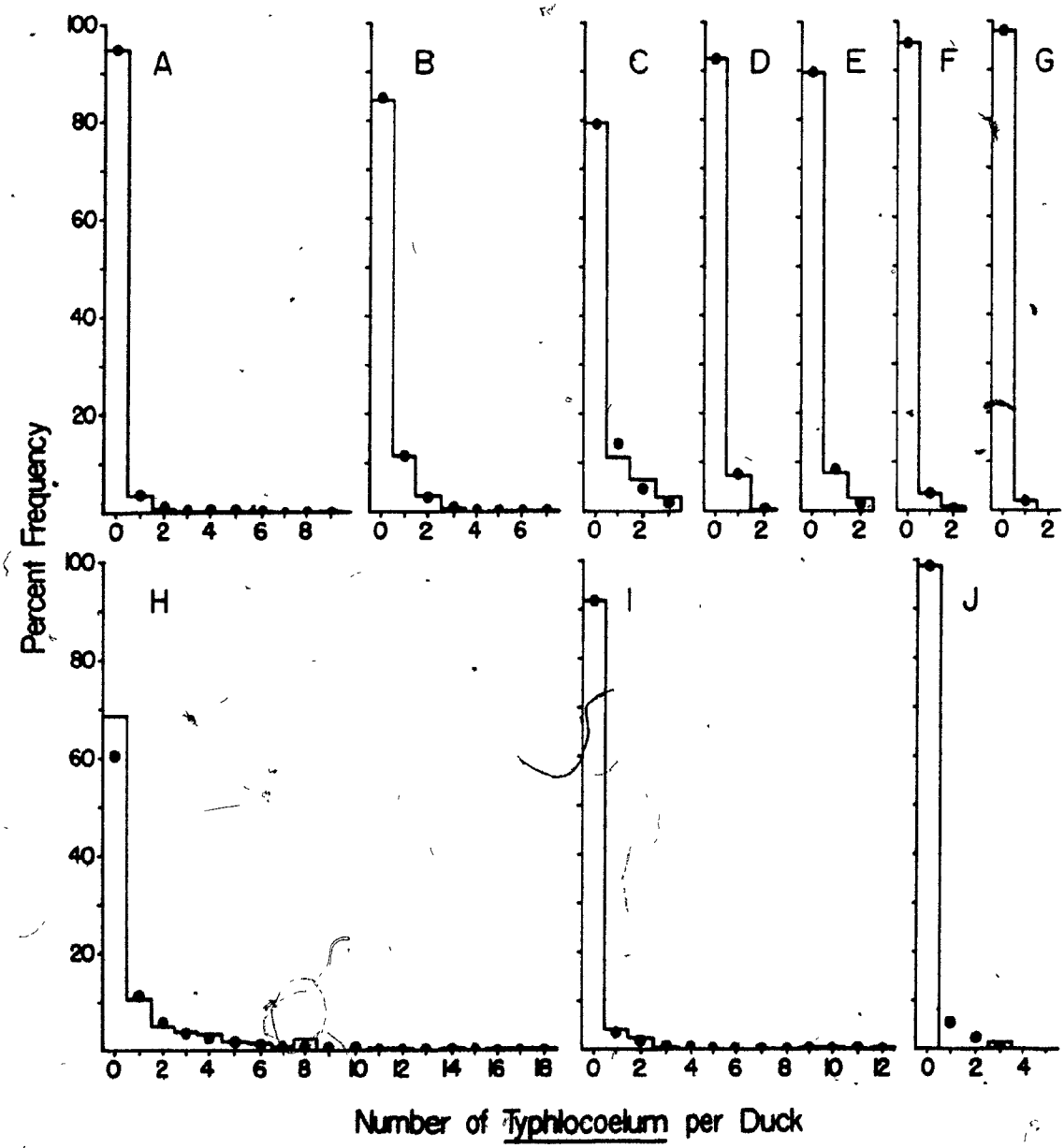
[2] Mean abundance.

[3] Variance to mean ratio.

Figure 8-7. Observed and theoretical frequency distributions for abundance of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* in various species of ducks shot in southern Manitoba (1977, 1978, 1979).

FIGURE	DUCK SPECIES	PARASITE	DISTRIBUTION
A	LSC	*T. c. cucumerinum*	-VE BINOMIAL
B	MAL	*T. c. sisowi*	-VE BINOMIAL
C	PIN	*T. c. sisowi*	-VE BINOMIAL
D	SHO	*T. c. sisowi*	POISSON
E	BWT	*T. c. sisowi*	-VE BINOMIAL
F	GWT	*T. c. sisowi*	-VE BINOMIAL
G	WIG	*T. c. sisowi*	POISSON
H	CAN	*T. c. cucumerinum*	-VE BINOMIAL
I	RED	*T. c. cucumerinum*	-VE BINOMIAL
J	RIN	*T. c. cucumerinum*	-VE BINOMIAL

The bars represent the observed distributions and the dots represent the theoretical distributions.
The abbreviations for host species are explained in the "List of Abbreviations".


A
B
C
D
E
F
G
H
I
J
Percent Frequency
Number of Typhlocoelum per Duck

Dr. Murray Lankester, Lakehead University, personal communication); oldsquaw (Bezubik, 1956); and white-winged scoter (Gower, 1938b). The absence of infection in the large sample of gadwall (n = 282) examined from southern Manitoba suggests that, in this region, gadwall are not a common host of *T. cucumerinum*. This may be related to the paucity of animal matter in their diets (Buscher, 1965). The lack of infections in goldeneyes, oldsquaw and white-winged scoter may have been a function of the small sample sizes (26, 1 and 15, respectively).

The absence of infections in bufflehead, wood ducks, ruddy ducks and surf scoter may also be a function of sample size (25 of fewer ducks of each species). There are no records of *T. cucumerinum* in these duck species, but this may be due, in part, to the limited number of parasitological surveys of these ducks, and does not necessarily imply that they are not potential hosts of *T. cucumerinum*.

Two new host records were established as a result of this study: American wigeon and ring-necked duck. *Typhlocoelum cucumerinum sisowi* has been recorded from the European wigeon (*Anas penelope*) in Great Britain (Beverley-Burton, 1972), but until the present study, there had been no records of *T. cucumerinum* from the American wigeon (*Anas americana*).

2. COMPARISON OF SOUTHERN MANITOBA AND SOUTHERN QUEBEC DATA

Of the 12 species of duck infected with the two subspecies of *T. cucumerinum* in southern Manitoba, seven were also infected in southern Québec (black duck, blue-winged teal, green-winged teal, mallard, pintail, canvasback and greater scaup). The levels of infection in each species of duck were similar in the two provinces except for greater scaup, where both the prevalence and abundance of *T. c. cucumerinum* were significantly higher in Manitoba than in Québec (Fisher's exact probability test, $P = 0.018$; Mann-Whitney U test, $z = 3.603$, $P = 0.00032$, respectively).

Greater scaup are primarily salt water ducks (Johnsgard, 1965) and most of them breed in Alaska, migrate across Canada, and winter on the Atlantic coast (Bellrose, 1976), although some do breed on the large freshwater lakes of central Manitoba (Palmer, 1976). Consequently, greater scaup shot in Manitoba may have been feeding on freshwater snails during

the summer in areas frequented by the heavily infected canvasbacks and redheads. Therefore, they would have had the opportunity to acquire *T. c. cucumerinum* infections. In contrast, greater scaup shot in southern Québec may have been feeding in coastal areas of Alaska during the summer where the distribution of freshwater snails may be limited. This might conceivably explain the differential levels of infection detected between greater scaup shot in southern Manitoba and southern Québec.

3. COMPARISONS WITH OTHER STUDIES

Data on the levels of *T. c. sisowi* and *T. c. cucumerinum* infections from nine other studies (Table 8-6) have been compared with both the southern Québec and the southern Manitoba data.

Florida ducks, a race of mallard resident in Florida (Kinsella and Forrester, 1972), had a significantly higher prevalence of *T. c. sisowi* than mallards shot in southern Québec ($\chi^2 = 10.12$, df = 1, $P < 0.01$) and southern Manitoba ($\chi^2 = 24.65$, df = 1, $P < 0.001$). Florida ducks are non-migratory (Bellrose, 1976) and have a high intake of gastropods (Palmer, 1976). In a situation where a local population of ducks exists for a prolonged period of time, a pool of metacercariae could be built up and maintained in the snails resulting, ultimately, in a higher prevalence of infection in the local ducks; this might explain the high prevalence of *T. c. sisowi* in Florida ducks.

The prevalence and abundance of *T. c. cucumerinum* in greater scaup were significantly higher in Michigan than in southern Québec (Fisher's exact probability test, $P = 0.002$; Mann-Whitney U test, $z = 3.91$, $P = 0.0001$, respectively). The lesser scaup examined by Town (1960) had a significantly higher prevalence (Fisher's exact probability test, $P = 0.0003$), abundance (Mann-Whitney U test, $z = 4.76$, $P = 0.00006$) and intensity (Mann-Whitney U test, $z = 2.93$, $P = 0.0036$) of *T. c. cucumerinum* than those examined in southern Manitoba. All ducks examined by Town (1960) were found sick or dead on the Lower Detroit River. *Typhlocoelum cucumerinum* has been cited as the cause of death in ducks (see Chapter V). It is conceivable that this might account, in part, for the higher levels of *T. c. cucumerinum* in the greater scaup and lesser scaup from Michigan.

Table 8-6. Summary of previous reports on levels of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* infections.

LOCATION	DUCK[1]	PARASITE	n	PREVALENCE	INTENSITY	REFERENCE
EASTERN CANADA	BLA	*T. c. sisowi*	34	6%	1 - 2	Mahoney and Threlfall (1978)
DELTA, MANITOBA	BWT	*T. c. sisowi*	114	5%	6	Buscher (1966)
MANITOBA & MICHIGAN	CAN	*T. c. cucumerinum*	180	23%	-	Cornwell and Cowan (1963)
MICHIGAN	LSC[2]	*T. c. cucumerinum*	52	21%	2.7 (1-7)	Town (1960)
MICHIGAN	GSC[2]	*T. c. cucumerinum*	15	40%	8.7 (1-23)	Town (1960)
MICHIGAN	CAN[2]	*T. c. cucumerinum*	22	36%	4.8 (1-14)	Town (1960)
MICHIGAN	GOL[2]	*T. c. cucumerinum*	8	12%	1.0 (1)	Town (1960)
IOWA	BWT	*T. c. sisowi*	23	4%	-	Taft (1971)
FLORIDA	MAL[3]	*T. c. sisowi*	78	38%	4 (1-20)	Kinsella and Forrester (1972)
TEXAS	SHO[4]	*T. c. sisowi*	18	6%	3 (3)	Broderson *et al.* (1977)
BRITAIN	MAL	*T. c. sisowi*	54	7%	1 - 3	Beverley-Burton (1972)
BRITAIN	PIN	*T. c. sisowi*	15	13%	1 - 3	Beverley-Burton (1972)
BRITAIN	SHO	*T. c. sisowi*	11	0%	-	Beverley-Burton (1972)
SLOVAQUIE	MAL	*T. c. sisowi*	37	5%	1	Macko and Buša (1960)
SLOVAQUIE	PIN	*T. c. sisowi*	11	27%	2.3 (2-3)	Macko and Buša (1960)

1 The abbreviations for host species are explained in the "List of Abbreviations".

2 Individual data on each duck enables comparisons of abundance and intensity with the present study.

3 The Florida duck is a race of mallard.

4 Spring sample; 20 shovelers examined in a fall sample were uninfected.

Canvasbacks examined by Cornwell and Cowan (1963) from both the Detroit River and southern Manitoba, had a significantly lower prevalence of *T. c. cucumerinum* than those examined from southern Manitoba in this study ($\chi^2 = 4.19$, df = 1, $P < 0.05$). In the present study, the levels of infections in canvasbacks varied between 1977 and 1978 and the differences between this study and that of Cornwell and Cowan (1963) probably reflect additional variation among years.

4. AGE AS A FACTOR INFLUENCING LEVELS OF INFECTION

Age has frequently been shown to influence the levels of parasitism in waterfowl. Juvenile canvasbacks from southern Manitoba and Michigan had higher numbers of worms than adults (Cornwell and Cowan, 1963). Similarly, juvenile blue-winged teal have been shown to have heavier parasite infections at the Delta Marsh, Manitoba than adults (Buscher, 1966). Bakke (1972) suggested that higher levels of infection in juvenile birds may be related to the greater intake of food or a poorly developed physiological resistance.

In this study, juvenile mallards and pintails from southern Manitoba were more heavily infected with *T. c. sisowi* than adults. Crichton and Welch (1972) found similar results for helminths in mallards and pintails on the Delta Marsh. Cornwell (1966) found that juvenile canvasbacks had a higher prevalence of *T. c. cucumerinum* than adults, but this was not shown to be the case for *T. c. cucumerinum* in canvasbacks in this study.

5. SEX AS A FACTOR INFLUENCING LEVELS OF INFECTION

Higher levels of parasitism in female ducks have been reported for mallards and pintails (Crichton and Welch, 1972) and canvasbacks (Cornwell, 1966) obtained from the Delta Marsh, Manitoba. Bakke (1972) listed a number of sources of sexual influences on parasitism, including hormonal influences, food habits, sensitivity to climate, activity in breeding season and immune response, but suggested that different feeding habits of males and females are probably the most important. Data from this study suggest that sex is not a factor influencing levels of *T. cucumerinum* infections in ducks.

6. -SEASONAL AND YEARLY FLUCTUATIONS IN LEVELS OF INFECTION

There are a number of reports in the literature showing that the levels of helminth infections vary with the season. Beverley-Burton (1972) suggested that the prevalence and intensity of helminth infections in waterfowl are highest in the summer. Kinsella and Forrester (1972) followed the levels of parasitism in Florida ducks and found that they increased through the summer, peaked in November and fell to a low in February and March. They related this to a decreased intake of animal matter in winter. Gower (1938a) found that the prevalence of *T. cucumerinum* was highest in the spring and lowest in the winter. He suggested that this may have been related to the specificity and distribution of the intermediate host.

Adult mallards shot in the summer in this study were more heavily infected with *T. c. sisowi* than those shot in the fall. The summer mallard samples were taken from a breeding population on the Delta Marsh whereas ducks shot in the fall represented mallards that were migrating through southern Manitoba to the wintering grounds. These migrants presumably came from a number of different breeding areas, some of which may have been important areas for the transmission of *T. c. sisowi* while others may have not. Such mixing during migration might result in a reduction of the overall levels of infection and might explain the higher prevalence and abundance observed in the local mallard population in the summer.

It could also be suggested that the short duration of *T. c. sisowi* infections in mallards (see Chapters V and VI) contributed to the seasonal differences. Mallards are infected when they arrive in southern Manitoba in the spring (personal observations). Based on the short duration of the infection observed in the laboratory, it is reasonable to suggest that they loose the infections during the summer. Perhaps the adult ducks have a refractory period after infection, or perhaps their snail intake decreases during the summer. This would result in a total decrease in the levels of infection in adult mallards in the fall.

Three species of ducks (mallard, shoveler and canvasback) had higher levels of infection in the fall of 1978 than in the fall of 1977. The severe drought in the spring and summer of 1977 may have influenced the dynamics of *T. cucumerinum* transmission and therefore may have contributed

to the low levels of infection that fall.

7. OVERDISPERSION OF *Typhlocoelum cucumerinum* POPULATIONS

The distribution of the two subspecies of *T. cucumerinum* in the host population is overdispersed in all but two duck species. This is in agreement with current host-parasite population studies (Crofton, 1971; Pennycuick, 1971; Anderson, 1978). Overdispersion, as exemplified by the negative binomial distribution, has been suggested to result from a number of factors. Among these are clumping of infective stages within the intermediate hosts in their environment, and multiple exposures to the parasites (Crofton, 1971). The cercariae of both subspecies of *T. cucumerinum* encyst within the first intermediate host, resulting in an accumulation of metacercariae in infected snails. Clumping of infected snails in the field may also occur and would presumably lead to multiple exposures. These two factors would contribute to the overdispersed distribution observed in most duck species.

The distribution in wigeon and shovelers was not overdispersed, however. This could conceivably be explained by the infrequent occurrence of snails in their diets (Bartonek, 1972; Mr. Paul DuBowy, Delta Waterfowl Research Station, personal communication; Mr. Rick Wishart, University of Manitoba, personal communication), which would reduce the chances for exposure to *T. c. sisowi* and also reduce the degree of overdispersion.

8. DISTRIBUTION OF *Typhlocoelum cucumerinum sisowi* AND *Typhlocoelum cucumerinum cucumerinum* AMONG VARIOUS DUCK SPECIES

One of the most interesting aspects of the survey data presented in this chapter is the distribution of the two subspecies among the various duck hosts. The results of this survey clearly show that the two subspecies of *T. cucumerinum* are segregated into dabbling and diving ducks, and that within each of these groups of ducks, the levels of infection vary among the different duck species. These differences in distribution of *T. cucumerinum* may result from differential susceptibilities of the host species to infection with the two different subspecies, from different feeding habits of the various duck species, or from an ecological separation of the hosts

in the field.

a) ROLE OF DUCK SUSCEPTIBILITY IN DETERMINING LEVELS OF INFECTION

In an attempt to determine whether different duck species are equally susceptible to the initial stages of *T. c. sisowi* infections, four species of ducks (mallard, blue-winged teal, gadwall and canvasback) were infected with metacercariae from *Physa gyrina* and necropsied at 20 hours post-infection. The results of these infections (Table 8-7) revealed that *T. c. sisowi* metacercariae are more successful in excysting and penetrating the intestinal wall of mallards than of canvasbacks. Similar results were obtained at later days post-infection (see Chapter IV) and these data suggest that the segregation of *T. c. sisowi* and *T. c. cucumerinum* in the field may be, in part, a function of differential susceptibilities of dabbling and diving ducks to the two subspecies of *T. cucumerinum*.

Among the three dabbling duck species used in the experimental infections, there were no significant differences in host susceptibility (Table 8-7). Therefore, the absence of infection in gadwall in the field is apparently not the result of a lower susceptibility to infection.

b) FEEDING ECOLOGY OF VARIOUS DUCK SPECIES

A second factor that may contribute to the different levels of infection in the field is the relative importance of snails in the diet of the various duck species. In order for a duck to become infected, it must eat an infected snail. One would predict that, the higher the abundance of snails in the diet of a particular species of duck, the higher the probability of *T. cucumerinum* infections.

Data from the feeding analysis undertaken by Korschgen (1955) were used to investigate this hypothesis. Korschgen's study was selected because it was the only feeding study that included all duck species relevant to the present study. Pearson product-moment correlation coefficients were calculated using the prevalence, abundance and intensity data from southern Manitoba, and Korschgen's values for percent occurrence of snails in the diet (arc sine transformation). In all cases, significant positive correlations were obtained: arc sine prevalence ($r = 0.747$,

Table 8-7. Results of susceptibility experiment using *Typhlocoelum cucumerinum sisowi* metacercariae from *Physa gyrina*.*

HOST	n	% INFECTED[1]	MEAN % RECOVERY OF *T. c. sisowi*[1]
MAL	6	77.8% a	11.25 b
BWT	6	100.0% a	9.67 b c
GAD	6	83.3% a	6.67 b c
CAN †	4	100.0% a	6.50 c

* Ducks were necropsied at 20 hours post-infection.

1 Values with the same letter are not significantly different.

† Diving duck species.

$P < 0.001$); square root abundance ($r = 0.825$, $P < 0.001$); intensity ($r = 0.736$, $P < 0.001$). This supports the hypothesis that the distribution of *T. cucumerinum* among the various duck hosts is, in part, related to the occurrence of snails in their diet (Figure 8-8).

c) ECOLOGY OF VARIOUS DUCK SPECIES AND RELATIONSHIP TO THE DISTRIBUTION OF *Typhlocoelum cucumerinum*

A third hypothesis proposed to explain the distribution of *T. c. sisowi* and *T. c. cucumerinum* in the field was that the hosts may be ecologically segregated. Dabbling ducks feed at the surface of the water and are frequently found in ponds and along shallow shores of lakes and marshes whereas diving ducks feed at the bottom of the pond or lake and are more commonly found in larger bays and deeper water (Godfrey, 1966). Although the ecological separation of dabbling and diving ducks is not complete, it may be sufficient to maintain two separate pools of infective metacercariae. In fact, even within the dabbling and diving ducks, those species that have similar habits can be shown to have similar levels of infection.

Within the dabbling ducks, mallards, pintails and black ducks have many similar habits and frequently are found together (Sowls, 1955; Bellrose, 1976; Palmer, 1976). One might expect that the levels of *T. c. sisowi* infections in these three species of ducks would be similar, and, in both the southern Québec and southern Manitoba data, they were.

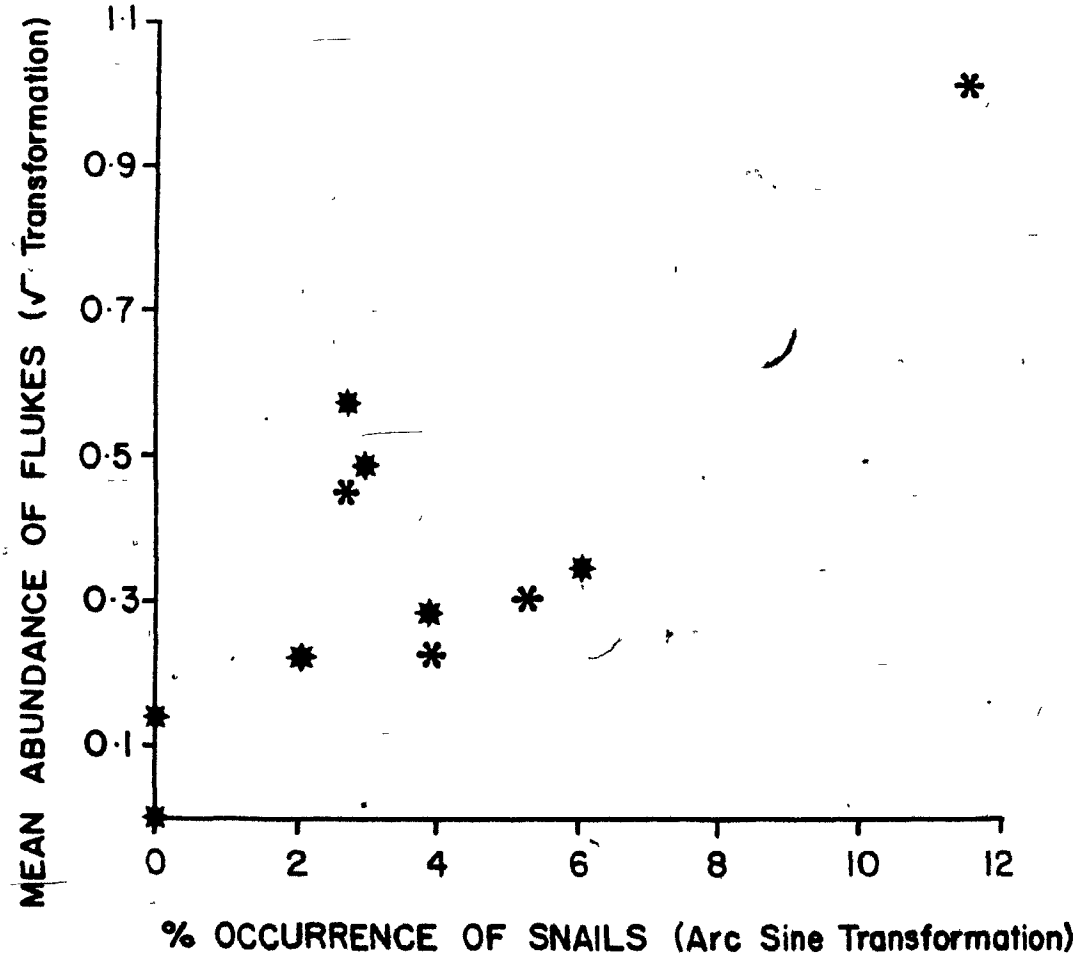
According to Palmer (1976) blue-winged teal, shovelers and green-winged teal share similar habitats and are often seen together. These three species were also shown to have similar levels of *T. c. sisowi* infections (Figures 8-1 through 8-6). It was also shown in Chapter III that these two groups of dabbling ducks harbour morphologically distinguishable forms of *T. c. sisowi*. Wigeon are the most terrestrial of the dabbling ducks (Palmer, 1976). As a result, they would probably feed on fewer aquatic snails than the other dabbling duck species and this might account for their low levels of infection with *T. c. sisowi*.

Among the diving ducks, canvasbacks, redheads and lesser scaup occur together (Palmer, 1976; Siegfried, 1976) and they have similar intensities of *T. c. cucumerinum* (Figure 8-6). Ring-necked ducks are

Figure 8-8. Comparison between abundance of *Typhlocoelum cucumerinum* and percent occurrence of snails (Korschgen, 1955) in various duck species. A square root transformation was used for abundance; an arc sine transformation was used for percent occurrence of snails.

✸ dabbling duck species;

✻ diving duck species.


MEAN ABUNDANCE OF FLUKES (√ Transformation)
1·1
0·9
0·7
0·5
0·3
0·1
0
2
4
6
8
10
12
% OCCURRENCE OF SNAILS (Arc Sine Transformation)

frequently seen with lesser scaup (Palmer, 1976). Although only a few ring-necked ducks were examined in this study, the intensity of *T. c. cucumerinum* in this species was similar to that in lesser scaup.

Neraasen and Holmes (1975) used the term "infective pool" to describe the population of infective stages of parasites. They suggested that, based on the relative abundance of different host species, each would contribute different kinds and numbers of parasites to the infective pool, and each would draw different proportions of infective stages from the pool. The above discussion has demonstrated that the levels of infection in different duck species can be related to some extent to their ecological relationships. These observations are made, however, with little regard to the relative abundance of the different duck species. This becomes very important when assessing the roles of various host species in the transmission of the parasites.

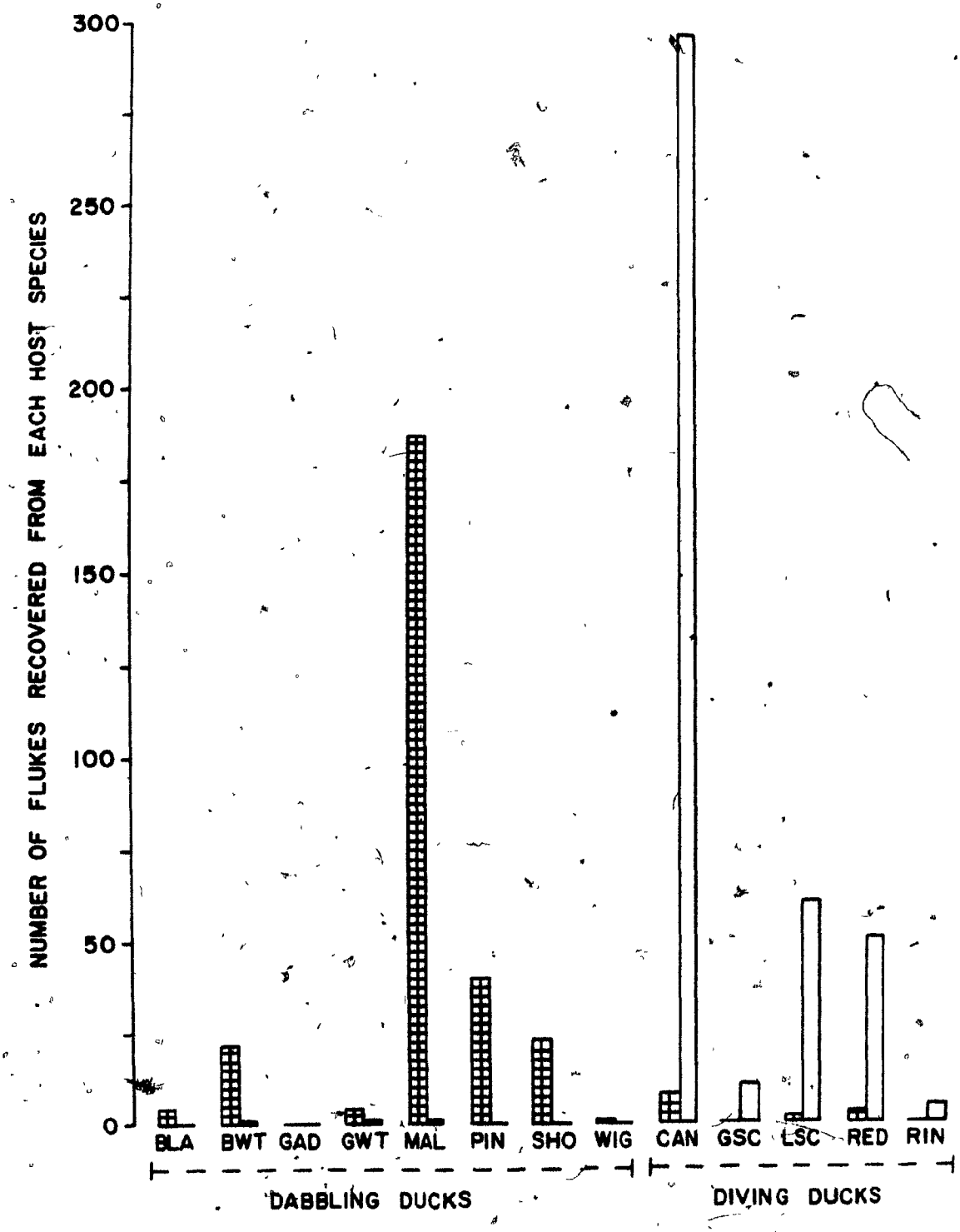
In an attempt to determine which species of ducks contribute the most, overall, to the *T. c. eisowi* and *T. c. cucumerinum* infective pools, the total number of specimens obtained from each host species in southern Manitoba during 1977, 1978 and 1979 were plotted (Figure 8-9). If it is assumed that the sample of ducks examined in this study accurately reflects the relative frequencies of the ducks that migrate through or breed in southern Manitoba, then the total number of specimens recovered from any single host species in this survey reflects the proportional contribution of that host species to the total pool of infective material. When the relative frequencies of ducks examined in this study were compared with the relative frequencies of each duck species in the bag checks on the Delta Marsh during the first two weeks of October, 1977, and 1978, only three species of ducks had different relative frequencies (blue-winged teal, lesser scaup and redhead), and the maximum difference was only 5%. Although the bag check data may reflect a certain bias as a result of selection on the part of the duck hunter for certain species of ducks, or as a result of limits on the numbers of mallards, canvasbacks and redheads shot per hunter per day, these two sources of bias would tend to cancel each other, as the limited species also tend to be the preferred species.

Figure 8-9. Graph of the total number of *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum* obtained from various species of ducks shot in southern Manitoba (1977, 1978, 1979).

Typhlocoelum cucumerinum sisowi

Typhlocoelum cucumerinum cucumerinum

The abbreviations for host species are explained in the "List of Abbreviations".


NUMBER OF FLUKES RECOVERED FROM EACH HOST SPECIES
300
250
200
150
100
50
0
BLA
BWT
GAD
GWT
MAL
PIN
SHO
WIG
CAN
GSC
LSC
RED
RIN
DABBLING DUCKS
DIVING DUCKS

From Figure 8-9, it is immediately obvious that the highest number of *T. c. sisowi* were obtained from mallards and that the highest number of *T. c. cucumerinum* were obtained from canvasbacks. Therefore, it could be suggested that in the area of southern Manitoba in general, mallards may be the major contributor to the *T. c. sisowi* pool and that canvasbacks may be the major contributor to the *T. c. cucumerinum* pool. However, this generalization can not be applied to localized areas within this region. If, on a certain pond, pintails are the predominant dabbling duck, these would be more important for *T. c. sisowi* transmission than mallards, and it is only in a global sense that mallards and canvasbacks can be regarded as the important hosts.

It thus appears that whether or not a duck species becomes infected with *T. cucumerinum* is largely dependent on the occurrence of snails in the diet, but that differential susceptibilities of dabbling and diving ducks to the two subspecies of *T. cucumerinum* together with an ecological separation of the two groups of ducks, contribute to the segregation of *T. c. sisowi* into dabbling ducks and *T. c. cucumerinum* into diving ducks.

CHAPTER IX. OVERVIEW

In the preceding six chapters, a large volume of data has been presented and a number of comparisons have been made between *Typhlocoelum cucumerinum sisowi* and *Typhlocoelum cucumerinum cucumerinum*. At the outset, it was shown conclusively that the two parasites must be considered as separate populations within the same species. However, because of the number of biological differences detected between the two subspecies, for all practical purposes, they are different organisms. It is appropriate now to relate the variability in morphology and life cycle strategies detected in the laboratory and the observations made in the field in an attempt to understand the broader aspects of the biology of these two organisms.

A most significant observation made in the field was the segregation of *T. c. sisowi* into dabbling ducks and *T. c. cucumerinum* into diving ducks. After investigating potential means by which this segregation could be maintained, it was proposed that, in the first instance, a degree of ecological isolation of dabbling and diving ducks might tend to perpetuate two separate pools of infective material, thereby maintaining the separation in the field. Cross-infection experiments in the laboratory together with field data revealed that *T. c. sisowi* establishes more successfully in mallards than in canvasbacks and that *T. c. cucumerinum* establishes more successfully in canvasbacks than in mallards. This may be attributed to differences intrinsic to the ducks and/or differences intrinsic to the parasites, and might also contribute to the segregation of the two parasites in the field.

The role of the snail phase in segregating the two parasites is not fully understood. Differences have been demonstrated in the infectivity of *T. c. sisowi* and *T. c. cucumerinum* miracidia, depending on the snail species; the snail host has also been shown to influence the infectivity of metacercariae to ducks. On the basis of experimental data, *Physa gyrina* is apparently the "best" host for both *T. c. sisowi* and *T. c. cucumerinum*. However, this may not be the most important host in the field for both parasites. Neraasen and Holmes (1975) noted that a knowledge of the relative abundance of various host species was very important in determining

which of these contribute the most to transmission. *Typhlocoelum cucumerinum sisowi* and *T. c. cucumerinum* are capable of developing in at least three different families of snails (Physidae, Lymnaeidae, Planorbidae) and would therefore be good models for studying the role of various host species in the transmission in the field. It may be shown, for instance, that in some localities, one host is important whereas, in other localities, another host is important. In fact, this is undoubtedly true with respect to the duck hosts as well. Field and laboratory studies suggest that mallards and canvasbacks are the most important hosts for *T. c. sisowi* and *T. c. cucumerinum*, respectively. However, on any individual pond, transmission of the parasites may depend on any of the species of ducks that abound in the area, and it is only over large geographical areas that mallards and canvasbacks can be seen as the important hosts.

Presumably the segregation of *T. c. sisowi* into dabbling ducks and *T. c. cucumerinum* into diving ducks would allow for adaptations of the two parasites to the environment provided by their respective duck hosts; the adaptations could be perpetuated because of the isolation of the gene pools.

The recent literature on speciation has acknowledged that reproductive isolation is not always related to geographical isolation (Gould and Johnston, 1972; Bush, 1975) and that the species may not be the unit on which natural selection acts. Ehrlich and Raven (1969) suggested that gene flow is rarely strong enough to unite the local populations into a larger, integrated species and considered that the local population, not the abstract species, is the "real" unit in nature. The observations made in this study certainly indicate that both *T. c. sisowi* and *T. c. cucumerinum* are adapted to their respective duck hosts and that they may be the target for natural selection. They may be undergoing speciation, or they may gradually intermix and become less distinct. Although it is impossible to predict what will happen to these parasites in the future, their present variability and different survival strategies can be compared.

Detailed morphological examination of *T. c. sisowi* and *T. c. cucumerinum* obtained from wild ducks revealed a large degree of variability in both parasites. This has also been substantiated in the laboratory. The

greatest degree of morphological variability is a function of the host species, although the age of the host and its geographical location are also potential sources of variability. According to Schiller (1959), "obtaining reliable data concerning the kinds of variants and the frequency with which they occur, is prerequisite to the proper evaluation of the relative stability of morphological characters". In both *T. c. sisowi* and *T. c. cucumerinum*, it is the frequency of the variants that differs. In most of the character variables investigated, all possible character states can be found in specimens of either subspecies, but the frequencies do vary between the two parasites, and can be used to distinguish populations. However, testis shape is the only morphological character that could be used to distinguish individuals of the two subspecies.

The field data gathered in this research indicated that there may be differences in the survival strategies of the two parasites. The intensity of *T. c. cucumerinum* infections in diving ducks was much higher than that of *T. c. sisowi* in dabbling ducks, but the proportion of mature flukes in the trachea was higher in dabbling ducks than in diving ducks. Experimental studies revealed four important differences in the migration and development of *T. c. sisowi* and *T. c. cucumerinum* in the ducks. Firstly, *T. c. cucumerinum* reach the trachea of canvasbacks much sooner than *T. c. sisowi* do in mallards, and secondly, *T. c. cucumerinum* reach maturity much later than *T. c. sisowi*. These two observations may explain the higher proportion of immature *T. c. cucumerinum* seen in wild diving ducks. A third important difference uncovered through experimental investigations was that *T. c. sisowi* remain in the trachea for less than one month whereas *T. c. cucumerinum* remain in the trachea for up to five months. Also, a large proportion of *T. c. sisowi* is unsuccessful in reaching the trachea and persist in the abdominal cavity and air sacs for up to seven months. This does not occur in *T. c. cucumerinum* infections. Therefore, the number of flukes reaching the trachea is lower in mallards than in canvasbacks. The difference in duration of the infection in the trachea and the relative success of the two parasites in reaching the trachea undoubtedly contribute to the higher intensities of infection in diving ducks than in dabbling ducks.

In addition to the differences noted above in migration and

development of *T. c. sisowi* and *T. c. cucumerinum*, there are also important differences in fecundity and in the minimum time required for completion of the life cycle. Evidence suggests that *T. c. cucumerinum* produces more eggs than *T. c. sisowi*, but that *T. c. cucumerinum* has a longer developmental time than *T. c. sisowi*. These two differences may compensate for each other.

As a result of this research, the interrelationship between *T. c. sisowi* and *T. c. cucumerinum* has been studied in detail. Although it has been shown that both are contained within the same species, so many differences in all aspects of their biology have been demonstrated that, for all practical purposes, they might be considered as separate species. The variability of these two subspecies together with their ecological isolation into different types of definitive hosts may not be a phenomenon restricted to *Typhlocoelum cucumerinum*. Natural isolation of local populations and subsequent variability undoubtedly occurs in other digenean species. An improved understanding of the interpopulation variability of host-parasite associations in general may reveal the extent to which distinctions between species and subspecies are relevant, and may also help to clarify the parasitologist's conception of the roles of species and populations in the evolution of parasitic organisms.

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APPENDIX 3-1. SPECIMENS OBTAINED FROM INDIVIDUALS OR MUSEUMS.

In addition to material obtained from wild ducks in southern Québec and southern Manitoba, specimens of the subfamily Typhlocoelinae were provided by individuals or museums throughout the world.

Dr. Murray Lankester, Lakehead University, Thunder Bay, Ontario, provided six specimens from the trachea of a goldeneye from Ontario.

Mrs. Mary Hanson Pritchard, Harold W. Manter Laboratory, University of Nebraska, Lincoln, Nebraska kindly provided three specimens:

Typhlocoelum americanum Manter and Williams, 1928

ex. *Spatula clypeata* (L.) "Type specimen"

Typhlocoelum cucumerinum (Rudolphi, 1809)

ex. *Marila affinis* (Eyton)

Typhlocoelum cymbium (Diesing, 1850)

ex. *Dafila acuta* (L.)

Dr. Ralph Lichtenfels, Animal Parasitology Institute, Beltsville Agricultural Research Center, Beltsville, Maryland loaned me specimens of the subfamily Typhlocoelinae:

Typhlocoelum americanum (Cat. # 28169)

Nettion carolinense, trachea (?), Texas (1 slide)

Typhlocoelum americanum (Cat. # 29178)

Pintail, larynx, California (1 vial of specimens)

Typhlocoelum americanum (Cat. # 43147)

Gadwall, oral cavity, Bear River Migratory Refuge, Utah (1 slide)

Typhlocoelum americanum (Cat. # 29251)

Mallard, trachea, Oregon (1 slide)

Typhlocoelum americanum (Cat. # 29254)

Pintail, trachea, Oregon (1 slide)

Typhlocoelum cucumerinum (Cat. # 28157)

Scaup, trachea, Washington, D.C. (1 slide)

Typhlocoelum cucumerinum (Cat. # 25961)

Marila americana, thoracic cavity, Maryland (1 slide plus sections)

Typhlocoelum flavum (Cat. # 25120)

Marila valisineria, trachea, North Carolina (1 vial of specimens)

Typhlocoelum cymbium (Cat. # 44097)

Domestic duck, upper respiratory passage, North Dakota (1 slide)

Typhlocoelum cymbium (Cat. # 44430)

Anas platyrhynchos, trachea (3 slides)

Typhlocoelum cymbium (Cat. # 46348)

Nyroca valisineria, trachea, Oregon (1 vial of specimens)

Typhlocoelum cymbium (Cat. # 55834)

Mallard, trachea, Pennsylvania (1 vial of specimens)

Typhlocoelum cymbium (Cat. # 37333

Muscovy duck, larynx, upper trachea, Delaware (1 vial of specimens)

Typhlocoelum sp. (Cat. # 25935)

Marila americana, lungs, Maryland (1 vial of specimens)

Typhlocoelum sp. (Cat. # 31768)

Wild duck, trachea, Michigan (1 slide)

Typhlocoelum sp. (Cat. # 41023)

Swan, trachea, Montana (1 slide)

Tracheophilus sp. (Cat. # 66797)

Snipe, body cavity, Panama (1 vial of specimens)

Typhlocoelum sp. (?) (Cat. # 49009)

Scarlet ibis, trachea, New York State (2 slides)

Neiva Lisete Schafranski, Universidade Federal de Minas Gerais, Brazil, provided two specimens of *Typhlocoelum cucumerinum* from Brazil.

Dr. Mary Beverley-Burton, University of Guelph, Guelph, Ontario, allowed me to examine the specimens of *Typhlocoelum sisowi* that she had collected in Britain (four specimens from mallards, three from pintails and two from European wigeon).

Dr. Gibson of the British Museum (Natural History), London, England, provided me with two specimens:

Typhlocoelum cucumerinum (Reg. # 1961.4.24.1)

ex. *Nyroca fuligula*, Hertfordshire, England

Typhlocoelum sisowi (Reg. # 1977.8.25.134-141)

Duck, Iran

Dr. Khalil of the Commonwealth Institute of Helminthology, St. Albans, England, kindly loaned me a specimen, *Tracheophilus sisowi*

(C.I.H. # 2830) from the trachea and bronchi of a domestic duck in Thailand (donor, Dr. Muangyai).

Dr. Claude Vaucher of the Muséum d'Histoire naturelle, Genève, provided a specimen, *Typhlocoelum cymbium*, from Charles Joyeux's collection. This specimen had also been examined by Georges Dubois. Dr. Vaucher also provided a second specimen, from André's collection, that had been deposited by Dr. Dubois.

Dr. J. Daniel McLaughlin, Concordia University, Montreal, Québec, collected a number of specimens of *Typhlocoelum* from ducks in South Africa which he made available to me. In addition, material was provided by Dr. David Skead, Barberspan Ornithological Station, Transvaal, Republic of South Africa. In total, 15 specimens from yellow-billed ducks (*Anas undulata*), five from red-billed pintails (*Anas erythrorhyncha*) and two from African pochards (*Aythya erythropthalma*) were examined.

APPENDIX 3-2. MEASUREMENTS MADE ON SPECIMENS OF *Typhlocoelum* OBTAINED FROM INDIVIDUALS AND MUSEUMS THROUGHOUT THE WORLD.

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Appendix 3-2-1. Measurements made on specimens of *Typhlocoelum* obtained from ducks in Ontario, Canada and from the United States (see Appendix 3-1) (Mean (mm) ± S.E.).

VARIABLE*	ONTARIO	UNITED STATES					
HOST*	GOL	CAN		RED		SC	SWA
n	6	n		n		2	1
BL	3.943 ± 0.083	3	7.86 ± 0.742	11	5.62 ± 0.24	7.34	9.32
BW	1.340 ± 0.055	4	3.01 ± 0.216	11	2.43 ± 0.08	2.36	3.43
PL	0.156 ± 0.007	3	0.310 ± 0.008	11	0.209 ± 0.010	0.190	0.381
PW	0.151 ± 0.002	3	0.282 ± 0.021	11	0.208 ± 0.013	0.155	0.451
OL	0.122 ± 0.001	4	0.324 ± 0.048	11	0.151 ± 0.022	0.204	0.248
OW	0.119 ± 0.011	4	0.360 ± 0.036	11	0.173 ± 0.017	0.268	0.479
SRL	0.081 ± 0.003	2	0.182 ± 0.007	8	0.148 ± 0.022	0.134	0.169
SRW	0.088 ± 0.006	2	0.332 ± 0.106	8	0.222 ± 0.025	0.268	0.226
ATL	0.116 ± 0.004	3	0.998 ± 0.560	7	0.715 ± 0.092	0.543	0.192
ATW	0.099 ± 0.008	3	0.320 ± 0.055	7	0.234 ± 0.020	0.430	0.210
PTL	0.136 ± 0.006	3	0.564 ± 0.212	9	0.346 ± 0.029	0.395	0.324
PTW	0.111 ± 0.018	3	0.630 ± 0.228	9	0.608 ± 0.038	0.451	0.649
EGGA	- -	3	0.00 ± 0.00	9	1.26 ± 0.50	0.21	0.00
EYEA	- -	3	2.73 ± 0.45	1	2.26 -	-	4.68
PA	0.343 ± 0.010	3	0.522 ± 0.028	11	0.428 ± 0.026	0.394	0.395
OA	2.808 ± 0.117	3	5.91 ± 0.58	10	4.30 ± 0.19	3.60	7.78
SRA	2.943 ± 0.126	2	5.79 ± 1.09	1	4.10 -	3.78	8.10
ATA	2.707 ± 0.156	2	5.51 ± 0.97	5	4.47 ± 0.27	3.36	7.13
PTA	3.112 ± 0.134	2	6.28 ± 1.02	5	5.03 ± 0.31	3.98	8.34
OES	0.056 ± 0.008	-	- -	2	0.063 ± 0.007	-	0.028
CL	- -	1	0.790 -	3	0.341 ± 0.075	0.338	-

* The abbreviations for host species and variables are explained in the "List of Abbreviations".

Appendix 3-2-2. Measurements made on specimens of *Typhlocoelum* obtained from ducks in the United States (see Appendix 3-1) (Mean (mm) ± S.E.).

VARIABLE*	UNITED STATES								
HOST*	DOM	DUC	GAD	MAL		MUS		PIN	
n	1	1	1	n		3		n	
BL	6.08	7.86	5.99	3	7.56 ± 0.80	5.25 ± 0.31		3	6.13 ± 0.21
BW	2.96	3.16	3.51	3	2.82 ± 0.58	3.06 ± 0.12		3	2.97 ± 0.12
PL	0.282	0.423	0.324	3	0.357 ± 0.021	0.305 ± 0.009		2	0.318 ± 0.050
PW	0.310	0.409	0.324	3	0.315 ± 0.034	0.268 ± 0.008		2	0.282 ± 0.042
OL	0.212	0.282	0.197	2	0.261 ± 0.021	0.183 ± 0.029		2	0.241 ± 0.001
OW	0.310	0.282	0.282	2	0.352 ± 0.000	0.244 ± 0.031		2	0.232 ± 0.050
SRL	0.240	0.197	0.113	2	0.317 ± 0.035	0.193 ± 0.019		2	0.141 ± 0.028
SRW	0.282	0.310	0.169	2	0.338 ± 0.014	0.244 ± 0.038		2	0.247 ± 0.035
ATL	0.099	0.212	0.169	2	0.332 ± 0.036	0.141 ± 0.021		2	0.170 ± 0.084
ATW	0.169	0.212	0.212	2	0.289 ± 0.049	0.226 ± 0.014		2	0.204 ± 0.008
PTL	0.155	0.226	0.254	2	0.310 ± 0.098	0.193 ± 0.042		2	0.190 ± 0.050
PTW	0.282	0.254	0.324	2	0.345 ± 0.007	0.329 ± 0.047		2	0.317 ± 0.021
EGGA	0.00	0.00	0.00	3	0.13 ± 0.13	0.00 ± 0.00		3	0.00 ± 0.00
EYEA	2.85	2.92	2.34	3	2.85 ± 0.41	-	-	3	2.19 ± 0.60
PA	0.564	0.606	0.536	3	0.644 ± 0.070	0.498 ± 0.046		2	0.466 ± 0.080
OA	4.68	5.83	4.46	2	5.84 ± 0.24	3.81 ± 0.26		2	4.58 ± 0.20
SRA	4.86	6.08	4.60	2	6.24 ± 0.16	4.08 ± 0.22		2	4.82 ± 0.20
ATA	4.21	5.35	4.21	2	5.63 ± 0.53	3.43 ± 0.19		2	4.29 ± 0.00
PTA	5.02	6.24	4.78	2	6.56 ± 0.32	4.16 ± 0.27		2	4.94 ± 0.24
NID	-	-	11.0	-	-	-	-	-	-
OES	0.056	0.183	0.028	3	0.108 ± 0.045	0.037 ± 0.020		1	0.042 -
CL	-	0.395	-	-	-	-	-	-	-

* The abbreviations for host species and variables are explained in the "List of Abbreviations".

Appendix 3-2-3. Measurements made on specimens of *Typhlocoelum* obtained from ducks in Brazil and Britain (see Appendix 3-1) (Mean (mm) ± S.E.).

VARIABLE*	BRAZIL	BRITAIN				
HOST*	DOM	MAL		PIN	WIG	TUF
n	2	n		n	2	1
BL	10.88	4	7.13 ± 0.78	3	6.61 ± 0.70	7.29	9.40
BW	4.67	4	2.94 ± 0.20	3	2.55 ± 0.32	2.79	4.02
PL	0.374	4	0.268 ± 0.019	3	0.277 ± 0.020	0.296	0.320
PW	0.430	4	0.233 ± 0.019	3	0.230 ± 0.025	0.324	0.256
OL	0.402	4	0.306 ± 0.020	3	0.315 ± 0.009	0.360	0.416
OW	0.437	4	0.324 ± 0.021	3	0.334 ± 0.040	0.374	0.320
SRL	0.451	3	0.183 ± 0.021	2	0.212 ± 0.000	0.254	-
SRW	0.599	3	0.362 ± 0.033	2	0.169 ± 0.000	0.388	-
ATL	2.552	4	0.222 ± 0.049	3	0.231 ± 0.019	0.268	0.640
ATW	1.053	4	0.260 ± 0.042	3	0.221 ± 0.033	0.303	0.448
PTL	1.571	4	0.201 ± 0.011	3	0.249 ± 0.070	0.260	0.480
PTW	2.410	4	0.377 ± 0.032	3	0.245 ± 0.026	0.444	0.880
EGGA	-	4	0.00 ± 0.00	3	0.87 ± 0.87	-	-
EYEA	-	4	2.33 ± 0.51	2	2.79 ± 0.45	-	-
PA	0.585	4	0.458 ± 0.067	3	0.540 ± 0.033	0.585	0.416
OA	8.00	4	5.49 ± 0.47	2	4.67 ± 1.08	6.44	-
SRA	8.36	3	6.13 ± 0.32	2	4.76 ± 1.32	6.76	-
ATA	7.12	4	4.94 ± 0.58	2	4.36 ± 1.16	6.20	-
PTA	8.85	4	5.89 ± 0.60	2	5.15 ± 1.17	6.97	-
NID	-	4	10.5 ± 0.9	2	9.5 ± 0.5	-	-
OES	0.085	4	0.049 ± 0.017	3	0.136 ± 0.031	0.084	-

* The abbreviations for host species and variables are explained in the "List of Abbreviations".

Appendix 3-2-4. Measurements made on specimens of *Typhlocoelum* obtained from ducks in Iran, Thailand and South Africa (see Appendix 3-1) (Mean (mm) ± S.E.).

VARIABLE*	IRAN			THAILAND	SOUTH AFRICA					
HOST*	DUC			DOM	RBT		YBT		SOP	
	n			1	n		n		n	
BL	4	7.56	± 0.62	7.21	5	7.79 ± 0.26	15	8.27 ± 0.19	2	8.94
BW	4	3.43	± 0.32	3.20	5	3.54 ± 0.10	15	3.86 ± 0.09	2	4.06
PL	4	0.312	± 0.056	0.300	5	0.361 ± 0.011	14	0.325 ± 0.008	2	0.282
PW	4	0.248	± 0.014	0.180	5	0.361 ± 0.006	14	0.281 ± 0.009	2	0.282
OL	3	0.336	± 0.009	0.320	5	0.299 ± 0.014	15	0.335 ± 0.010	1	0.130
OW	3	0.320	± 0.000	0.340	5	0.310 ± 0.021	15	0.405 ± 0.009	1	0.122
SRL	–	–	–	–	4	0.211 ± 0.039	15	0.225 ± 0.015	1	0.141
SRW	–	–	–	–	4	0.289 ± 0.023	15	0.395 ± 0.024	1	0.268
ATL	3	0.288	± 0.061	0.300	5	0.220 ± 0.025	15	0.250 ± 0.017	2	0.926
ATW	3	0.256	± 0.018	0.200	5	0.228 ± 0.019	15	0.317 ± 0.010	2	0.310
PTL	3	0.299	± 0.056	0.240	5	0.217 ± 0.019	15	0.266 ± 0.010	2	0.472
PTW	3	0.357	± 0.056	0.380	5	0.302 ± 0.028	15	0.423 ± 0.021	2	0.908
EGGA	–	–	–	1.56	5	0.04 ± 0.04	15	0.09 ± 0.05	1	0.00
EYEA	–	–	–	–	5	3.00 ± 0.18	14	3.23 ± 0.20	1	2.34
PA	4	0.548	± 0.088	0.640	5	0.668 ± 0.050	15	0.597 ± 0.038	2	0.635
OA	–	–	–	–	5	5.48 ± 0.20	15	6.10 ± 0.13	2	6.46
SRA	–	–	–	–	4	5.68 ± 0.25	15	6.35 ± 0.14	1	4.06
ATA	–	–	–	–	5	5.25 ± 0.20	15	5.71 ± 0.14	2	5.93
PTA	–	–	–	–	5	5.94 ± 0.21	15	6.64 ± 0.15	1	4.21
NID	–	–	–	–	5	11.0 ± 0.0	15	10.9 ± 0.2	2	11.5
OES	–	–	–	–	4	0.028 ± 0.024	15	0.148 ± 0.014	2	0.162
CL	–	–	–	–	1	0.423 –	5	0.640 ± 0.039	2	0.726

* The abbreviations for host species and variables are explained in the "List of Abbreviations".

APPENDIX 3-3. MORPHOMETRICS OF SPECIMENS FROM VARIOUS SPECIES OF WILD DUCKS.

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Appendix 3-3-1. Numerical variables of mature specimens of *Typhlocoelum* obtained from black ducks and blue-winged teal from southern Québec and southern Manitoba.

NUMERICAL VARIABLE*	BLACK DUCK n	BLACK DUCK MEAN (mm) ± 95% C.L.	BLUE-WINGED TEAL n	BLUE-WINGED TEAL MEAN (mm) ± 95% C.L.
BL	7	8.35 ± 0.50	16	7.68 ± 0.31
BW	8	3.50 ± 0.11	22	2.97 ± 0.08
PL	8	0.326 ± 0.012	18	0.262 ± 0.009
PW	8	0.314 ± 0.011	18	0.286 ± 0.009
OL	7	0.285 ± 0.018	21	0.275 ± 0.011
OW	7	0.301 ± 0.023	21	0.322 ± 0.016
SRL	4	0.356 ± 0.073	14	0.257 ± 0.019
SRW	4	0.409 ± 0.069	14	0.350 ± 0.032
ATL	6	0.262 ± 0.024	18	0.388 ± 0.041
ATW	6	0.262 ± 0.028	17	0.356 ± 0.038
PTL	6	0.256 ± 0.031	19	0.314 ± 0.032
PTW	6	0.342 ± 0.034	19	0.588 ± 0.057
PA	8	0.636 ± 0.036	17	0.541 ± 0.019
OA	7	6.16 ± 0.36	15	6.22 ± 0.25
SRA	6	6.70 ± 0.28	12	6.60 ± 0.29
ATA	6	5.80 ± 0.42	15	5.82 ± 0.22
PTA	7	6.69 ± 0.41	15	6.75 ± 0.27
NID	6	10.3 ± 0.3	7	10.6 ± 0.2
OES	8	0.183 ± 0.049	14	0.120 ± 0.024

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 3-3-2. Numerical variables of mature specimens of *Typhlocoelum* obtained from green-winged teal and mallards from southern Québec and southern Manitoba.

NUMERICAL VARIABLE*	GREEN-WINGED TEAL n	GREEN-WINGED TEAL MEAN (mm) ± 95% C.L.	MALLARD n	MALLARD MEAN (mm) ± 95% C.L.
BL	4	8.61 ± 0.96	126	8.00 ± 0.09
BW	7	3.50 ± 0.28	134	3.40 ± 0.04
PL	5	0.274 ± 0.019	127	0.318 ± 0.004
PW	5	0.302 ± 0.019	127	0.297 ± 0.005
OL	6	0.326 ± 0.013	123	0.286 ± 0.005
OW	6	0.370 ± 0.025	123	0.323 ± 0.005
SRL	4	0.230 ± 0.013	95	0.283 ± 0.010
SRW	4	0.363 ± 0.021	95	0.330 ± 0.011
ATL	5	0.380 ± 0.061	113	0.236 ± 0.007
ATW	5	0.316 ± 0.048	113	0.276 ± 0.008
PTL	5	0.293 ± 0.028	123	0.267 ± 0.013
PTW	5	0.723 ± 0.122	123	0.356 ± 0.016
PA	4	0.550 ± 0.026	127	0.634 ± 0.010
OA	4	7.20 ± 0.95	122	5.93 ± 0.08
SRA	2	9.06 ± 0.37	94	6.34 ± 0.08
ATA	4	6.93 ± 0.97	114	5.64 ± 0.07
PTA	3	8.09 ± 1.33	120	6.48 ± 0.08
NID	3	11.3 ± 0.3	69	10.5 ± 0.1
OES	5	0.121 ± 0.016	106	0.094 ± 0.008

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 3-3-3. Numerical variables of mature specimens of *Typhlocoelum* obtained from pintails and shovelers from southern Québec and southern Manitoba.

NUMERICAL VARIABLE*	PINTAIL n	PINTAIL MEAN (mm) ± 95% C.L.	SHOVELER n	SHOVELER MEAN (mm) ± 95% C.L.
BL	19	7.54 ± 0.33	14	8.27 ± 0.28
BW	19	3.11 ± 0.16	15	2.76 ± 0.10
PL	19	0.281 ± 0.008	14	0.278 ± 0.009
PW	19	0.256 ± 0.009	14	0.288 ± 0.011
OL	18	0.278 ± 0.014	14	0.273 ± 0.015
OW	18	0.295 ± 0.013	14	0.310 ± 0.015
SRL	12	0.190 ± 0.018	13	0.209 ± 0.015
SRW	12	0.258 ± 0.019	13	0.346 ± 0.020
ATL	17	0.217 ± 0.018	15	0.322 ± 0.030
ATW	17	0.249 ± 0.016	15	0.271 ± 0.023
PTL	18	0.242 ± 0.019	15	0.248 ± 0.040
PTW	18	0.301 ± 0.015	15	0.594 ± 0.029
PA	19	0.557 ± 0.023	13	0.520 ± 0.026
OA	18	5.45 ± 0.23	14	6.59 ± 0.21
SRA	14	5.66 ± 0.27	13	6.75 ± 0.23
ATA	16	4.71 ± 0.26	14	6.24 ± 0.21
PTA	18	5.92 ± 0.24	14	7.11 ± 0.23
NID	9	10.7 ± 0.3	12	11.0 ± 0.3
OES	19	0.119 ± 0.020	12	0.096 ± 0.015

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 3-3-4. Numerical variables of mature specimens of *Typhlocoelum* obtained from canvasbacks and greater scaup from southern Québec and southern Manitoba.

NUMERICAL VARIABLE*	CANVASBACK n	CANVASBACK MEAN (mm) ± 95% C.L.	GREATER SCAUP n	GREATER SCAUP MEAN (mm) ± 95% C.L.
BL	102	8.70 ± 0.17	8	8.81 ± 0.40
BW	104	3.33 ± 0.08	8	3.73 ± 0.16
PL	101	0.282 ± 0.005	8	0.279 ± 0.016
PW	101	0.264 ± 0.005	8	0.271 ± 0.011
OL	101	0.319 ± 0.008	7	0.320 ± 0.016
OW	101	0.354 ± 0.008	7	0.357 ± 0.013
SRL	85	0.292 ± 0.011	7	0.274 ± 0.028
SRW	85	0.398 ± 0.013	7	0.445 ± 0.034
ATL	100	1.094 ± 0.049	7	1.337 ± 0.084
ATW	100	0.383 ± 0.020	7	0.450 ± 0.080
PTL	98	0.609 ± 0.022	7	0.633 ± 0.054
PTW	98	0.995 ± 0.040	7	1.471 ± 0.055
PA	101	0.560 ± 0.011	7	0.512 ± 0.047
OA	98	6.46 ± 0.13	7	6.03 ± 0.56
SRA	88	6.79 ± 0.14	7	6.78 ± 0.34
ATA	97	6.24 ± 0.13	7	6.16 ± 0.34
PTA	97	7.27 ± 0.15	7	7.40 ± 0.40
NID	93	11.6 ± 0.1	7	11.4 ± 0.4
OES	96	0.086 ± 0.006	7	0.062 ± 0.011

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 3-3-5. Numerical variables of mature specimens of *Typhlocoelum* obtained from lesser scaup and redheads from southern Québec and southern Manitoba.

NUMERICAL VARIABLE*	LESSER SCAUP n	LESSER SCAUP MEAN (mm) ± 95% C.L.	REDHEAD n	REDHEAD MEAN (mm) ± 95% C.L.
BL	16	6.83 ± 0.31	26	8.82 ± 0.35
BW	17	2.92 ± 0.10	27	3.43 ± 0.18
PL	15	0.234 ± 0.006	24	0.280 ± 0.005
PW	16	0.227 ± 0.009	24	0.251 ± 0.007
OL	16	0.266 ± 0.016	25	0.329 ± 0.018
OW	16	0.281 ± 0.020	25	0.354 ± 0.017
SRL	11	0.219 ± 0.021	23	0.294 ± 0.031
SRW	11	0.387 ± 0.024	23	0.378 ± 0.028
ATL	15	0.958 ± 0.051	24	0.951 ± 0.081
ATW	15	0.232 ± 0.027	24	0.379 ± 0.030
PTL	16	0.426 ± 0.021	25	0.569 ± 0.061
PTW	16	0.851 ± 0.046	25	1.011 ± 0.077
PA	16	0.500 ± 0.021	24	0.562 ± 0.020
OA	15	5.10 ± 0.23	23	6.78 ± 0.31
SRA	10	5.71 ± 0.23	21	7.19 ± 0.33
ATA	15	4.85 ± 0.21	22	6.63 ± 0.33
PTA	15	5.69 ± 0.26	23	7.55 ± 0.34
NID	13	11.6 ± 0.4	21	11.9 ± 0.2
OES	14	0.087 ± 0.011	21	0.075 ± 0.012

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

APPENDIX 3-4. RESULTS FROM ANALYSES OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TESTS ON NUMERICAL VARIABLES OF SPECIMENS FROM VARIOUS SPECIES OF WILD DUCKS.

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Appendix 3-4-1. Body length of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN BODY LENGTH (mm) **
RED †	26	8.82
GSC †	8	8.81
CAN †	102	8.70
GWT	4	8.61
BLA	7	8.36
SHO	14	8.27
MAL	126	8.00
BWT	16	7.68
PIN	19	7.54
LSC †	16	6.83

$F = 4.98$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-2. Body width of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN BODY WIDTH (mm) **
GSC †	8	3.73
GWT	7	3.50
BLA	8	3.50
RED †	27	3.43
MAL	134	3.40
CAN †	104	3.33
PIN	19	3.11
BWT	22	2.97
LSC †	17	2.92
SHO	15	2.76

$F = 3.96$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-3. Pharynx length of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN PHARYNX LENGTH (mm) **
BLA	8	0.326
MAL	127	0.318
CAN †	101	0.282
PIN	19	0.281
RED †	24	0.280
GSC †	8	0.279
SHO	14	0.278
GWT	5	0.274
BWT	18	0.262
LSC †	15	0.234

$F = 10.97$; $df = 9$; $P > F = 0.0001$

*. The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-4. Pharynx width of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN PHARYNX WIDTH (mm) **
BLA	8	0.314
GWT	5	0.302
MAL	127	0.297
SHO	14	0.288
BWT	18	0.286
GSC †	8	0.271
CAN †	101	0.264
PIN	19	0.256
RED †	24	0.251
LSC †	16	0.227

F = 7.14; df = 9; P > F = 0.0001

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-5. Ovary length of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN OVARY LENGTH (mm) **
RED †	25	0.330
GWT	6	0.326
GSC †	7	0.320
CAN †	101	0.319
MAL	123	0.286
BLA	7	0.285
PIN	18	0.278
BWT	21	0.275
SHO	14	0.273
LSC †	16	0.266

$F = 3.37$; $df = 9$; $P > F = 0.0006$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-6. Ovary width of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN OVARY WIDTH (mm) **
GWT	6	0.370
GSC †	7	0.357
RED †	25	0.354
CAN †	101	0.354
MAL	123	0.323
BWT	21	0.322
SHO	14	0.310
BLA	7	0.301
PIN	18	0.295
LSC †	16	0.281

$F = 3.73$; $df = 9$; $P > F = 0.0002$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-7. Seminal receptacle length of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN SEMINAL RECEPTACLE LENGTH (mm) **
BLA	4	0.356
RED †	23	0.294
CAN †	85	0.292
MAL	95	0.283
GSC †	7	0.274
BWT	14	0.257
GWT	4	0.230
LSC †	11	0.219
SHO	13	0.209
PIN	12	0.190

F = 2.85; df = 9; P > F = 0.0033

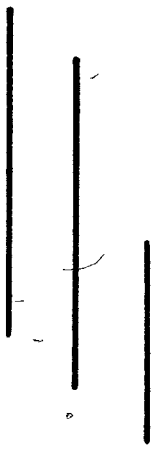
* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-8. Seminal receptacle width of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN SEMINAL RECEPTACLE WIDTH (mm) **
GSC †	7	0.445
BLA	4	0.409
CAN †	85	0.398
LSC †	11	0.387
RED †	23	0.378
GWT	4	0.363
BWT	14	0.350
SHO	13	0.346
MAL	95	0.330
PIN	12	0.258



$F = 3.72$; $df = 9$; $P > F = 0.0002$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-9. Anterior testis width of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN ANTERIOR TESTIS WIDTH (mm) **
GSC †	7	0.450
CAN †	100	0.383
RED †	24	0.379
BWT	17	0.356
GWT	5	0.316
MAL	113	0.276
SHO	15	0.271
BLA	6	0.262
PIN	17	0.249
LSC †	15	0.232

$F = 6.00$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-10. Posterior testis length of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN POSTERIOR TESTIS LENGTH (mm) **
GSC †	7	0.633
CAN †	98	0.609
RED †	25	0.569
LSC †	16	0.426
BWT	19	0.314
GWT	5	0.293
MAL	123	0.267
BLA	6	0.256
SHO	15	0.248
PIN	18	0.242

$F = 29.41$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-11. Distance from pharynx to anterior in mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN DISTANCE FROM PHARYNX TO ANTERIOR (mm) **
BLA	8	0.636
MAL	127	0.634
RED †	24	0.562
CAN †	101	0.560
PIN	19	0.557
GWT	4	0.550
BWT	17	0.541
SHO	13	0.520
GSC †	7	0.512
LSC †	16	0.500

$F = 6.85$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-12. Distance from ovary to pharynx in mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN DISTANCE FROM OVARY TO PHARYNX (mm) **
GWT	4	7.20
RED †	23	6.78
SHO	14	6.59
CAN †	98	6.46
BWT	15	6.22
BLA	7	6.16
GSC †	7	6.03
MAL	122	5.93
PIN	18	5.45
LSC †	15	5.10

$F = 4.98$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-13. Distance from seminal receptacle to pharynx in mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN DISTANCE FROM SEMINAL RECEPTACLE TO PHARYNX (mm) **
GWT	2	9.06
RED †	21	7.19
CAN †	88	6.79
GSC †	7	6.78
SHO	13	6.75
BLA	6	6.70
BWT	12	6.60
MAL	94	6.34
LSC †	10	5.71
PIN	14	5.66

$F = 4.59$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-14. Distance from anterior testis to pharynx in mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN DISTANCE FROM ANTERIOR TESTIS TO PHARYNX (mm) **
GWT	4	6.93
RED †	22	6.63
CAN †	97	6.24
SHO	14	6.24
GSC †	7	6.16
BWT	15	5.82
BLA	6	5.81
MAL	114	5.64
LSC †	15	4.84
PIN	16	4.71

$F = 7.35$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-15. Distance from posterior testis to pharynx in mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN DISTANCE FROM POSTERIOR TESTIS TO PHARYNX (mm) **
GWT	3	8.09
RED †	23	7.55
GSC †	7	7.40
CAN †	97	7.27
SHO	14	7.11
BWT	15	6.75
BLA	7	6.69
MAL	120	6.48
PIN	18	5.92
LSC †	15	5.69

$F = 6.85$; $df = 9$; $P > F = 0.0001$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

Appendix 3-4-16. Oesophagus length of mature *Typhlocoelum* obtained from various species of wild ducks.

HOST SPECIES*	n	MEAN OESOPHAGUS LENGTH (mm) **
BLA	8	0.183
GWT	5	0.121
BWT	14	0.120
PIN	19	0.119
SHO	12	0.096
MAL	106	0.094
LSC †	14	0.087
CAN †	96	0.086
RED †	21	0.075
GSC †	7	0.062

$F = 2.39$; $df = 9$; $P > F = 0.0128$

* The abbreviations for host species are explained in the "List of Abbreviations".

** Means connected with the same line are not significantly different.

† Diving duck species.

APPENDIX 3-5. DATA ON CHARACTER VARIABLES FOR *Typhlocoelum* OBTAINED FROM VARIOUS SPECIES OF WILD DUCKS

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A.

HOST SPECIES	ANTERIOR TESTIS SHAPE ROUND	LOBED
DABBLING DUCKS	181	0
DIVING DUCKS	10	138

$\chi^2 = 290.71$; df = 1; $P < 0.001$

B.

HOST SPECIES	POSTERIOR TESTIS SHAPE ROUND	LOBED
DABBLING DUCKS	187	0
DIVING DUCKS	10	139

$\chi^2 = 297.54$; df = 1; $P < 0.001$

Appendix 3-5-2. Number of mature *Typhlocoelum* obtained from various host species demonstrating various character states for uterine loops between testes.

HOST SPECIES*	UTERINE LOOPS BETWEEN TESTES: SEVERAL	ONE	NONE
BLA	7	0	0
BWT	19	1	0
GWT	4	0	1
MAL	122	2	1
PIN	20	0	0
SHO	15	0	0
CAN †	11	16	74
GSC †	1	5	1
LSC †	3	4	9
RED †	2	2	21
DABBLING DUCKS	187	3	2
DIVING DUCKS	17	27	105

$\chi^2 = 258.71$; df = 2; P = 0.0001

* The abbreviations for host species are explained in the "List of Abbreviations".

† Diving duck species.

Appendix 3-5-3. Number of mature *Typhlocoelum* obtained from various host species demonstrating various character states for anterior extent of vitellaria.

HOST SPECIES*	ANTERIOR EXTENT OF VITELLARIA: INTESTINAL BIFURCATION	PHARYNX	ASYMMETRICAL
BLA	0	7	1
BWT	1	15	1
GWT	2	3	0
MAL	10	111	5
PIN	3	16	0
SHO	1	12	1
CAN †	11	81	6
GSC †	0	7	0
LSC †	5	5	2
RED †	0	24	1
DABBLING DUCKS	17	164	8
DIVING DUCKS	16	117	9

$\chi^2 = 1.303$; df = 2; P = 0.5213

* The abbreviations for host species are explained in the "List of Abbreviations".

† Diving duck species.

Appendix 3-5-4. Number of mature *Typhlocoelum* obtained from various host species demonstrating various character states for posterior extent of vitellaria.

	POSTERIOR EXTENT OF VITELLARIA:		
HOST SPECIES*	INTESTINAL ARC	EXCRETORY BLADDER	ASYMMETRICAL
BLA	2	5	0
BWT	4	18	0
GWT	4	1	0
MAL	22	94	11
PIN	6	11	3
SHO	2	11	2
CAN †	49	35	13
GSC †	5	2	0
LSC †	10	3	1
RED †	8	11	6
DABBLING DUCKS	40	140	16
DIVING DUCKS	72	51	20

$\chi^2 = 43.844$; df = 2; P = 0.0001

* The abbreviations for host species are explained in the "List of Abbreviations".

† Diving duck species.

Appendix 3-5-5. Number of mature *Typhlocoelum* obtained from various host species demonstrating various character states for ventral surface topography.

	VENTRAL SURFACE TOPOGRAPHY:		
HOST SPECIES*	NO INDENTATIONS	SMALL INDENTATIONS	LARGE INDENTATIONS
BLA	0	0	8
BWT	4	5	15
GWT	0	3	3
MAL	4	4	125
PIN	1	4	15
SHO	4	6	5
CAN †	39	49	15
GSC †	4	1	1
LSC †	9	5	2
RED †	12	11	3
DABBLING DUCKS	13	22	171
DIVING DUCKS	64	66	21

$\chi^2 = 168.49$; df = 2; P = 0.0001

* The abbreviations for host species are explained in the "List of Abbreviations".

† Diving duck species.

Appendix 3-5-6. Number of mature *Typhlocoelum* obtained from various host species demonstrating various character states for position of genital pore.

	POSITION OF GENITAL PORE:		
HOST SPECIES*	ANTERIOR TO MID-PHARYNX	MID-PHARYNX	POSTERIOR TO MID-PHARYNX
BLA	2	0	0
BWT	1	0	0
MAL	21	1	0
PIN	1	1	1
SHO	0	1	3
CAN †	2	11	8
RED †	1	1	1
DABBLING DUCKS	25	3	4
DIVING DUCKS	3	12	9

$\chi^2 = 24.00$; df = 2; P = 0.0001

* The abbreviations for host species are explained in the "List of Abbreviations".

† Diving duck species.

Appendix 3-5-7. Number of mature *Typhlocoelum* obtained from various host species demonstrating various character states for posterior extent of cirrus sac.

HOST SPECIES*	POSTERIOR EXTENT OF CIRRUS SAC: POSTERIOR TO INTESTINAL BIFURCATION	NOT POSTERIOR TO INTESTINAL BIFURCATION
BLA	1	0
BWT	2	0
MAL	12	3
PIN	4	1
SHO	4	0
CAN †	49	1
GSC †	5	0
LSC †	3	0
RED †	8	0
DABBLING DUCKS	23	4
DIVING DUCKS	65	1

P = 0.0477

* The abbreviations for host species are explained in the "List of Abbreviations".

† Diving duck species.

Appendix 3-5-8. Number of mature *Typhlocoelum* obtained from various host species demonstrating various numbers of inner diverticula on the intestinal caeca.

HOST SPECIES*	NUMBER OF INNER DIVERTICULA: 8 - 11	12 - 15
MAL	66	7
PIN	10	1
SHO	11	4
CAN †	94	109
GSC †	4	8
LSC †	15	21
RED †	7	18
$\chi^2 = 91.35$; df = 6; $P < 0.001$		
DABBLING DUCKS	103	14
DIVING DUCKS	122	157
$\chi^2_{adj} = 65.95$; df = 1; $P < 0.001$		

* The abbreviations for host species are explained in the "List of Abbreviations".

† Diving duck species.

APPENDIX 6-1. NUMERICAL VARIABLES OF SPECIMENS OF *Typhlocoelum cucumerinum sisowi* RECOVERED FROM VARIOUS SITES IN MALLARDS AT VARIOUS DAYS POST-INFECTION.

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Appendix 6-1-1. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum sisowi* recovered from various sites in mallards at (A) 1 day post-infection and (B) 3 days post-infection.

A.

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	ABDOMINAL CAVITY MEAN (mm) ± S.E.	LUNGS n	LUNGS MEAN (mm) ± S.E.
BL	28	0.220 ± 0.006	2	0.249 ± 0.057
BW	24	0.091 ± 0.003	1	0.127 -
PL	13	0.024 ± 0.001	1	0.034 -
PW	13	0.020 ± 0.001	1	0.031 -
PA	10	0.059 ± 0.002	-	- -
OSL	21	0.047 ± 0.001	1	0.062 -
OSW	21	0.049 ± 0.001	1	0.057 -
VSL	19	0.024 ± 0.001	-	- -
VSW	19	0.027 ± 0.001	-	- -
VSA	10	0.084 ± 0.006	-	- -

B.

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	ABDOMINAL CAVITY MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	ANTERIOR AIR SACS MEAN (mm) ± S.E.	LUNGS n	LUNGS MEAN (mm) ± S.E.
BL	18	0.319 ± 0.014	15	0.362 ± 0.016	2	0.370 ± 0.020
BW	18	0.151 ± 0.006	13	0.163 ± 0.011	1	0.195 -
PL	19	0.035 ± 0.001	13	0.038 ± 0.002	-	- -
PW	19	0.025 ± 0.001	13	0.032 ± 0.002	-	- -
PA	14	0.076 ± 0.003	-	- -	-	- -
OSL	20	0.057 ± 0.002	14	0.064 ± 0.003	1	0.075 -
OSW	20	0.063 ± 0.002	14	0.073 ± 0.003	1	0.083 -
VSL	18	0.045 ± 0.001	15	0.044 ± 0.003	-	- -
VSW	18	0.047 ± 0.001	15	0.047 ± 0.002	-	- -
VSA	14	0.113 ± 0.008	10	0.124 ± 0.006	-	- -

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-1-2. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum sisowi* recovered from various sites in mallards at 6 days post-infection.

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.	LUNGS n	MEAN (mm) ± S.E.
BL	5	0.562 ± 0.055	15	0.643 ± 0.030	4	0.716 ± 0.035
BW	5	0.299 ± 0.015	13	0.324 ± 0.009	4	0.377 ± 0.013
PL	3	0.064 ± 0.002	11	0.064 ± 0.003	2	0.066 ± 0.010
PW	3	0.058 ± 0.001	11	0.065 ± 0.002	2	0.074 ± 0.001
PA	3	0.109 ± 0.019	11	0.117 ± 0.006	2	0.120 ± 0.002
OSL	2	0.088 ± 0.011	11	0.097 ± 0.003	2	0.094 ± 0.010
OSW	2	0.096 ± 0.002	11	0.096 ± 0.007	2	0.104 ± 0.024
VSL	5	0.070 ± 0.005	13	0.074 ± 0.003	4	0.081 ± 0.004
VSW	5	0.071 ± 0.002	13	0.082 ± 0.004	4	0.085 ± 0.006
VSA	3	0.231 ± 0.021	9	0.231 ± 0.016	2	0.225 -

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-1-3. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum sisowi* recovered from various sites in mallards at 12 days post-infection.

NUMERICAL	ABDOMINAL CAVITY		POSTERIOR AIR SACS		ANTERIOR AIR SACS		LUNGS	
VARIABLE*	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.
BL	10	0.838 ± 0.127	6	0.912 ± 0.127	16	0.963 ± 0.061	3	1.380 ± 0.119
BW	10	0.461 ± 0.057	6	0.451 ± 0.034	16	0.527 ± 0.026	3	0.559 ± 0.023
PL	10	0.078 ± 0.007	6	0.081 ± 0.005	15	0.092 ± 0.004	3	0.109 ± 0.001
PW	10	0.072 ± 0.006	6	0.074 ± 0.005	15	0.086 ± 0.003	3	0.093 ± 0.008
PA	10	0.121 ± 0.012	6	0.121 ± 0.008	15	0.142 ± 0.009	3	0.182 ± 0.017
NID	8	10.1 ± 0.5	5	11.2 ± 0.4	13	10.0 ± 0.2	2	9.5 ± 0.5
OSL	8	0.095 ± 0.008	5	0.097 ± 0.002	8	0.122 ± 0.005	2	0.118 ± 0.012
OSW	10	0.109 ± 0.007	5	0.108 ± 0.005	10	0.129 ± 0.005	2	0.135 ± 0.009
VSL	10	0.073 ± 0.002	6	0.079 ± 0.003	15	0.077 ± 0.002	3	0.085 ± 0.006
VSW	10	0.077 ± 0.004	6	0.081 ± 0.004	15	0.088 ± 0.002	3	0.084 ± 0.006
VSA	10	0.306 ± 0.044	6	0.316 ± 0.052	15	0.313 ± 0.023	3	0.475 ± 0.048

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-1-4. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum sisowi* recovered from various sites in mallards at 21 days post-infection.

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	POSTERIOR AIR SACS n	MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.	LUNGS n	MEAN (mm) ± S.E.
BL	14	1.991 ± 0.116	2	2.187 ± 0.607	3	1.363 ± 0.095	7	2.852 ± 0.565
BW	14	0.840 ± 0.043	2	0.948 ± 0.266	3	0.522 ± 0.055	7	1.246 ± 0.204
PL	14	0.127 ± 0.007	2	0.156 ± 0.029	3	0.107 ± 0.010	7	0.160 ± 0.022
PW	14	0.122 ± 0.004	2	0.135 ± 0.021	3	0.094 ± 0.010	7	0.142 ± 0.018
OL	7	0.062 ± 0.011	1	0.107 -	-	- -	4	0.176 ± 0.023
OW	7	0.070 ± 0.018	1	0.127 -	-	- -	4	0.196 ± 0.012
SRL	4	0.048 ± 0.002	1	0.042 -	-	- -	4	0.139 ± 0.040
SRW	4	0.060 ± 0.009	1	0.040 -	-	- -	4	0.218 ± 0.117
ATL	7	0.087 ± 0.021	1	0.083 -	-	- -	4	0.137 ± 0.013
ATW	7	0.063 ± 0.014	1	0.112 -	-	- -	4	0.182 ± 0.006
PTL	7	0.080 ± 0.020	1	0.086 -	-	- -	4	0.142 ± 0.027
PTW	7	0.111 ± 0.028	1	0.104 -	-	- -	4	0.186 ± 0.016
EGGA	6	0.976 ± 0.064	-	- -	-	- -	1	2.810 -
PA	14	0.184 ± 0.007	2	0.241 ± 0.041	3	0.172 ± 0.009	7	0.330 ± 0.055
OA	7	1.373 ± 0.135	1	1.999 -	-	- -	2	2.035 ± 0.393
SRA	5	1.564 ± 0.095	1	2.106 -	-	- -	2	2.133 ± 0.437
ATA	7	1.344 ± 0.126	1	1.892 -	-	- -	3	2.023 ± 0.254
PTA	8	1.507 ± 0.129	1	2.320 -	-	- -	2	2.214 ± 0.500
NID	14	11.5 ± 0.4	2	12.0 ± 0.0	3	10.7 ± 0.3	5	11.0 ± 0.6
OES	14	0.030 ± 0.005	2	0.021 ± 0.021	3	0.004 ± 0.002	7	0.068 ± 0.017

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Appendix 6-1-4. ... CONTINUED ...

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	POSTERIOR AIR SACS n	MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.	LUNGS n	MEAN (mm) ± S.E.
OSL	4	0.109 ± 0.009	2	0.127 ± 0.028	2	0.108 ± 0.010	2	0.084 ± 0.006
OSW	4	0.127 ± 0.007	2	0.134 ± 0.025	2	0.099 ± 0.000	2	0.096 ± 0.022
VSL	6	0.064 ± 0.004	2	0.075 ± 0.000	2	0.068 ± 0.006	2	0.064 ± 0.004
VSW	6	0.081 ± 0.002	2	0.086 ± 0.002	2	0.074 ± 0.009	2	0.072 ± 0.002
VSA	6	0.597 ± 0.072	2	0.762 ± 0.212	2	0.500 ± 0.022	2	0.395 ± 0.000

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-1-5. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum sisowi* recovered from various sites in mallards at 30 days post-infection.

NUMERICAL	ABDOMINAL CAVITY		POSTERIOR AIR SACS		ANTERIOR AIR SACS		LUNGS		TRACHEA	
VARIABLE*	n	MEAN (mm) ± S.E.	n	MEAN (mm)	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.
BL	6	1.343 ± 0.224	1	3.360	13	1.196 ± 0.048	6	3.323 ± 1.040	9	5.09 ± 0.51
BW	6	0.739 ± 0.097	1	1.340	13	0.562 ± 0.019	7	1.601 ± 0.355	9	2.37 ± 0.17
PL	6	0.109 ± 0.013	1	0.211	13	0.094 ± 0.003	6	0.164 ± 0.032	9	0.247 ± 0.009
PW	6	0.097 ± 0.014	1	0.174	13	0.091 ± 0.004	6	0.147 ± 0.028	9	0.190 ± 0.011
OL	1	0.065 -	1	0.130	-	- -	4	0.254 ± 0.009	9	0.244 ± 0.016
OW	1	0.070 -	1	0.146	-	- -	4	0.270 ± 0.013	9	0.254 ± 0.019
SRL	1	0.038 -	1	0.070	-	- -	2	0.226 ± 0.000	6	0.175 ± 0.021
SRW	1	0.058 -	1	0.065	-	- -	2	0.268 ± 0.056	6	0.195 ± 0.030
ATL	1	0.039 -	1	0.107	-	- -	2	0.233 ± 0.021	9	0.190 ± 0.014
ATW	1	0.052 -	1	0.146	-	- -	2	0.246 ± 0.064	9	0.223 ± 0.025
PTL	1	0.065 -	1	0.143	-	- -	3	0.229 ± 0.013	9	0.172 ± 0.016
PTW	1	0.078 -	1	0.231	-	- -	3	0.302 ± 0.004	9	0.273 ± 0.034
EGGA	-	- -	-	-	-	- -	2	1.07 ± 1.07	8	0.60 ± 0.12
EYEA	-	- -	-	-	-	- -	-	- -	1	1.36 -
PA	6	0.157 ± 0.020	1	0.324	11	0.169 ± 0.013	8	0.328 ± 0.062	9	0.487 ± 0.028
OA	1	1.464 -	1	2.178	-	- -	3	3.899 ± 0.658	9	3.87 ± 0.31
SRA	-	- -	1	2.320	-	- -	2	4.740 ± 0.260	7	3.99 ± 0.41
ATA	1	1.357 -	1	2.142	-	- -	2	3.335 ± 0.765	9	3.71 ± 0.28
PTA	1	1.571 -	1	2.356	-	- -	3	4.249 ± 0.708	9	4.22 ± 0.34
NID	5	11.4 ± 0.2	1	11.0	10	10.6 ± 0.3	6	10.8 ± 0.2	7	10.6 ± 0.3

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Appendix 6-1-5.

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NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	POSTERIOR AIR SACS n	MEAN (mm)	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.	LUNGS n	MEAN (mm) ± S.E.	TRACHEA n	MEAN (mm) ± S.E.
OES	5	0.008 -	1	0.042	11	0.018 ± 0.004	6	0.049 ± 0.023	9	0.147 ± 0.054
OSL	3	0.105 ± 0.009	1	0.070	11	0.118 ± 0.004	3	0.112 ± 0.021	-	- -
OSW	3	0.114 ± 0.013	1	0.127	11	0.124 ± 0.005	3	0.154 ± 0.042	-	- -
VSL	5	0.066 ± 0.004	-	-	12	0.072 ± 0.003	4	0.054 ± 0.007	-	- -
VSW	5	0.067 ± 0.005	-	-	12	0.080 ± 0.002	4	0.069 ± 0.011	-	- -
VSA	5	0.426 ± 0.065	-	-	13	0.440 ± 0.018	4	0.540 ± 0.114	-	- -

* The abbreviations for numerical varibles are described in the "List of Abbreviations".

Appendix 6-1-6. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum sisowi* recovered from various sites in mallards at 40 days post-infection.

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.	LUNGS n	MEAN (mm) ± S.E.	TRACHEA n	MEAN (mm) ± S.E.
BL	4	1.220 ± 0.071	6	1.177 ± 0.069	3	1.274 ± 0.005	17	6.73 ± 0.71
BW	4	0.747 ± 0.116	6	0.738 ± 0.041	3	0.635 ± 0.061	17	2.67 ± 0.27
PL	4	0.104 ± 0.005	6	0.100 ± 0.006	3	0.095 ± 0.005	17	0.234 ± 0.017
PW	4	0.090 ± 0.001	6	0.110 ± 0.022	3	0.090 ± 0.006	17	0.220 ± 0.016
OL	-	- -	-	- -	-	- -	14	0.273 ± 0.015
OW	-	- -	-	- -	-	- -	14	0.314 ± 0.017
SRL	-	- -	-	- -	-	- -	14	0.262 ± 0.028
SRW	-	- -	-	- -	-	- -	14	0.367 ± 0.024
ATL	-	- -	-	- -	-	- -	14	0.250 ± 0.014
ATW	-	- -	-	- -	-	- -	14	0.296 ± 0.020
PTL	-	- -	-	- -	-	- -	14	0.295 ± 0.022
PTW	-	- -	-	- -	-	- -	14	0.381 ± 0.024
EGGA	-	- -	-	- -	-	- -	13	0.000 ± 0.000
EYEA	-	- -	-	- -	-	- -	12	3.14 ± 0.22
PA	4	0.150 ± 0.008	6	0.169 ± 0.010	3	0.168 ± 0.017	17	0.542 ± 0.051
OA	-	- -	-	- -	-	- -	14	5.70 ± 0.26
SRA	-	- -	-	- -	-	- -	14	5.94 ± 0.27
ATA	-	- -	-	- -	-	- -	14	5.48 ± 0.26
PTA	-	- -	-	- -	-	- -	14	6.22 ± 0.29

... CONTINUED ...

Appendix 6-1-6. ... CONTINUED ...

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.	LUNGS n	MEAN (mm) ± S.E.	TRACHEA n	MEAN (mm) ± S.E.
NID	4	10.2 ± 0.5	5	9.8 ± 0.7	2	9.5 ± 0.5	14	9.9 ± 0.2
OES	4	0.021 ± 0.002	6	0.023 ± 0.005	3	0.021 ± 0.014	17	0.073 ± 0.017
OSL	4	0.100 ± 0.007	6	0.118 ± 0.004	2	0.114 ± 0.006	2	0.117 ± 0.009
OSW	4	0.134 ± 0.005	6	0.122 ± 0.005	2	0.119 ± 0.003	2	0.118 ± 0.002
VSL	4	0.065 ± 0.002	6	0.070 ± 0.003	3	0.064 ± 0.001	3	0.066 ± 0.007
VSW	4	0.071 ± 0.005	6	0.084 ± 0.002	3	0.076 ± 0.006	3	0.077 ± 0.002
VSA	4	0.409 ± 0.006	6	0.423 ± 0.043	3	0.475 ± 0.031	3	0.380 ± 0.042

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-1-7. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum sisowi* recovered from various sites in mallards at 45 days post-infection.

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.	TRACHEA n	MEAN (mm) ± S.E.
BL	2	1.392 ± 0.072	4	1.344 ± 0.052	13	7.92 ± 0.204
BW	2	0.634 ± 0.107	4	0.642 ± 0.037	13	2.97 ± 0.079
PL	2	0.091 ± 0.011	4	0.099 ± 0.006	13	0.254 ± 0.007
PW	2	0.108 ± 0.028	4	0.095 ± 0.005	13	0.260 ± 0.005
OL	-	-	-	-	12	0.271 ± 0.015
OW	-	-	-	-	12	0.314 ± 0.012
SRL	-	-	-	-	9	0.224 ± 0.022
SRW	-	-	-	-	9	0.392 ± 0.024
ATL	-	-	-	-	13	0.354 ± 0.042
ATW	-	-	-	-	13	0.399 ± 0.028
PTL	-	-	-	-	12	0.333 ± 0.021
PTW	-	-	-	-	12	0.583 ± 0.043
EGGA	-	-	-	-	13	0.17 ± 0.09
EYEA	-	-	-	-	11	3.01 ± 0.19
PA	2	0.166 ± 0.002	4	0.156 ± 0.009	13	0.573 ± 0.010
OA	-	-	-	-	12	5.99 ± 0.16
SRA	-	-	-	-	10	6.31 ± 0.14
ATA	-	-	-	-	13	5.58 ± 0.14
PTA	-	-	-	-	12	6.38 ± 0.17
NID	2	10.0 ± 0.0	4	9.8 ± 0.5	9	10.4 ± 0.2
OES	2	0.023 ± 0.005	4	0.029 ± 0.005	9	0.091 ± 0.038
OSL	-	-	2	0.099 ± 0.013	-	-
OSW	-	-	2	0.126 ± 0.000	-	-
VSL	2	0.067 ± 0.007	4	0.064 ± 0.004	-	-
VSW	2	0.082 ± 0.012	4	0.082 ± 0.004	-	-
VSA	2	0.486 ± 0.022	4	0.508 ± 0.039	-	-

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

APPENDIX 6-2. NUMBER OF *Typhlocoelum cucumerinum sisowi* RECOVERED FROM MALLARDS AT VARIOUS DAYS POST-INFECTION DEMONSTRATING VARIOUS CHARACTER STATES FOR VARIOUS CHARACTER VARIABLES.

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Appendix 6-2-2. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for uterine loops between testes. 315

Appendix 6-2-3. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for anterior extent of vitellaria. 316

Appendix 6-2-4. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for posterior extent of vitellaria. 316

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Appendix 6-2-6. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for position of genital pore 318

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Appendix 6-2-1. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for the shape of the testes.

	TESTIS SHAPE:		
DAYS POST-INFECTION	ROUND	LOOSELY-LOBED	DEEPLY-LOBED
21	15	0	0
30	13	0	0
40	14	0	0
45	13	0	0

Appendix 6-2-2. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for uterine loops between testes.

	UTERINE LOOPS BETWEEN TESTES:		
DAYS POST-INFECTION	SEVERAL	ONE	NONE
21	6	1	0
30	11	0	0
40	12	1	0
45	13	0	0

Appendix 6-2-3. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for anterior extent of vitellaria.

	ANTERIOR EXTENT OF VITELLARIA:		
DAYS POST-INFECTION	INTESTINAL BIFURCATION	PHARYNX	ASYMMETRICAL
21	0	2	1
30	0	11	0
40	2	11	0
45	0	13	0

Appendix 6-2-4. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for posterior extent of vitellaria.

	POSTERIOR EXTENT OF VITELLARIA:		
DAYS POST-INFECTION	INTESTINAL ARC	EXCRETORY BLADDER	ASYMMETRICAL
30	0	10	0
40	2	9	2
45	2	11	0

Appendix 6-2-5. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for ventral surface topography.

	VENTRAL SURFACE TOPOGRAPHY:		
DAYS POST-INFECTION	NO INDENTATIONS	SMALL INDENTATIONS	LARGE INDENTATIONS
1	30	0	0
3	39	0	0
6	20	7	0
12	28	5	2
21	9	11	3
30	5	4	9
40	5	2	10
45	0	7	6

Appendix 6-2-6. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for position of genital pore.

	POSITION OF GENITAL PORE:		
DAYS POST-INFECTION	ANTERIOR TO MID-PHARYNX	MID-PHARYNX	POSTERIOR TO MID-PHARYNX
21	0	0	1
30	5	1	0
40	1	0	1
45	3	0	0

Appendix 6-2-7. Number of *Typhlocoelum cucumerinum sisowi* recovered from mallards at various days post-infection demonstrating various character states for posterior extent of cirrus sac.

	POSTERIOR EXTENT OF CIRRUS SAC:	
DAYS POST-INFECTION	POSTERIOR TO INTESTINAL BIFURCATION	NOT POSTERIOR TO INTESTINAL BIFURCATION
21	2	0
30	3	0
45	1	3

APPENDIX 6-3. NUMERICAL VARIABLES OF SPECIMENS OF *Typhlocoelum cucumerinum cucumerinum* RECOVERED FROM VARIOUS SITES IN CANVASBACKS AT VARIOUS DAYS POST-INFECTION.

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Appendix 6-3-1. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 1 day post-infection and 3 days post-infection. 320

Appendix 6-3-2. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 6 days post-infection 321

Appendix 6-3-3. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 12 days post-infection. 322

Appendix 6-3-4. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 21 days post-infection. 323

Appendix 6-3-5. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 30 days post-infection. 325

Appendix 6-3-6. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 52 days post-infection. 327

Appendix 6-3-1. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at (A) 1 day post-infection and (B) 3 days post-infection.

A.

NUMERICAL VARIABLE*	n	ABDOMINAL CAVITY MEAN (mm) ± S.E.
BL	6	0.196 ± 0.007
BW	6	0.095 ± 0.009
PL	6	0.022 ± 0.001
PW	6	0.017 ± 0.001
PA	6	0.056 ± 0.001
OSL	6	0.042 ± 0.002
OSW	6	0.044 ± 0.002
VSL	5	0.026 ± 0.001
VSW	5	0.029 ± 0.002
VSA	6	0.065 ± 0.003

B.

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	ABDOMINAL CAVITY MEAN (mm) ± S.E.	POSTERIOR AIR SACS n	POSTERIOR AIR SACS MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	ANTERIOR AIR SACS MEAN (mm)
BL	51	0.299 ± 0.006	9	0.314 ± 0.009	1	0.324
BW	44	0.144 ± 0.003	5	0.140 ± 0.007	-	-
PL	35	0.032 ± 0.001	3	0.036 ± 0.003	-	-
PW	35	0.024 ± 0.000	3	0.031 ± 0.001	-	-
PA	29	0.072 ± 0.002	3	0.080 ± 0.007	-	-
OSL	42	0.058 ± 0.001	5	0.057 ± 0.002	-	-
OSW	41	0.061 ± 0.001	5	0.062 ± 0.003	-	-
VSL	44	0.050 ± 0.001	6	0.050 ± 0.002	-	-
VSW	44	0.051 ± 0.001	6	0.053 ± 0.004	-	-
VSA	34	0.101 ± 0.002	3	0.113 ± 0.006	-	-

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-3-2. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 6 days post-infection.

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	POSTERIOR AIR SACS n	MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.
BL	4	0.624 ± 0.088	12	0.654 ± 0.039	4	0.670 ± 0.043
BW	4	0.276 ± 0.016	12	0.318 ± 0.019	4	0.292 ± 0.019
PL	4	0.052 ± 0.008	12	0.055 ± 0.002	4	0.057 ± 0.003
PW	4	0.046 ± 0.004	12	0.044 ± 0.002	4	0.046 ± 0.003
PA	3	0.129 ± 0.017	12	0.114 ± 0.007	4	0.121 ± 0.007
OL	4	0.091 ± 0.014	12	0.090 ± 0.004	4	0.090 ± 0.003
OW	4	0.088 ± 0.006	12	0.096 ± 0.003	4	0.094 ± 0.008
VSL	4	0.086 ± 0.009	12	0.093 ± 0.004	4	0.099 ± 0.004
VSW	4	0.092 ± 0.006	12	0.099 ± 0.003	4	0.100 ± 0.003
VSA	4	0.192 ± 0.031	12	0.213 ± 0.011	4	0.229 ± 0.012

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-3-3. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 12 days post-infection.

NUMERICAL	ABDOMINAL CAVITY		POSTERIOR AIR SACS		ANTERIOR AIR SACS		LUNGS		TRACHEA	
VARIABLE*	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm)
BL	20	1.469 ± 0.065	38	1.407 ± 0.042	5	1.642 ± 0.106	3	1.440 ± 0.031	1	1.321
BW	20	0.618 ± 0.024	38	0.604 ± 0.014	5	0.684 ± 0.056	3	0.653 ± 0.069	1	0.564
PL	15	0.085 ± 0.002	35	0.089 ± 0.001	5	0.096 ± 0.003	3	0.094 ± 0.002	1	0.084
PW	15	0.069 ± 0.003	34	0.075 ± 0.002	5	0.077 ± 0.004	3	0.077 ± 0.002	1	0.077
OL	14	0.030 ± 0.006	24	0.022 ± 0.001	4	0.028 ± 0.003	3	0.023 ± 0.002	1	0.021
OW	14	0.034 ± 0.008	24	0.026 ± 0.001	4	0.034 ± 0.002	3	0.034 ± 0.004	1	0.024
SRL	14	0.026 ± 0.001	29	0.028 ± 0.001	5	0.027 ± 0.002	3	0.025 ± 0.003	1	0.024
SRW	14	0.039 ± 0.003	29	0.040 ± 0.001	5	0.045 ± 0.005	3	0.034 ± 0.003	1	0.028
PA	14	0.189 ± 0.009	35	0.177 ± 0.006	5	0.207 ± 0.017	3	0.184 ± 0.006	1	0.161
OA	11	0.956 ± 0.057	25	0.958 ± 0.031	4	1.198 ± 0.064	3	1.010 ± 0.026	1	1.043
SRA	11	0.988 ± 0.059	30	0.999 ± 0.031	5	1.179 ± 0.073	3	1.034 ± 0.029	1	1.072
NID	19	11.5 ± 0.2	36	11.4 ± 0.1	5	11.8 ± 0.2	3	11.0 ± 0.0	1	11.0
CL	6	0.092 ± 0.006	10	0.101 ± 0.005	1	0.102 -	2	0.114 ± 0.026	1	0.112
OSL	13	0.110 ± 0.005	30	0.112 ± 0.003	3	0.122 ± 0.004	2	0.116 ± 0.004	1	0.098
OSW	13	0.103 ± 0.004	30	0.108 ± 0.002	3	0.108 ± 0.008	2	0.103 ± 0.009	1	0.112
VSL	19	0.109 ± 0.003	37	0.104 ± 0.002	5	0.113 ± 0.003	3	0.110 ± 0.003	1	0.126
VSW	19	0.118 ± 0.003	37	0.115 ± 0.002	5	0.121 ± 0.001	3	0.113 ± 0.007	1	0.126
VSA	17	0.483 ± 0.023	38	0.456 ± 0.013	5	0.524 ± 0.028	3	0.517 ± 0.021	1	0.550

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-3-4. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 21 days post-infection.

NUMERICAL	ABDOMINAL CAVITY		POSTERIOR AIR SACS		ANTERIOR AIR SACS		TRACHEA	
VARIABLE*	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.
BL	2	2.336 ± 1.364	6	2.777 ± 0.234	18	3.351 ± 0.135	31	2.765 ± 0.115
BW	2	1.116 ± 0.384	6	1.320 ± 0.063	19	1.505 ± 0.043	31	1.484 ± 0.026
PL	2	0.114 ± 0.026	6	0.144 ± 0.002	18	0.147 ± 0.003	30	0.148 ± 0.003
PW	2	0.092 ± 0.022	6	0.114 ± 0.006	18	0.128 ± 0.002	30	0.117 ± 0.003
OL	1	0.052 -	6	0.060 ± 0.004	18	0.073 ± 0.003	28	0.059 ± 0.003
OW	1	0.088 -	6	0.068 ± 0.007	18	0.096 ± 0.005	28	0.086 ± 0.004
SRL	1	0.063 -	6	0.054 ± 0.003	18	0.076 ± 0.013	28	0.052 ± 0.002
SRW	1	0.158 -	6	0.149 ± 0.013	18	0.151 ± 0.018	28	0.131 ± 0.006
ATL	1	0.409 -	5	0.237 ± 0.031	14	0.357 ± 0.018	25	0.242 ± 0.018
ATW	1	0.169 -	5	0.064 ± 0.015	14	0.127 ± 0.008	25	0.135 ± 0.015
PTL	1	0.240 -	6	0.179 ± 0.031	15	0.220 ± 0.011	26	0.154 ± 0.011
PTW	1	0.409 -	6	0.282 ± 0.027	15	0.338 ± 0.019	26	0.377 ± 0.013
PA	2	0.256 ± 0.126	6	0.308 ± 0.013	18	0.370 ± 0.014	30	0.329 ± 0.010
OA	1	2.42 -	6	1.904 ± 0.172	17	2.276 ± 0.096	28	1.865 ± 0.084
SRA	1	2.50 -	6	1.976 ± 0.176	17	2.358 ± 0.099	29	1.927 ± 0.084
ATA	1	2.34 -	5	1.925 ± 0.166	14	2.231 ± 0.109	29	1.829 ± 0.079
PTA	1	2.77 -	6	2.128 ± 0.187	15	2.605 ± 0.122	29	2.073 ± 0.094
NID	2	12.0 ± 0.0	6	12.0 ± 0.4	17	12.0 ± 0.2	31	11.6 ± 0.2

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Appendix 6-3-4. ... CONTINUED ...

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	POSTERIOR AIR SACS n	MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.	TRACHEA n	MEAN (mm) ± S.E.
OES	2	0.040 ± 0.016	6	0.047 ± 0.013	17	0.057 ± 0.005	30	0.047 ± 0.004
CL	1	0.254 -	4	0.201 ± 0.023	16	0.251 ± 0.012	28	0.217 ± 0.009
OSL	-	- -	-	- -	-	- -	2	0.160 ± 0.010
OSW	-	- -	-	- -	-	- -	2	0.138 ± 0.016
VSL	2	0.080 ± 0.018	1	0.085 -	18	0.098 ± 0.003	27	0.083 ± 0.001
VSW	2	0.100 ± 0.008	1	0.099 -	18	0.111 ± 0.002	27	0.111 ± 0.003
VSA	2	0.677 ± 0.310	1	0.818 -	18	0.992 ± 0.039	28	0.811 ± 0.033

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-3-5. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 30 days post-infection.

NUMERICAL	ABDOMINAL CAVITY		POSTERIOR AIR SACS		ANTERIOR AIR SACS		LUNGS		TRACHEA	
VARIABLE*	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.
BL	4	3.915 ± 0.488	4	4.735 ± 0.190	16	4.160 ± 0.214	26	4.693 ± 0.254	80	4.058 ± 0.078
BW	4	1.729 ± 0.139	4	2.115 ± 0.027	16	1.933 ± 0.082	26	2.020 ± 0.050	80	1.913 ± 0.035
PL	4	0.162 ± 0.013	4	0.171 ± 0.008	16	0.166 ± 0.005	26	0.188 ± 0.003	79	0.169 ± 0.002
PW	4	0.130 ± 0.012	4	0.164 ± 0.003	16	0.148 ± 0.006	26	0.157 ± 0.003	79	0.148 ± 0.002
OL	3	0.147 ± 0.025	4	0.158 ± 0.010	16	0.116 ± 0.010	26	0.130 ± 0.006	80	0.137 ± 0.005
OW	3	0.127 ± 0.021	4	0.183 ± 0.130	16	0.146 ± 0.010	26	0.164 ± 0.008	80	0.156 ± 0.005
SRL	3	0.113 ± 0.029	4	0.096 ± 0.007	16	0.097 ± 0.009	26	0.098 ± 0.006	78	0.108 ± 0.005
SRW	3	0.202 ± 0.019	4	0.278 ± 0.009	16	0.229 ± 0.017	26	0.246 ± 0.011	78	0.229 ± 0.008
ATL	3	0.517 ± 0.066	4	0.599 ± 0.034	16	0.477 ± 0.044	26	0.496 ± 0.018	77	0.497 ± 0.020
ATW	3	0.202 ± 0.057	4	0.268 ± 0.033	16	0.214 ± 0.018	26	0.207 ± 0.014	77	0.195 ± 0.009
PTL	3	0.291 ± 0.056	4	0.338 ± 0.079	16	0.242 ± 0.016	26	0.263 ± 0.014	78	0.272 ± 0.009
PTW	3	0.409 ± 0.071	4	0.546 ± 0.059	16	0.479 ± 0.030	26	0.514 ± 0.019	78	0.469 ± 0.013
EGGA	-	- -	-	- -	-	- -	-	- -	1	0.282 -
PA	4	0.360 ± 0.041	4	0.331 ± 0.019	16	0.357 ± 0.025	25	0.400 ± 0.011	77	0.368 ± 0.007
OA	3	2.990 ± 0.430	4	3.422 ± 0.151	16	2.905 ± 0.160	26	3.033 ± 0.082	79	2.831 ± 0.057
SRA	3	3.110 ± 0.452	4	3.520 ± 0.161	16	3.015 ± 0.164	26	3.149 ± 0.085	78	2.955 ± 0.060
ATA	3	2.887 ± 0.402	4	3.332 ± 0.167	16	2.829 ± 0.162	26	2.952 ± 0.075	77	2.740 ± 0.057
PTA	3	3.313 ± 0.472	4	3.782 ± 0.182	16	3.258 ± 0.177	26	3.389 ± 0.086	77	3.192 ± 0.065

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Appendix 6-3-5. ... CONTINUED ...

NUMERICAL VARIABLE*	ABDOMINAL CAVITY n	MEAN (mm) ± S.E.	POSTERIOR AIR SACS n	MEAN (mm) ± S.E.	ANTERIOR AIR SACS n	MEAN (mm) ± S.E.	LUNGS n	MEAN (mm) ± S.E.	TRACHEA n	MEAN (mm) ± S.E.
NID	4	11.2 ± 0.5	4	11.8 ± 0.5	14	11.5 ± 0.3	19	11.3 ± 0.3	76	11.6 ± 0.1
OES	4	0.046 ± 0.009	4	0.044 ± 0.014	16	0.063 ± 0.008	26	0.075 ± 0.006	78	0.053 ± 0.003
CL	2	0.268 ± 0.028	1	0.332 -	14	0.276 ± 0.021	19	0.336 ± 0.015	60	0.276 ± 0.007
VSL	2	0.076 ± 0.002	-	- -	8	0.078 ± 0.002	1	0.049 -	23	0.074 ± 0.002
VSW	2	0.100 ± 0.012	-	- -	8	0.099 ± 0.004	1	0.091 -	23	0.094 ± 0.003
VSA	2	0.874 ± 0.113	-	- -	9	1.191 ± 0.097	1	1.114 -	23	0.995 ± 0.030

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

Appendix 6-3-6. Descriptive statistics of numerical variables of specimens of *Typhlocoelum cucumerinum cucumerinum* recovered from various sites in canvasbacks at 52 days post-infection.

NUMERICAL	ANTERIOR AIR SACS		LUNGS		TRACHEA	
VARIABLE*	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.	n	MEAN (mm) ± S.E.
BL	2	4.235 ± 0.135	6	5.797 ± 0.816	53	8.49 ± 0.349
BW	3	1.993 ± 0.145	6	2.520 ± 0.270	53	3.32 ± 0.122
PL	3	0.164 ± 0.012	6	0.198 ± 0.013	48	0.247 ± 0.007
PW	3	0.150 ± 0.005	6	0.174 ± 0.018	50	0.223 ± 0.007
OL	1	0.080 -	6	0.228 ± 0.053	51	0.297 ± 0.015
OW	1	0.119 -	6	0.301 ± 0.063	51	0.323 ± 0.016
SRL	-	- -	6	0.172 ± 0.028	49	0.242 ± 0.016
SRW	-	- -	6	0.329 ± 0.040	49	0.453 ± 0.028
ATL	-	- -	6	0.632 ± 0.223	49	1.146 ± 0.076
ATW	-	- -	6	0.301 ± 0.047	48	0.500 ± 0.030
PTL	-	- -	6	0.346 ± 0.092	50	0.662 ± 0.039
PTW	-	- -	6	0.708 ± 0.163	50	1.152 ± 0.072
EGGA	1	2.38 -	2	0.292 ± 0.292	45	1.11 ± 0.19
EYEA	-	- -	-	- -	14	4.67 ± 0.35
PA	3	0.442 ± 0.037	6	0.451 ± 0.017	50	0.597 ± 0.013
OA	-	- -	6	4.188 ± 0.652	51	6.29 ± 0.28
SRA	-	- -	6	4.328 ± 0.692	50	6.40 ± 0.28
ATA	-	- -	6	4.093 ± 0.621	49	6.14 ± 0.26
PTA	-	- -	6	4.663 ± 0.748	50	7.09 ± 0.31
NID	2	11.5 ± 1.5	5	11.4 ± 0.4	53	11.3 ± 0.1
OES	3	0.019 ± 0.011	6	0.080 ± 0.017	48	0.051 ± 0.011
CL	2	0.226 ± 0.070	5	0.383 ± 0.026	12	0.489 ± 0.037

* The abbreviations for numerical variables are explained in the "List of Abbreviations".

APPENDIX 6-4. NUMBER OF *Typhlocoelum cucumerinum cucumerinum* RECOVERED FROM CANVASBACKS AT VARIOUS DAYS POST-INFECTION DEMONSTRATING VARIOUS CHARACTER STATES FOR VARIOUS CHARACTER VARIABLES.

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Appendix 6-4-1. Number of *Typhlocoelum cucumerinum cucumerinum* recovered from canvasbacks at various days post-infection demonstrating various character states for the shape of the testes.

	TESTIS SHAPE:		
DAYS POST-INFECTION	ROUND	LOOSELY-LOBED	DEEPLY-LOBED
21	0	52	2
30	0	7	122
52	0	1	50

Appendix 6-4-2. Number of *Typhlocoelum cucumerinum cucumerinum* recovered from canvasbacks at various days post-infection demonstrating various character states for uterine loops between testes.

	UTERINE LOOPS BETWEEN TESTES:		
DAYS POST-INFECTION	SEVERAL	ONE	NONE
21	0	4	44
30	0	11	114
52	1	3	42

Appendix 6-4-3. Number of *Typhlocoelum cucumerinum cucumerinum* recovered from canvasbacks at various days post-infection demonstrating various character states for anterior extent of vitellaria.

	ANTERIOR EXTENT OF VITELLARIA:		
DAYS POST-INFECTION	INTESTINAL BIFURCATION	PHARYNX	ASYMMETRICAL
30	3	8	2
52	12	35	1

Appendix 6-4-4. Number of *Typhlocoelum cucumerinum cucumerinum* recovered from canvasbacks at various days post-infection demonstrating various character states for posterior extent of vitellaria.

	POSTERIOR EXTENT OF VITELLARIA:		
DAYS POST-INFECTION	INTESTINAL ARC	EXCRETORY BLADDER	ASYMMETRICAL
30	7	1	0
52	23	19	6

Appendix 6-4-5. Number of *Typhlocoelum cucumerinum cucumerinum* recovered from canvasbacks at various days post-infection demonstrating various character states for ventral surface topography.

	VENTRAL SURFACE TOPOGRAPHY:		
DAYS POST-INFECTION	NO INDENTATIONS	SMALL INDENTATIONS	LARGE INDENTATIONS
1	6	0	0
3	2	49	0
6	15	5	0
12	46	21	0
21	43	15	0
30	65	65	0
52	47	6	0

Appendix 6-4-6. Number of *Typhlocoelum cucumerinum cucumerinum* recovered from canvasbacks at various days post-infection demonstrating various character states for position of genital pore.

	POSITION OF GENITAL PORE:		
DAYS POST-INFECTION	ANTERIOR TO MID-PHARYNX	MID-PHARYNX	POSTERIOR TO MID PHARYNX
12	0	0	29
21	0	1	50
30	0	3	111
52	2	6	4

Appendix 6-4-7. Number of *Typhlocoelum cucumerinum cucumerinum* recovered from canvasbacks at various days post-infection demonstrating various character states for posterior extent of cirrus sac.

	POSTERIOR EXTENT OF CIRRUS SAC:	
DAYS POST-INFECTION	POSTERIOR TO INTESTINAL BIFURCATION	NOT POSTERIOR TO INTESTINAL BIFURCATION
12	21	0
21	49	0
30	109	1
52	31	2

APPENDIX 7-1. RESULTS OF LINEAR REGRESSION ANALYSES ON THE NUMBER OF *Typhlocoelum cucumerinum cucumerinum* METACERCARIAE.

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Appendix 7-1-1. Parameters of linear regression analyses between the number of *Typhlocoelum cucumerinum cucumerinum* metacercariae and the number of *Typhlocoelum cucumerinum cucumerinum* rediae. 334

Appendix 7-1-2. Parameters of linear regression analyses between the number of *Typhlocoelum cucumerinum cucumerinum* metacercariae and the length of the snails. 334

Appendix 7-1-1. Parameters of linear regression analyses between the number of *Typhlocoelum cucumerinum cucumerinum* metacercariae and the number of *Typhlocoelum cucumerinum cucumerinum* rediae.

DAY	SNAIL	n	r	Y INT	SLOPE	SIGNIFICANCE
30	*P. gyrina*	9	0.385	3.45	1.09	
30	*L. elodes*	3	-0.721	3.85	-0.69	
60	*H. trivolvis*	7	0.468	9.33	4.54	

Appendix 7-1-2. Parameters of linear regression analyses between the number of *Typhlocoelum cucumerinum cucumerinum* metacercariae and the length of the snails.

DAY	SNAIL	n	r	Y INT	SLOPE	SIGNIFICANCE
30	*P. gyrina*	10	0.430	2.66	1.35	
30	*L. elodes*	5	0.026	1.68	0.36	
60	*H. trivolvis*	7	0.615	2.70	4.06	