GEOGRAPHIC AND HOST-INDUCED VARIATIONS OF *HAEMATOLOECHUS BUTTENSIS* AND A RE-EVALUATION OF REPRESENTATIVES OF THE GENUS IN CANADA AND THE UNITED STATES

by

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ABSTRACT

Of the fifteen species of flukes referred to the genus Haematoloechus Looss, 1899, in Canada and the United States, nine are considered to be synonyms of one or another of the six species considered to be valid. Species identification is primarily based on the ratio of the transverse diameter of the oral sucker to the acetabulum (O/A), the anterior extent of the extracaecal loops along the ovary or testes, and orientation of the testes. The species considered valid are: H. longiplexus Stafford, 1902; H. breviplexus Stafford, 1902; H. varioplexus Stafford, 1902 (= H. similiplexus, = H. parviplexus, = H. buttensis, = H. floedae, = H. uniplexus); H. medioplexus Stafford, 1902; H. complexus (Seely, 1906) (= H. coloradensis, = H. confusus, = H. oxyorchis); H. kernensis Ingles, 1932 (= H. tumidus). Flukes are discussed using the name previously considered valid.

H. buttensis, experimentally reared in the laboratory, was used to study morphological variations resulting from changes in technical procedures and environment.

Techniques used to prepare study specimens of flukes affected some taxonomic characters previously used to separate species. The presence of spines, position of oral sucker, and size of flukes were affected by temperature of fixative, and the use of distilled water. Pressure added to the coverslip affected length and width of ovary, testes, sucker, and body.

The size of flukes, experimentally reared in the frog, R. pretiosa, was affected by temperature at which the host was maintained, the numbers of metacercariae fed to frogs, and the

age of the worm. Host size and sex had no apparent influence on fluke morphology.

Sixty-day-old flukes were experimentally reared in either R. pretiosa, B. boreas, R. clamitans, or R. aurora. The effects of developing in different frog hosts were to alter the anterior extent of the extracaecal uterine loops relative to the posterior testis, the distribution of the vitellaria, and the size and shape of ovary, testes, and size of the body and suckers. The O/A ratio remained relatively uniform in flukes from all hosts.

Flukes recovered from frogs fed infected dragonflies had a larger body size (length and width), larger testes and ovaries, and had extracaecal loops reaching farther along the posterior testis than did flukes recovered from frogs fed infected damselflies.

The O/A ratios for H. longiplexus, H. breviplexus, H. complexus, H. buttensis, H. coloradensis, and H. medioplexus did not vary among specimens collected from different localities. No significant difference in this ratio occurred between flukes that had inhabited more than one host.

The O/A ratio of H. parviplexus was significantly greater when R. sylvatica was the definitive host than when R. catesbeiana was the host. This ratio also differed significantly between specimens collected from two localities in Nebraska, even though the host in both localities was R. catesbeiana.

Pooled data for H. varioplexus and H. similiplexus indicated that flukes from R. catesbeiana, R. clamitans,

R. pipiens, and B. woodhousei differed in their O/A ratios and egg lengths and widths.

Ovary and testes may be lobed or unlobed. Uterine loops extend from the posterior portion of the worm to near the anterior border of the anterior testis in H. varioplexus, H. buttensis, and H. parviplexus. The extracaecal loops reach to the posterior border of the ovary in H. breviplexus and beyond the anterior border of the ovary in H. longiplexus.

In flukes containing extracaecal uterine loops, the left loop was absent in 4.2% of the H. parviplexus and 8.3% of H. varioplexus examined. The right loop was absent in 9.0% of H. buttensis examined.

Egg size (length and width) did not vary geographically in H. longiplexus, H. complexus, H. breviplexus, H. buttensis, H. coloradensis, or H. medioplexus. Egg lengths of H. parviplexus differed in flukes from R. sylvatica and R. catesbeiana.

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INTRODUCTION

Morphology is the principal standard used by parasitological taxonomists in naming, identifying and classifying most groups of parasitic organisms (Crites,1962). Physiological changes developing in an organism as a result of environmental variation may parallel morphological changes, although the latter may or may not be obvious (Chandler, 1923).

As pointed out by Haley (1962), there has long been a need for studies to determine the influence of different host environments on parasite structure, physiology and behaviour. Intraspecific variation may result from the influence of varying environments encountered by a parasite among individuals of a single host species and, to a greater degree, from that of the various environments encountered by a parasite in hosts of different species.

Intraspecific variation among parasitic animals occurs frequently and, according to Wharton (1957), is similar to that known to exist among free-living animals. Wharton also noted that differences resulting from intraspecific variability present taxonomic problems. According to Stunkard (1957), among hermaphroditic self-fertilizing organisms such as helminths, the "genetic" species is valueless, and classification of these organisms must depend on the use of other criteria. "The concept of species", he states, "must be based on a correlation of larval structure, life history, physiology, host relationships and morphological comparisons of adults." However, he states that "morphology is the main factor defining species of

parasites." Wright (1960) disagreed with Stunkard in that he considered acceptance of the "genetic" species to be a more constructive approach toward trematode taxonomy. Mayr (1969) defined a species as: "groups of interbreeding natural populations that are reproductively isolated from other such groups."

Cross- and self-insemination have been demonstrated for some species of trematodes (Nollen, 1968; Moseley and Nollen, 1973). Reproductive isolation in concurrent infections has also been demonstrated (Nollen, Pyne, Moseley and Bunker, 1975). Therefore, the biological species concept could be applied to trematode taxonomy; but, the sheer volume of experimental study required for its adequate application makes it difficult to apply to more than a few thoroughly studied organisms. The use of morphology in defining species of helminth parasites will remain for quite some time.

The genus Haematoloechus comprises a group of widely distributed plagiorchioid flukes, parasitic in the lungs of Amphibia. No less than 56 species of trematodes have been referred to this genus world-wide and, at one time or another, have been allocated to as many as five genera. Odening (1958) assigned all species to the genus Haematoloechus and recognized three major groups: 1) Old World; 2) Australia; and 3) America, the latter divided into three subgroups: North, South, and Central America. He later (Odening, 1960b) employed four genera for the same assembly of species.

The validity of several species in the North American subgroup has been questioned by Cort (1915a), Manter (1938),

and Harwood (1932). These authors indicated the desirability of establishing experimental infections in the laboratory to ascertain species validity. The need for such studies on intraspecific variations was also emphasized by Haley (1962). Studies of this type have been undertaken for several genera e.g. Telorchis by Watertor (1965); Prosthogonimus by Boddeke (1960 a, b) and others.

The uncertain taxonomy of species of Haematoloechus seems to have arisen from primary reliance on morphological features of sexually mature worms for which the degree of variation was unknown; many species of this genus are known from only one type specimen. These taxonomic characters, defined by Mayr (1969: 121) as "any attribute of a member of a taxon by which it differs or may differ from a member of a different taxon." Are subject to varied interpretations and technical manipulations.

The complex life histories of digenetic trematodes include, in addition to the sexually mature adult in the definitive host, several larval stages whose morphology and developmental aspects provide additional taxonomic information. The development of "haematoloechid" taxonomy is hindered by the fact that life histories have been determined for only ten species. Krull (1930, 1931) described the life histories of H. medioplexus Stafford, 1902 and H. parviplexus (Irwin, 1929). In 1932 Krull reported on part of the life cycle of H. longiplexus Stafford, 1902 and, in 1933 and 1934 on all stages of H. complexus (Seely, 1906). The life histories of H. oxyorchis Ingles, 1932, H. breviplexus Stafford, 1902 and H. complexus were described by Ingles (1933), Schell (1965), and Dronen (1975) respectively.

The life history of H. similis (Looss, 1899) was described by Grabda-Kazubska (1960), Ginetsinkaya and Dobrovolskij (1968), and Smirnova and Ibrasheva (1967). Dollfus, Doby and Laurent (1960) investigated the life cycle of H. bombynae (Zeder, 1800). The life history of H. asper Looss, 1899 was described by Dobrovolskij (1965), and by Ginetsinkaya and Dobrovolskij (1968). Many of the above life cycles are incompletely known, and three are European.

Specimens of Haematoloechus sp. collected by the author in the spring and summer of 1974 in British Columbia indicated a great deal of morphological variation in characters used to separate species in this genus. My attempts to identify the worms led to the conclusion that either as many as six species or as few as three species occurred here.

The purpose of this thesis is to examine some of the variations in morphology of the fifteen species of Haematoloechus described from Canada and the United States and to use these data to assess the validity of the described species. This will be done in two parts.

Part I: Experimental

Laboratory experiments investigate the morphological variation which occur in this genus by using H. buttensis Ingles, 1936 as a model.

Rana pretiosa Baird and Girard, 1853 the usual definitive host of H. buttensis in British Columbia, is used to determine the effects on important taxonomic characters of host ambient temperature, size, sex and number of worms present. Variation in

flukes, developed in different definitive hosts, is also examined. As well, variations in flukes are examined when different insect second intermediate hosts or molluscan first intermediate hosts are used.

Aspects of preparing specimens for examination are investigated to establish their effects on adult morphology.

Part II: A. Geographical Variation:

Specimens of some species collected from various localities in Canada and the United States are compared and analyzed for morphological variation.

Data from this part of the thesis have been derived by examining specimens from new collections made by the author or cooperating colleagues. These data are supplemented by examining specimens borrowed from museums and other universities. Obvious errors in the identification of borrowed specimens have been corrected.

B. Examination of type specimens:

A re-examination of 9 out of the 15 type specimens available has been done to determine the accuracy o descriptions of the type material. All species are initially considered valid and are discussed under their appropriate names. A tentative taxonomic revision is made at the end of the thesis.

The results from the above investigations should provide a practical classification of the species from the United States and Canada. It should also contribute information on factors important in the delineation of species within other genera of

trematodes, as well as within the genus Haematoloechus.

LIFE CYCLE OF *HAEMATOLOECHUS BUTTENSIS*

Introduction:

Haematoloechus buttensis Ingles, 1936 was recovered from *Rana pretiosa* collected from a lake 6 miles east of Greenwood, B.C., during the summer of 1975. Frogs contained from one to ten lung flukes each. Examination of damselfly and dragonfly naiads from the same lake, revealed that only the damselfly naiad (*Ischnura perparva* Selys, 1876) was infected with a metacercaria similar to that of other *Haematoloechus* spp. Naiads of *I. cervula* Selys, 1876 and the dragonfly, *Aeschna palmata* Hagen, 1856 in the same lake, were not infected. *Physa nuttalli*, Lea, 1864 collected from the same location, contained cercariae, in sporocysts, which fit the general description of cercariae previously described for other frog lung flukes. *Stagnicola elodes* (Say, 1821) and *Helisoma trivolvis* (Say, 1816) were recovered from the lake, but did not contain any trematode infections. These observations suggested that *H. buttensis* had as intermediate hosts the snail *P. nuttalli* and the naiad *I. perparva* in B.C.

Since the life history of *H. buttensis* has not been reported, I had to determine the life-cycle before I could do other experimental work on host-induced variability. The results are presented here in brief. A detailed account will be published separately.

Materials and Methods:

Sources of Experimental Animals

Resources were not available for establishing and breeding

laboratory-reared stocks of all the hosts needed for the experimental work. However, precautions were taken to select stocks of hosts which were free of parasites. This was done by examining potential hosts from many localities in British Columbia. Localities found to contain uninfected stocks of potential hosts were sampled on at least two other subsequent occasions.

The closest localities to Vancouver which contained uninfected hosts were selected as sources for experimental animals. Part of the sample was rechecked every time a new collection was made. The localities and numbers of hosts examined are given below.

Physa nuttalli was collected from a stream 15 miles south of Penticton. One-hundred snails were selected at random and examined for trematode infections. No infection was found.

Naiads of Ischnura perparva were collected from a stream 15 miles east of Lumby. Fifty naiads were examined for trematode infections. All were negative for trematode infections of any kind.

R. pretiosa was collected from a stream 15 miles east of Lumby, B. C. Fifteen frogs were examined for lung fluke infections. All were negative. Frogs were kept an additional two weeks to allow any flukes that might have been acquired naturally to mature enough to be separated from experimental infections.

Damselfly and dragonfly larvae were identified by Robert Cannings, Provincial Museum, Victoria. Snails were identified by Mrs. M.F.I. Smith, National Museum of Canada, Ottawa.

Methods for Infecting Hosts

Fifty P. nuttalli were infected by allowing them to ingest eggs of H. buttensis from Manning Park for one hour, after which snails were washed to removed excess eggs and placed in finger bowls containing dechlorinated water. Two snails were dissected one hour after feeding on eggs. Two more snails were examined every hour for the first five hours, every three hours for the next 18 hours, then every five days until cercariae emerged. Snails were maintained in aquaria on a diet of boiled lettuce.

Ten cercariae, shed from experimentally infected Physa nuttalli, were placed in each of 50 petri dishes, each containing one I. perparva naiad. Two naiads were examined after five hours, and an additional two every day for 10 days.

Ten frogs were each fed ten metacercariae, which had been dissected from experimentally infected naiads. Two frogs were necropsied at 5, 7, 21, and 30 days. All frogs were maintained at 20°C on a diet of earthworms.

Results and Discussion:

First Intermediate Host

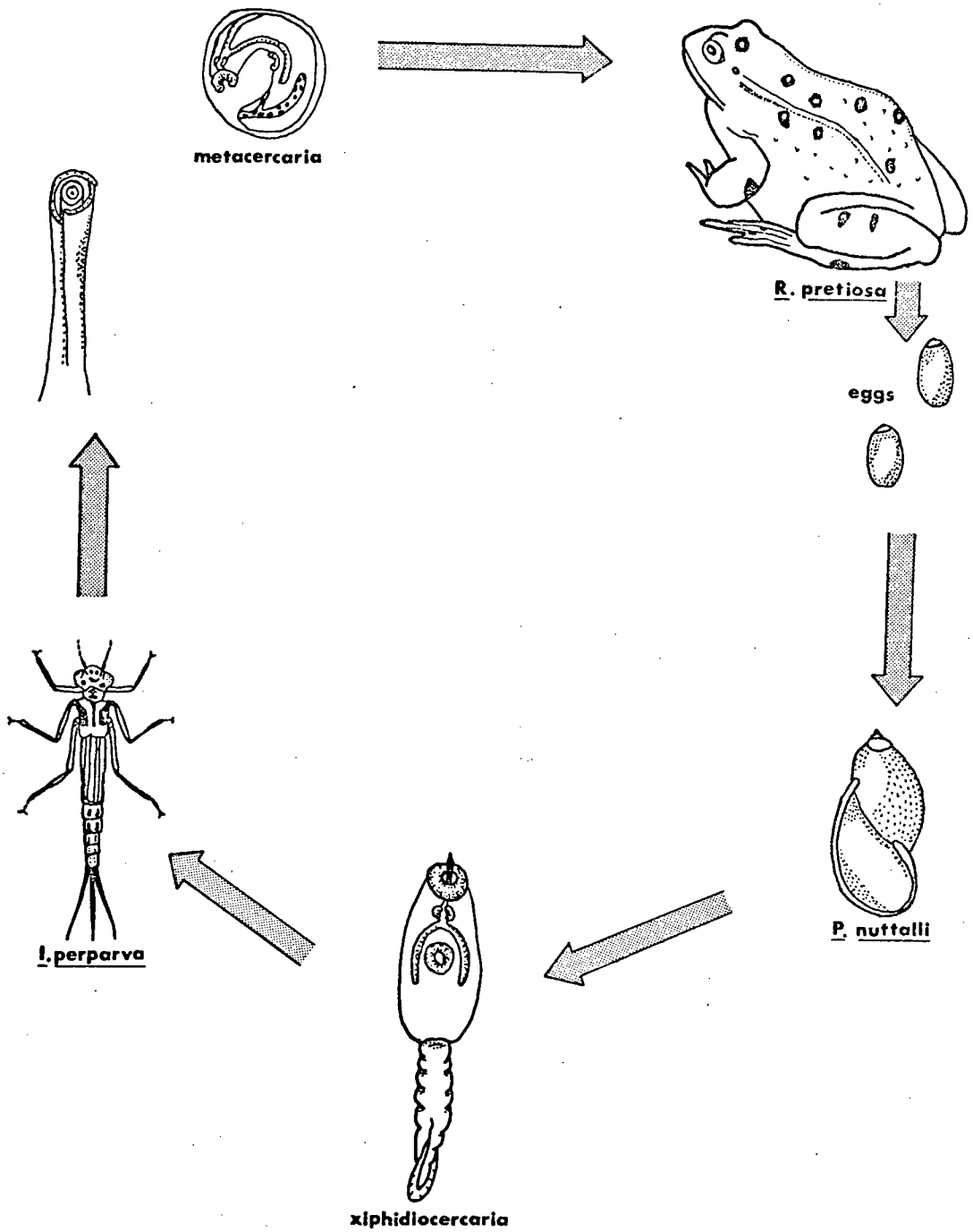
The eggs of H. buttensis were 0.024-0.029 (0.026) mm long by 0.012-0.017 (0.015) wide, operculate, light brown, and contained miracidia when laid.

Gravid worms deposit embryonated eggs in the lung cavity of Rana pretiosa. Eggs are carried from the lungs up the bronchioles and through the glottis by ciliary action of the lung and bronchiole cells (Krull, 1931). Eggs are swallowed and were found in faeces upon passing through the digestive tract.

They sink to the bottom of the pond, but do not hatch until they are swallowed by the snail Physa nuttalli (Fig. 1). Eggshells and some miracidia are passed in the faeces. Miracidia in P. nuttalli migrate through the intestinal wall. Mother sporocysts develop in the intestinal wall of the snail, or may be found free in the haemocoel. Cort et al. (1954) noted that the development of mother sporocysts in this way typically occurs in members of the superfamily Plagiorchioidea. Mother sporocysts have been observed in only three other species of Haematoloechus: Schell (1965) reported a mother sporocyst belonging to H. breviplexus in the intestinal wall of the snail Gyraulus similaris (Baker, 1919); Rankin (1939) demonstrated the existence of a mother sporocyst stage in H. merchanti; and in H. coloradensis, where Dronen (1975) demonstrated the development of a mother sporocyst on the intestinal surface in the snail Physa virgata.

Daughter sporocysts developed in the snail's hepatic gland and produced cercariae in approximately 30 days. Up to 250 cercariae were shed per night by a single heavily infected snail. Cercariae swam actively and survived up to 36 hours at 20°C.

Figure 1. Life cycle of *Haemataloechus buttensis*.


metacercaria
R. pretiosa
eggs
P. nuttalli
I. perparva
xiphidiocercaria

Second Intermediate Host

Under experimental conditions cercariae infected the larval stages of damselflies by penetrating the abdominal wall and passing through the the haemocoel, where they did not encyst. Metacercariae were also recovered from the head region, but were not seen to penetrate the cuticle of this area. Metacercariae never encysted on the rectal gills of larvae.

My field collections of potential intermediate hosts suggested that the damselfly Ischnura perparva is the usual insect host in British Columbia. Adult damselflies may harbor up to 73 metacercariae, though this is rare. My field observations indicate that two to three metacercariae per infected damselfly is more common.

Infection of Definitive Host

Frogs were experimentally infected through eating infected naiads, but they can also become infected by eating infected adult damselflies or dragonflies (Krull, 1931). The author has often seen tenerals, resting on stems of vegetation, captured and eaten by frogs. Experimental observations demonstrated that, upon being released from the damselfly, the young flukes migrate forward through the oesophagus, pass through the glottis and enter the lungs by way of the bronchi. Flukes matured in about 14 to 21 days. Lung flukes may remain in frogs for up to 15 months, after which they are lost and new ones take their place (Krull, 1930, 1931). I have found up to 74 H. buttensis in a single lung of R. pretiosa.

PART I. MORPHOLOGICAL VARIATION

1. EFFECTS OF FIXATION AND FLATTENING ON ADULT MORPHOLOGY

Introduction

Measurements of parasites have long been considered of significance in the characterization of new species (Ulmer, 1950). References to measurements in the literature often omit precise information about the techniques employed. Flattening trematodes prior to measurement, or fixing worms in solutions of different temperatures, may have a profound effect on their morphology. These changes in morphology may result in new species being described by different authors using different techniques. It is therefore important to examine the effects of various preparatory techniques on fluke morphology.

Several authors who described new species of Haematoloechus, did not record their prefixative and fixative techniques (Seely, 1906; Irwin, 1929; Harwood, 1932; Ingles, 1932, 1936). Cort (1915a) usually transferred flukes into distilled water before killing them. This was followed by killing the worm in either cold or in hot fluid. H.W.Manter (communication to G.J.Spencer, 1934) also used distilled water. Worms were then killed by placing them on a slide, adding a drop of killing solution (A.F.A.) to the worm, applying a coverslip and exerting pressure on the coverglass to flatten the specimen.

Krull (1931: 222) placed flukes in cold water to relax them and to get them to shed eggs before killing them. This required from ten to sixty minutes. He stated that "This treatment causes no serious changes in structure, except that parts of the

cuticle slough off." Worms were then plunged into hot 70% alcohol. Stafford (1902: 597) states only that "worms are killed in a mixture of glacial acetic acid and alcohol."

Characters considered by previous authors to be important in delineating species in this group are : the presence and extent of extracaecal longitudinal folds of the uterus, egg size, degree of lobation of testis and ovary, general shape and position of the testes, degree of spination of the tegument, and the ratio between the transverse diameters of the oral sucker and the acetabulum (Fig. 2). A description of these and other measurements is given in Appendix 1.

Materials and Methods

To determine the effects of fixation and flattening on the adult morphology of H. buttensis I fed metacercariae, developed in naiads of the damselfly Ischnura perparva, to each of ten male, adult Rana pretiosa of approximately the same length (55–59 mm snout urostyle length). Twenty-one days later, 157 gravid adults were recovered from the lungs of these experimentally infected definitive hosts. One-hundred thirty-five of these worms were used to determine the effects various preparatory methods have on some morphological characters.

All specimens were measured and drawn to scale. All drawings were made with the aid of a Bausch and Lomb microprojector. Measurements are in millimeters unless otherwise stated.

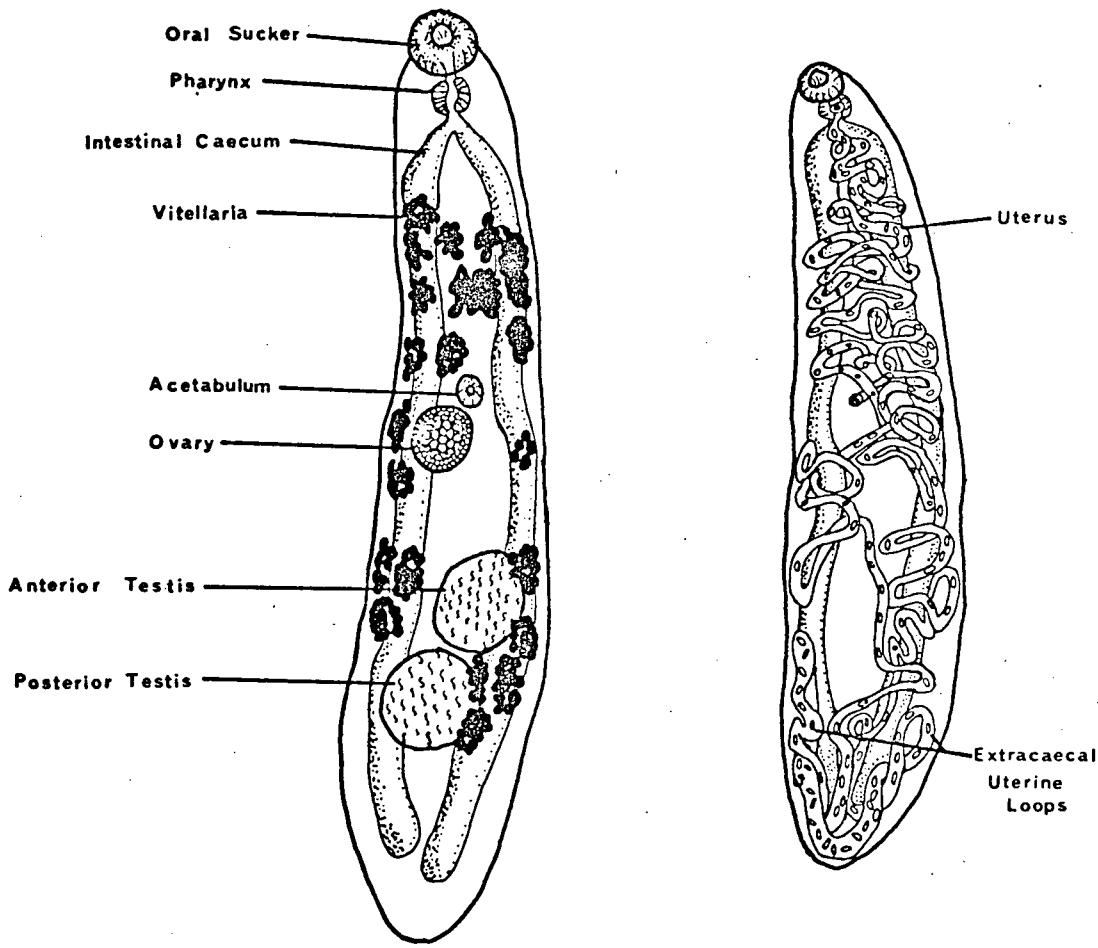
Preparation Method I

This method investigates morphological changes in flukes kept in one of two commonly used pre-fixative solutions: distilled water and amphibian Ringer's Solution (Appendix 2).

Three worms were placed in each of eight 90 mm round pyrex petri dishes. Four of the dishes contained 25 ml each of distilled water and the remaining four dishes contained 25 ml each of Ringer's solution (Appendix 2). All of the dishes were kept at 6°C. A sample of three worms from a dish of each solution was taken at 3, 5, 7 and 10 minutes. These samples were fixed in hot 70% ethanol, stained by the method given in Appendix 3 and mounted unflattened on a slide using a number 1 cover glass (18 mm sq).

The percent increase in a measurement is calculated by subtracting the measurement in Frog Ringer's solution (FR) from the corresponding measurement in distilled water (DW), dividing the difference by FR, then multiplying the quotient by 100 %.

Figure 2. Diagrammatic figure of adult Haematoloechus sp., showing principal parts of anatomy.


Oral Sucker
Pharynx
Intestinal Caecum
Vitellaria
Acetabulum
Ovary
Anterior Testis
Posterior Testis
Uterus
Extracaecal
Uterine
Loops

Results of Method I

Twenty-one-day old worms fixed and stained under similar conditions, but differing only in respect to treatment in different pre-fixative solutions, showed considerable variation in body length and width and the presence of spines on the tegument. Body length and width of adults treated in distilled water are greater than those of flukes placed in Ringer's solution by as much as 40%and 100% respectively. Frog Ringer's solution did not affect spination, but cuticular spines of worms maintained in distilled water were lost (Table 1).

Differences in measurements of flukes maintained in distilled water compared with those of flukes in Ringer's solution increased with time (Fig.3). The progressive difference in length and width is a result of an increase in these dimensions for flukes maintained in distilled water. No significant increase in size occurred in flukes kept in frog Ringer's solution for 3, 5, 7 or 10 minutes.

Preparation Method II

I tested the effect of the temperature of distilled water and of Ringer's solution on the morphology of H. buttensis by placing three worms in each of 24 petri dishes (described in method I). Groups of four dishes were kept at either 6°C, 20°C or 40°C. One dish from each group was sampled at 3, 5, 7 and 10 minutes. Post-fixative handling was as for Method I.

Results of method II

Body length and width did not differ in flukes maintained in Frog Ringer's solution at different temperatures (Table 2). No significant increase in length or width occurred when flukes from distilled water but at different temperatures were compared at corresponding times. Flukes maintained in distilled water at 6°C or 20°C lose their spines after three minutes. Spines were lost prior to three minutes in distilled water kept at 40°C.

The body length and width of worms in distilled water increased with time. The percent increase in length or width of flukes maintained in distilled water, compared with that of worms sampled at a corresponding time and temperature, but from Frog Ringer's solution, increased with time (Fig. 4). Worms, kept in Ringer's solution, and sampled at 3, 5, 7 and 10 minutes, had spines over their entire tegument.

Table 1. Effect of pre-fixative solution on some characters of 21-day-old H. buttensis.

Frog Ringer's				
Time (min.)	3	5	7	10
Character				
Spines	present	present	present	present
Body Length	3.14(2.78-3.29)	3.28(2.81-3.44)	2.95(2.73-3.30)	3.26(2.90-3.50)
Body Width	0.66(0.60-0.81)	0.63(0.61-0.71)	0.68(0.64-0.77)	0.65(0.53-0.70)

Distilled water				
Time (min.)	3	5	7	10
Character				
Spines	present	absent	absent	absent
Body Length	3.33(2.91-3.50)	3.41(3.17-3.56)	3.56(3.40-3.89)	4.57(4.30-4.81)
Body Width	0.68(0.57-0.75)	0.71(0.63-0.80)	0.89(0.76-0.98)	1.35(1.12 -1.44)

All solutions were used at 6°C.

Fig. 3 Percent increase in body length and width of flukes maintained in distilled water over those maintained in Frog Ringer's solution. The percent increase is calculated by subtracting the measurement in Frog Ringer's solution (FR) from the corresponding measurement in distilled water (DW), dividing the difference by FR, then multiplying the quotient by 100.

$$\%I = (DW - FR) / FR \times 100$$

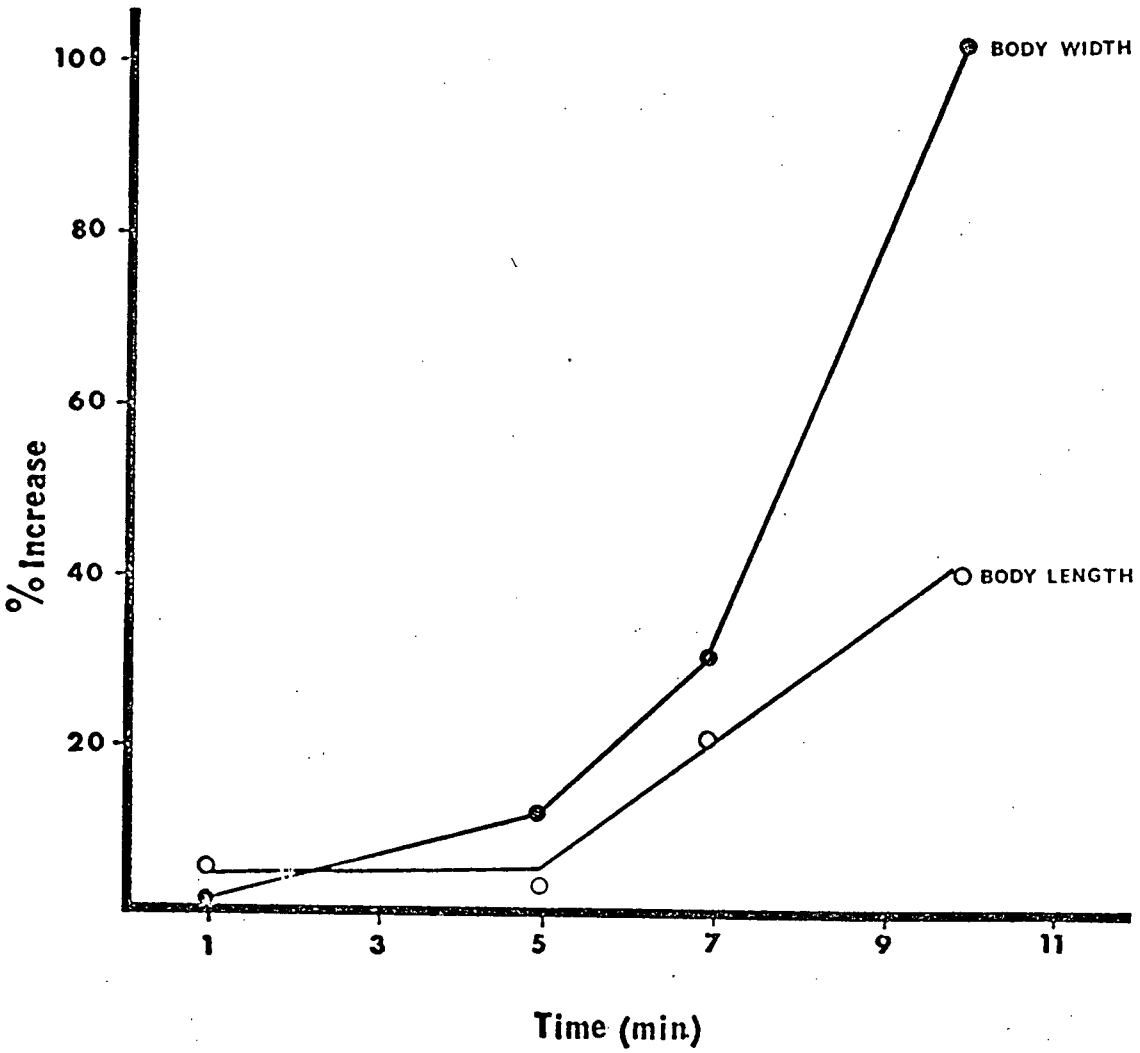
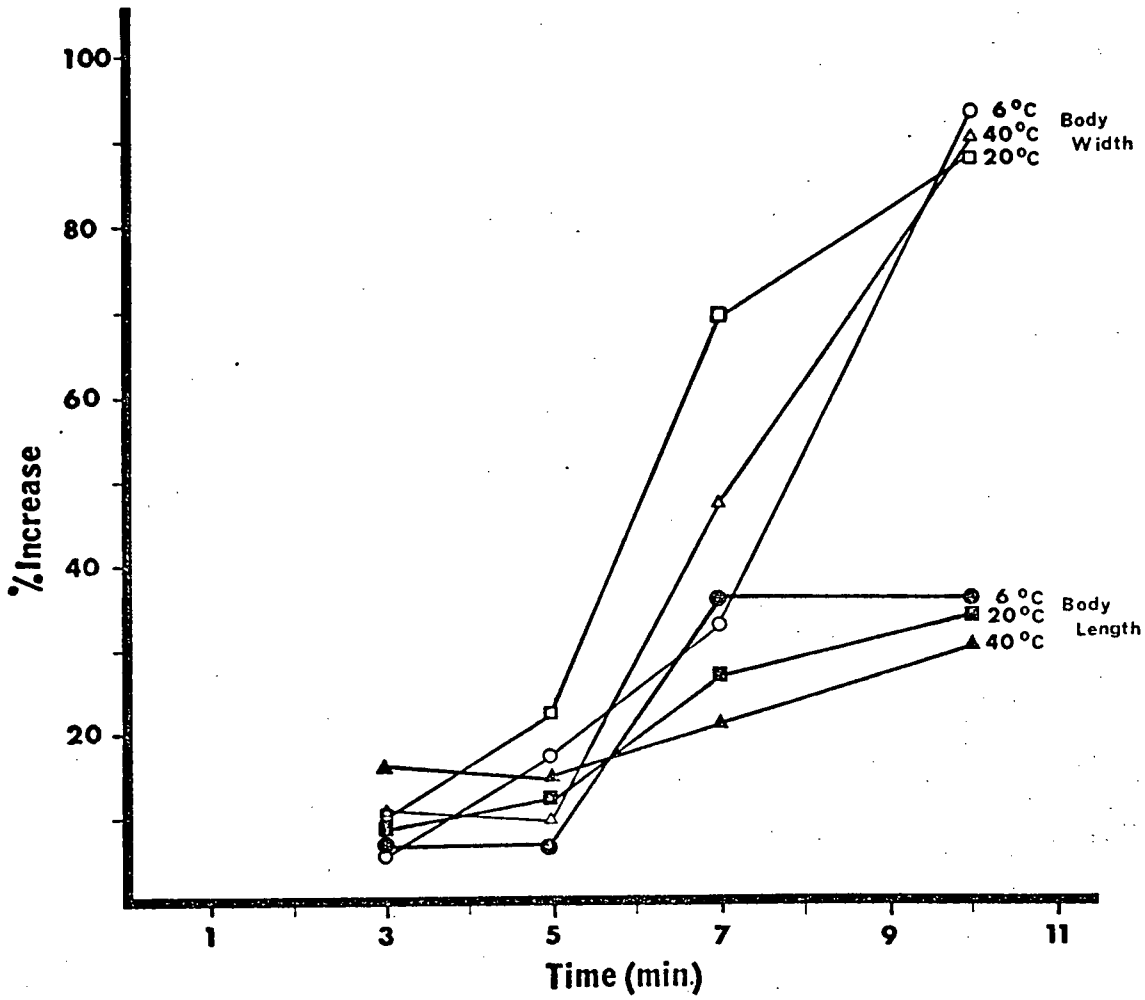

100
80
60
40
20
% Increase
BODY WIDTH
BODY LENGTH
1
3
5
7
9
11
Time (min)

Table 2. The effect of temperature on morphology of 21-day old *H. buttensis* reared in *R. pretiosa* maintained at 6 °C, 20 °C or 40 °C.

Frog Ringer's Time (min.)		3	5	7	10
Character	°C				
Spines	6	present	present	present	present
	20	present	present	present	present
	40	present	present	present	present
Body Length	6	3.47(3.28-3.67)	3.61(3.40-3.93)	3.40(3.22-3.69)	3.39(3.17-3.66)
	20	3.52(3.33-3.71)	3.57(3.33-3.77)	3.35(3.18-3.58)	3.46(3.21-3.72)
	40	3.26(3.19-3.51)	3.49(3.37-3.71)	3.55(3.25-3.79)	3.57(3.17-3.70)
Body Width	6	0.72(0.71-0.85)	0.69(0.60-0.73)	0.76(0.64-0.83)	0.66(0.59-0.75)
	20	0.75(0.69-0.87)	0.65(0.55-0.77)	0.67(0.61-0.76)	0.70(0.59-0.73)
	40	0.77(0.65-0.80)	0.73(0.61-0.81)	0.73(0.63-0.79)	0.71(0.65-0.88)
Distilled Water					
Spines	6	present	absent	absent	absent
	20	present	absent	absent	absent
	40	absent	absent	absent	absent
Body Length	6	3.69(3.41-3.92)	3.88(3.59-3.99)	4.11(3.76-4.23)	4.61(4.24-4.71)
	20	3.81(3.61-4.12)	4.00(3.72-4.18)	4.22(3.96-4.38)	4.73(4.66-5.03)
	40	3.77(3.52-4.18)	3.98(3.67-4.11)	4.33(4.01-4.45)	4.67(4.39-4.84)
Body Width	6	0.77(0.68-0.89)	0.81(0.70-0.86)	1.01(0.89-1.11)	1.27(1.15-1.35)
	20	0.81(0.73-0.88)	0.79(0.67-0.86)	1.13(1.05-1.22)	1.31(1.19-1.39)
	40	0.83(0.75-0.88)	0.80(0.71-0.94)	1.07(0.99-1.14)	1.35(1.28-1.47)

Fig. 4 Percent increase in body length and width of flukes maintained at different temperatures in distilled water over those maintained at a corresponding temperature but in Frog Ringer's solution. The percent increase is calculated by subtracting the measurement in Frog Ringer's solution (FW) from the corresponding measurement in distilled water (DW), dividing the difference by FR, then multiplying the quotient by 100.

$$\% I = (DW - FR) / FR \times 100$$


100
80
60
40
20
% Increase
1
3
5
7
9
11
Time (min.)
6°C
40°C
20°C
Body Width
6°C
20°C
40°C
Body Length

Preparation Method III

The effects of different fixatives used at 6°C or 70°C on fluke morhphology were investigated. Flukes were washed for one minute in Ringer's solution, and three worms were placed in each of ten petri dishes. The 10 dishes were separated into two equal groups. Each group contained a dish with one of the following five fixative solutions:

A.F.A. (Appendix 2)

70% ethanol

Schaudinn's fixative (Appendix 2)

Bouin's fixative (Appendix 2)

10% formalin

Fixatives in the first and second groups were used at 6°C and 70°C, respectively. The post-fixation process was the same in all cases (Appendix 3).

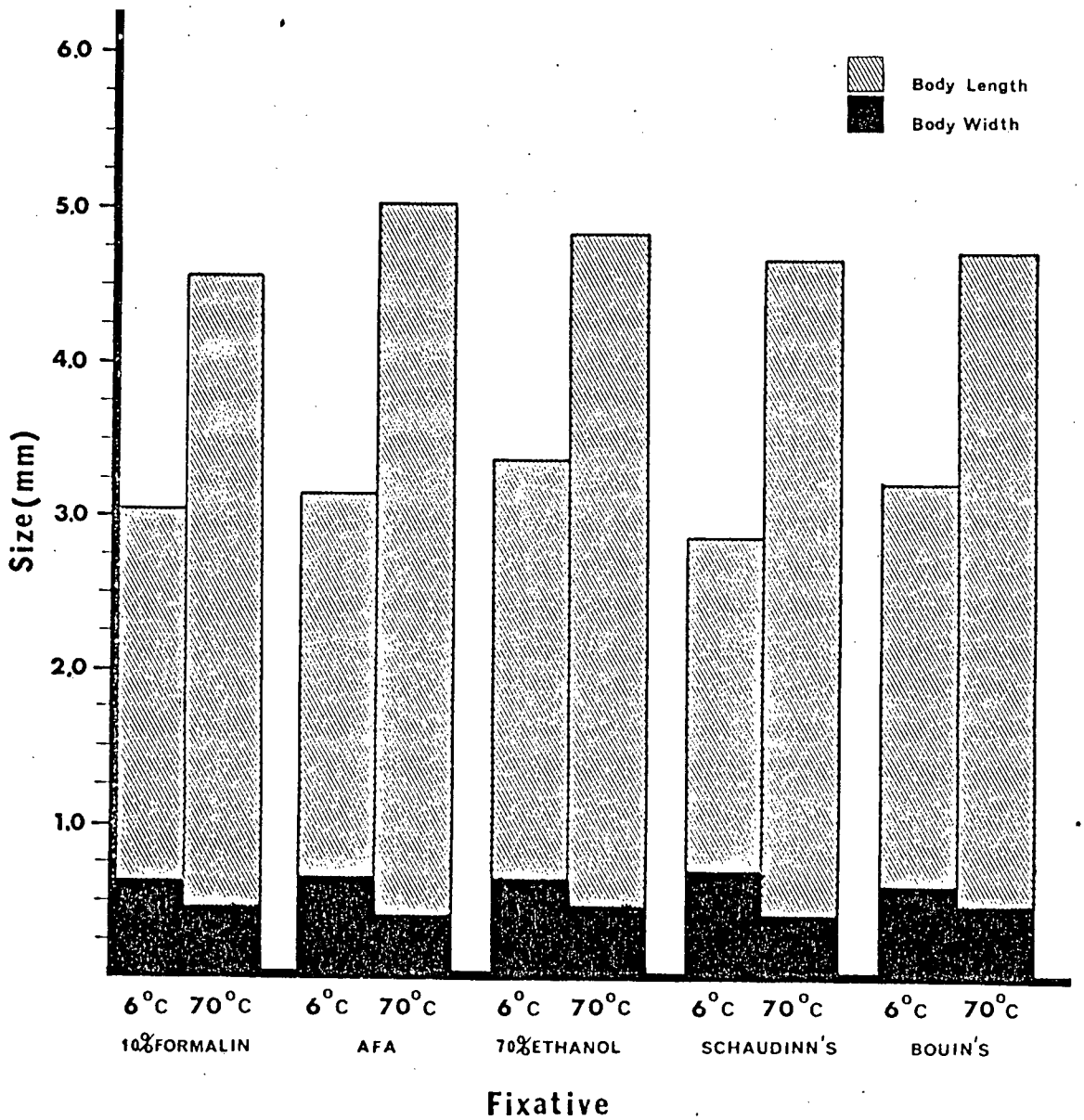
Results of Method III

Measurements of worms fixed in different solutions, but at the same temperature, did not differ significantly. However, worms from hot (70°C) fixatives were longer and narrower than worms fixed in the same solution at 6°C (Fig.5 and Table 3). Twenty percent (3/15) of the worms fixed at 6°C had subterminal suckers. The O/A and O/P ratios, lobing of testes and ovary, and extent of extracaecal loops were not affected by fixative solution or temperature.

Table 3. Effect of temperature and fixative on length and width of H. buttensis.

Character		Fixative Solution				
		10% Formalin	A.F.A.	70% Ethanol	Schaudin's	Bouin's
Length	Cold	3.02(2.80-3.14)	3.16(3.00-3.25)	3.32(3.23-3.47)	2.84(2.76-3.05)	3.23(3.11-3.41)
	Hot	4.57(4.21-4.62)	5.04(4.85-5.17)	4.83(4.67-4.92)	4.70(4.55-4.83)	4.74(4.65-5.02)
Width	Cold	0.63(0.59-0.68)	0.65(0.62-0.70)	0.61(0.57-0.63)	0.66(0.62-0.73)	0.59(0.56-0.68)
	Hot	0.41(0.37-0.45)	0.39(0.32-0.41)	0.45(0.40-0.52)	0.42(0.33-0.45)	0.44(0.40-0.51)

Fig. 5 The effects of fixative and temperature on body length and width of 21-day-old H. buttensis reared in R. pretiosa.


6.0
5.0
4.0
3.0
2.0
1.0
Size (mm)
Body Length
Body Width
6°C 70°C
10% FORMALIN
6°C 70°C
AFA
6°C 70°C
70% ETHANOL
6°C 70°C
SCHAUDINN'S
6°C 70°C
BOUIN'S
Fixative

Preparation Method IV

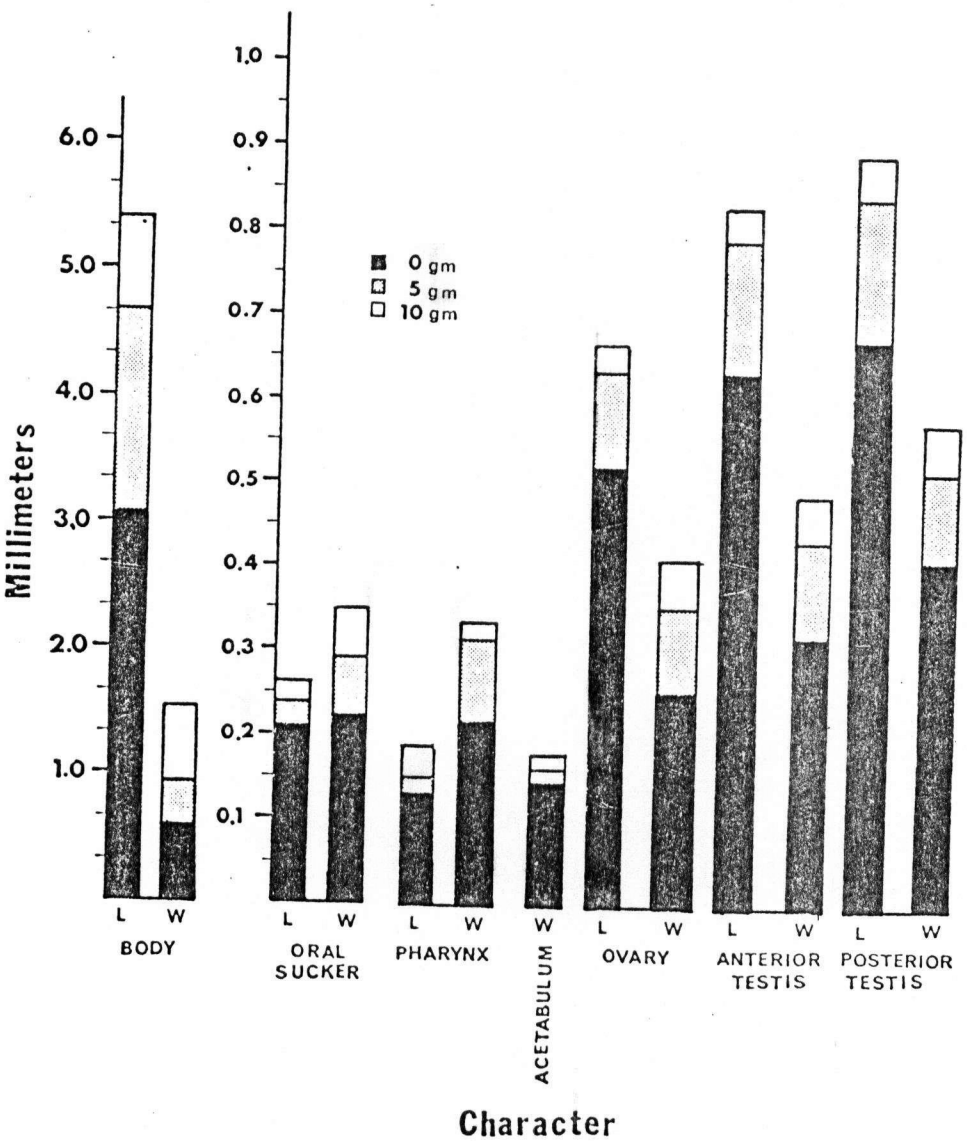
This method investigated the effects on fluke morphology of weight added to the coverslip while drying slides. Nine worms were prepared for mounting on slides in the manner described in Appendix 3. Each of the nine worms was mounted in permount on a separate slide. The slides were divided into three equal groups. A 5g weight was placed on the coverslip of each slide in group 1, a 10g weight on those of group 2 and no weight in group 3. Slides were dried for five days and measurements were taken on 15 characters (Appendix 4).

Results of Method IV

The size of a character increased with increased weight on the coverslip during drying (Fig. 6). Body length ranged from a mean of 3.05 in unflattened worms to 5.43 in worms flattened with 10g. Body widths varied from 0.63 to 1.51, anterior testis length ranged from 0.63 to 0.83. Measurements for other characters are given in Appendix 4.

The size ratio of the oral sucker to acetabulum diameters did not change markedly with increased weight during drying. This ratio varied between 2.5 and 2.8 .

Fig. 6 The effect of flattening on some characters of 21-day-old H. buttensis.


Millimeters
6.0
5.0
4.0
3.0
2.0
1.0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0 gm
5 gm
10 gm
L W
BODY
ORAL SUCKER
PHARYNX
ACETABULUM
OVARY
ANTERIOR TESTIS
POSTERIOR TESTIS
Character

2. VARIATION IN FLUKES DEVELOPED IN THE NATURAL DEFINITIVE HOST *RANA PRETIOSA*.

Introduction

More information is needed about host-dependent variation in parasitic helminths in order better to assess the stability of taxonomic characters used in identification (Haley, 1962).

The influences of different hosts, as well as of individuals of a single host species, on variations in parasite structure has received increased attention over the past fifteen years. Schiller (1959), Haley (1962), and others, have pointed out that this variation has caused considerable confusion in classifying helminth parasites.

Despite the cautioning of the above authors, many taxonomists continue to describe new species from a small number of specimens. A small sample size could result in a narrow range of variation in any particular character. Subsequent samples from the same or other host species could result in specimens with variations in characters different from those of the first sample. This would be most pronounced if the host in some way influenced parasite development. Accordingly, Watertor (1967) noted that the developmental rates of experimentally reared *Telorchis bonnerensis* Waitz, 1960 differed markedly when reared in different amphibian or reptilian hosts. Blankespoor (1974) experimenting with *Plagiorchis noblei* Park, 1936, Boddeke (1960a) with *Prosthogonimus ovatus*, and others, have noted similar host-dependent variations in development.

Factors such as host age, number of worms present, and

temperatures may also alter fluke morphology (Haley, 1962).

The purpose of this section of the thesis was to investigate morphological variation in H. buttensis developed in its normal host R. pretiosa. This study should provide information on the characters most suitable for use in separating species within the genus. As well, it should provide guide lines for studying variation in H. buttensis developed in different host species.

Materials and Methods

Source of Experimental Animals

Large numbers of uninfected or "clean" frogs, snails and naiads of Odonata were needed for this and other experiments. It was considered impractical to try to rear these animals in the laboratory and so natural sources of uninfected animals were sought.

Uninfected snails, Physa nuttalli, were collected on four separate occasions, over a two-year period, from a pond fifteen miles south of Penticton, B.C. Fifty snails from each sample were examined for natural infections with trematodes, but none was found. Some of the snails remaining from the first two collections were used as a source of "clean" hosts in subsequent experiments. The last two collections were examined as an additional assurance that snails from the collecting site were not infected.

A source of uninfected damselfly naiads, Ischnura perparva, was discovered in a stream fifteen miles east of Lumby, B. C.

Fifty naiads were collected and examined for natural trematode infections. Metacercariae are visible when live naiads are illuminated from beneath and observed under a stereoscopic microscope. Necropsies were performed on the fifty naiads to assure no metacercariae were present. No metacercariae were found. In addition, each naiad was microscopically examined prior to use in all experiments. This assured that no natural infections occurred in naiads used for experiments.

A stream located fifteen miles east of Lumby, B. C. served as a source of uninfected Rana pretiosa. Fifteen frogs, from a larger sample, were examined for helminth infections of the lungs, but none was found. Subsequent examination of R. pretiosa from the same locality in 1975 and 1976 demonstrated that lung flukes were not present in frogs in that area. Frogs to be used in experiments were kept an additional two weeks at 20°C. Faecal smears were taken during the two-week period and examined for fluke eggs. No eggs were present. The two-week quarantine would have allowed any flukes that were already in the lungs to mature enough to be separated from those from experimental infections.

Infected Rana pretiosa were collected from a beaver pond one mile east of Manning Park Lodge in Manning Park. Frogs were sampled from this pond on five different occasions during the spring and summer of 1974. Examination of these samples revealed that only one species of lung fluke was present. The morphology of this fluke coincided with the description of H. buttensis published by Ingles in 1936. Eggs from these worms served as the source for experimental infections.

Experimental Infections

Adult H. buttensis, obtained from the lungs of R. pretiosa, were placed in a dish of pond water for release of eggs. Groups of 25 snails were infected by allowing them to ingest eggs for one hour, after which the snails were removed and placed in aquaria containing pond water, where they were left for approximately 30 days for the cercariae to develop and emerge. Infected snails were maintained at 20°C.

Emerging cercariae were pipetted from fish tanks, and 200 cercariae were placed in each of 10 finger bowls filled with pond water. Ten naiads were placed in each bowl and left for 24 hours, after which they were transferred to plastic containers and maintained at 20°C. After five days naiads were dissected and metacercariae removed. Frogs were infected by wrapping ten metacercariae in moistened bread, stringing a thread through the bread and lowering the wad into a tank containing one frog. Frogs usually ate the bread within three minutes. Fifteen frogs were infected in this way. Samples of three frogs were examined and lung flukes collected at 5, 14, 21, 28 and 60 days. Specimens were prepared using the standardized procedure given in Appendix 3. Drawings of selected flukes were made with the aid of a Bausch and Lomb microprojector and camera lucida. All measurements were made as described in Appendix 1 and are in millimeters unless otherwise stated.

Results

The experiment yielded 700 specimens of H. buttensis of known ages. Comparative measurements are presented in

Appendix 5. Comparisons of the morphological features used by Ingles (1936) in his description of H. buttensis are presented with my experimental results.

Variations with Age, Growth and Maturity:

1. Maturity:

All worms were mature by 14 days after infecting frogs. Maturity is here defined as being reached when worms contain eggs in their uterus. My experiments indicate that worms may become mature when less than 2.0 mm long (Appendix 5). Ingles (1936) did not mention the size at which H. buttensis becomes mature. His type description is based on ten worms that varied in length from 3.2 mm to 10 mm. Therefore, they were probably all adults.

2. Body Size:

Type Description: "Length averages 7.4 mm and varies from 3.2 mm to 10 mm. Width averages 1.3 mm and varies between 0.7 mm and 2.2 mm" (Ingles, 1936, pp. 78-80).

Experimental Results: Flukes recovered from R. pretiosa 60 days after initial exposure were on average 3.4 times longer (6.01) than fourteen-day-old mature worms (1.75). Average body width increased from 0.51 in fourteen-day-old worms to 1.26 in 60-day-old worms. The fastest relative growth occurred between the metacercarial stage and five-day-old worms, and between 14 and 21 days (Fig. 7). Adult flukes thus ranged from 1.61 to 6.67 mm long.

3. Vitellaria:

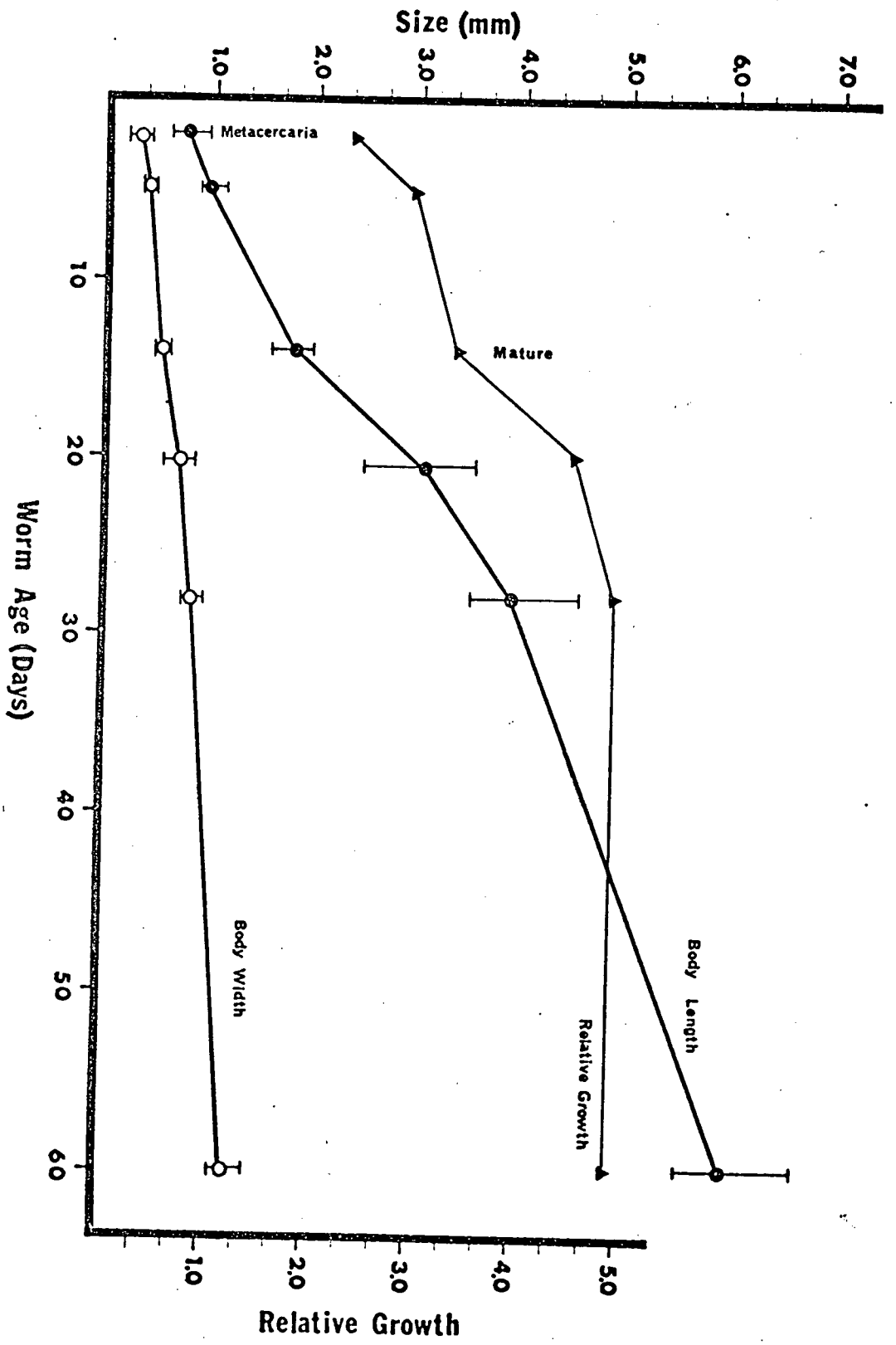
Type Description: "Vitellaria lateral and dorsal to caeca; extend across dorsal region anterior to ovary; seventeen groups were counted in one young fluke." (Ingles, 1936, p.80).

Experimental Results: Vitellaria are not evident until 14 days post infection. They extend across the dorsal region anterior to the ovary and down the lateral margins of the worms. The right vitellaria length is consistently greater than the left. However, the percent difference between the two changes with age, which indicates a difference in growth rate between the left and right vitellaria. At 14, 21, 28 and 60 days the percent difference is 21.4, 7.0, 34.0 and 39.6, respectively. Therefore, the left vitellaria grew much faster relative to the right side between 14 and 21 days, which corresponds to the fastest growth period of the fluke. The right side develops faster than the left side after 21 days.

4. Ovary and Testes:

Type Description: "Ovary kidney-shaped, never lobed, averages 0.44 in length by 0.32 in width; its range varies from 0.26 to 0.55 and from 0.20 to 0.37 respectively; usually on right side..... Testes nearly same size and shape; length averages 0.82; width averages 0.64; range from 0.45 to 1.03 and 0.48 to 0.87 respectively." (Ingles, 1936, p.80).

Fig. 7 Relationship between body length, body width, and relative growth, and age of worms developed in Rana pretiosa. The mean and range is given for the length and width of worms of different age groups.


Size (mm)
1.0
2.0
3.0
4.0
5.0
6.0
7.0
Metacercaria
Mature
Body Width
Relative Growth
Body Length
Worm Age (Days)
10
20
30
40
50
60
1.0
2.0
3.0
4.0
5.0
Relative Growth

Experimental Results: the ratio of the length of the testes (sum of both testes lengths) to that of the ovary remained fairly constant for 14, 21 and 28-day-old flukes, but increased for 60-day-old worms, the ratios being 2.7:1, 2.6:1, 2.5:1 and 3.3:1, respectively. This suggests an increase in egg production at this time.

The ovary changed from a smooth round organ at fourteen days to an elliptical, highly lobed form by 60 days (Figs. 8 to 13). The ovary increased in length from 0.08 to 0.71 and in width from 0.08 to 0.36 during the study period.

Testes changed from elliptical, smooth organs to elongate forms which either remained smooth or developed some degree of lobing (Figs. 14 to 18). The anterior testis increased in length from an average of 0.34 in 14- day-old worms to 1.12 in 60-day-old worms, and 0.22-0.48 in width. The posterior testis increased from 0.31-1.22 and 0.22-0.52 for length and width respectively.

5. Longitudinal Folds of the Uterus:

Type Description: "Uterus runs posteriorly between testes; extracaecally the folds may extend to anterior level of anterior testis on the same side as the ovary; on the opposite side they extend only half as far; it returns to anterior part of the body between the testes and has many folds anterior to the acetabulum." (Ingles, 1936, p.80).

Experimental Results: the right extracaecal uterine loop extends forward a distance of 1/4 to 3/4 the length along the posterior testis. The left loop extends forward 1/2 the distance

to the posterior testis, from the end of the worm, to 3/4 the distance along the posterior testis. The relative lengths of both uterine folds (body length/uterine loop length) decreased with age. The right fold decreased in relative length from 16.1:1 at 14 days to 4.0:1 at 60 days. The left fold decreased in relative length from 19.6:1 at 14 days to 5.6:1 at 60 days.

Fig. 8. Metacercaria from Ischnura perparva.

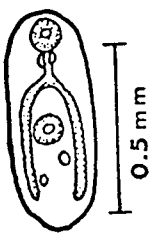
Fig. 9 Five-day-old fluke developed in Rana pretiosa.

Fig. 10 Fourteen-day-old fluke developed in Rana pretiosa.

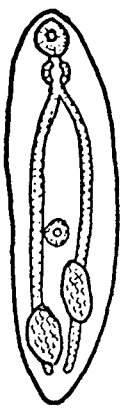
Fig. 11 Twenty-one-day-old worm developed in Rana pretiosa.

Fig. 12 Twenty-eight-day-old worm developed in Rana pretiosa.

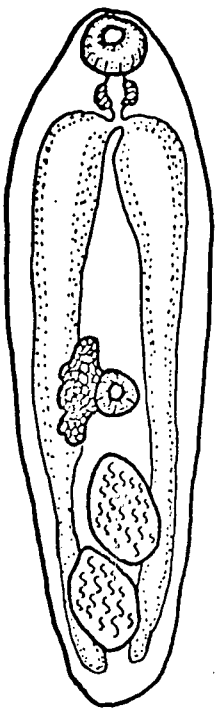
Fig. 13 Sixty-day-old fluke developed in Rana pretiosa.



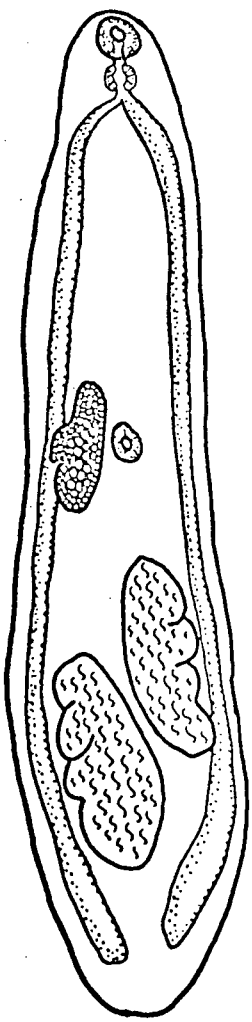
8



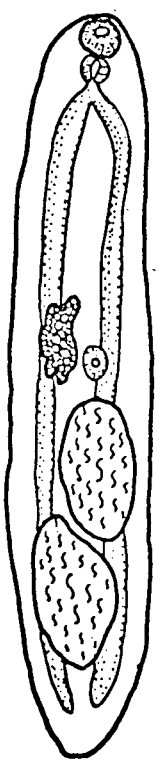
9



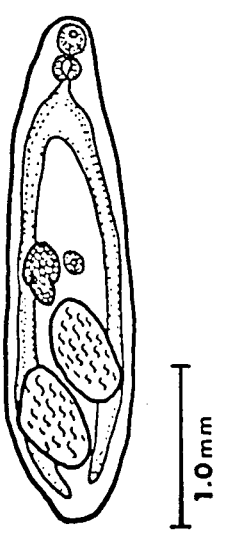
10



13



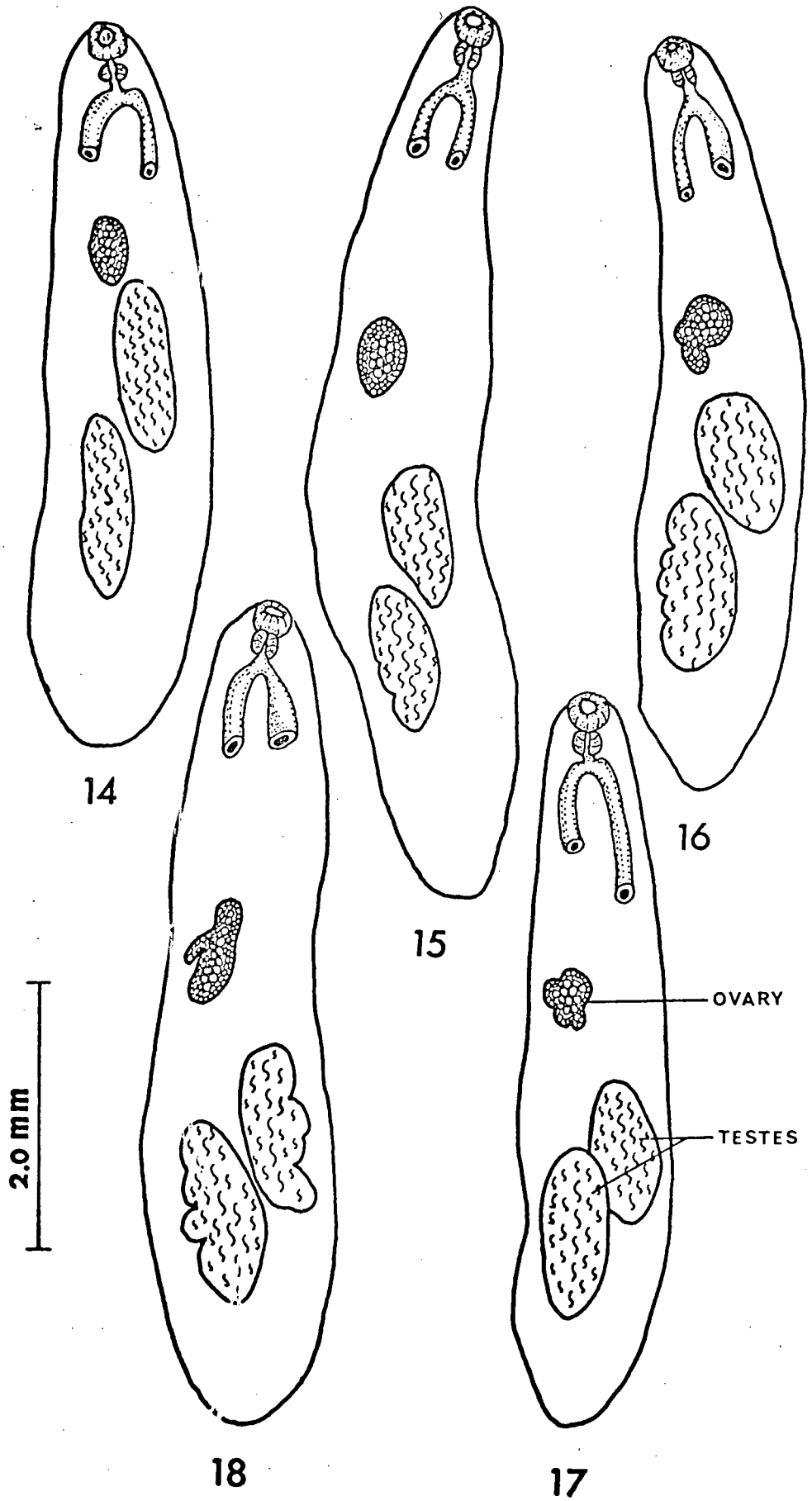
12



11

Figures 14 to 18.

Variation in size, shape and position of ovary and testes of 60-day-old worms developed in *Rana pretiosa*.


14
15
16
18
17
OVARY
TESTES
2.0 mm

6. Spination:

Type Description: "Cuticle armed with spines posteriorly to the acetabulum.: (Ingles, 1936, p. 78).

Experimental Results: Flukes of all ages had spines. Spines were uniformly distributed over the entire body surface. Spines appeared to be less numerous and were smaller on the posterior portion of the body.

7. Sucker ratio:

Type Description: "Oral sucker subterminal averages 0.33 mm in length by 0.46 mm in width; it ranges from 0.18 to 0.14 and from 0.29 to 0.47 respectively [sic]. Acetabulum located in first half of body and smaller than oral sucker; it averages 0.26 in length by 0.31 in width; it ranges from 0.15 to 0.36 in length and 0.24 to 0.37 in width. Ratio of length of oral sucker to acetabulum is 1:0.7, and range of variation of this ratio is 1:0.8 to 1:0.6 " (Ingles, 1936, pp.78-80).

Experimental Results: The oral sucker in 60-day-old worms was terminal and averaged 0.25 long by 0.29 wide. The acetabulum was median and always closer to the ovary than to the anterior end. Acetabulum diameter increased from an average of 0.07 in fourteen-day-old flukes to 0.11 in 60-day-old worms. Oral sucker diameter increased from 0.18 to 0.29 during the same time. The sucker ratio for post metacercarial worms remained constant for all age groups. Five-day-old worms had only a slightly smaller ratio (2.35:1) than mature worms (2.5:1 to 2.6:1) (Fig. 19). Metacercariae have an O/A ratio significantly smaller than that in worms recovered from frogs (Fig. 19). The smaller O/A ratio

for metacercariae is a reflection of the relatively larger acetabulum. The diameter of the acetabulum decreases for five-day-old worms, then begins to increase in size again (Fig. 20).

8. Eggs:

Type Description: "Eggs operculate, brown with completely formed miracidia when laid; average 0.027 by 0.014; range 0.025 to 0.030 and 0.011 to 0.017 for length and width respectively" (Ingles, 1936, p. 80).

Experimental Results: Eggs are present first in area "C" of the uterus (Appendix 6, Fig.8#) 14 days after initial exposure and average 0.017 by 0.015. Eggs increase in size as they pass along the uterus until, at 21 days, they average 0.024 by 0.016 and are found in area "A" (Appendix 6, Fig.8#). Thereafter egg size remains constant regardless of fluke age.

Fig. 19 Change in O/A ratio with age of worm. The mean and range is given for each age group.

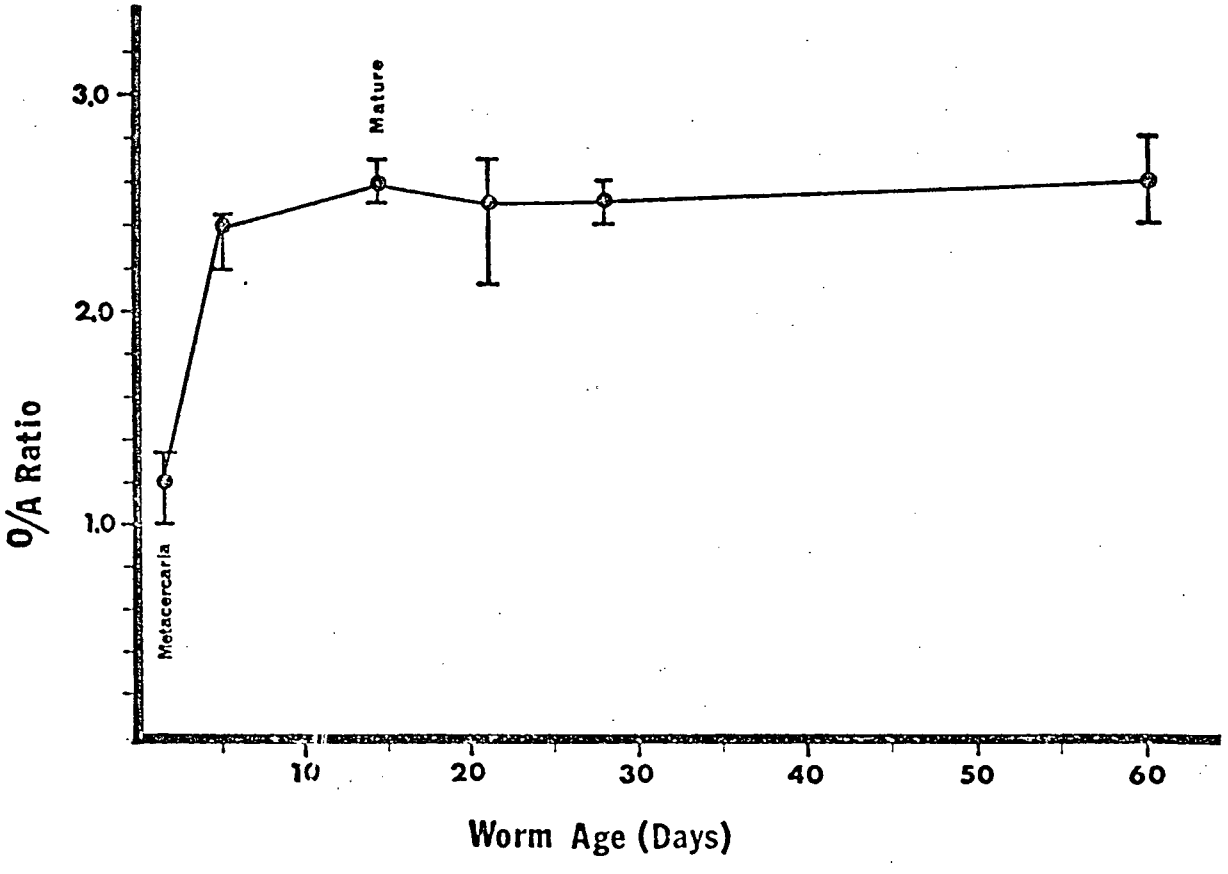
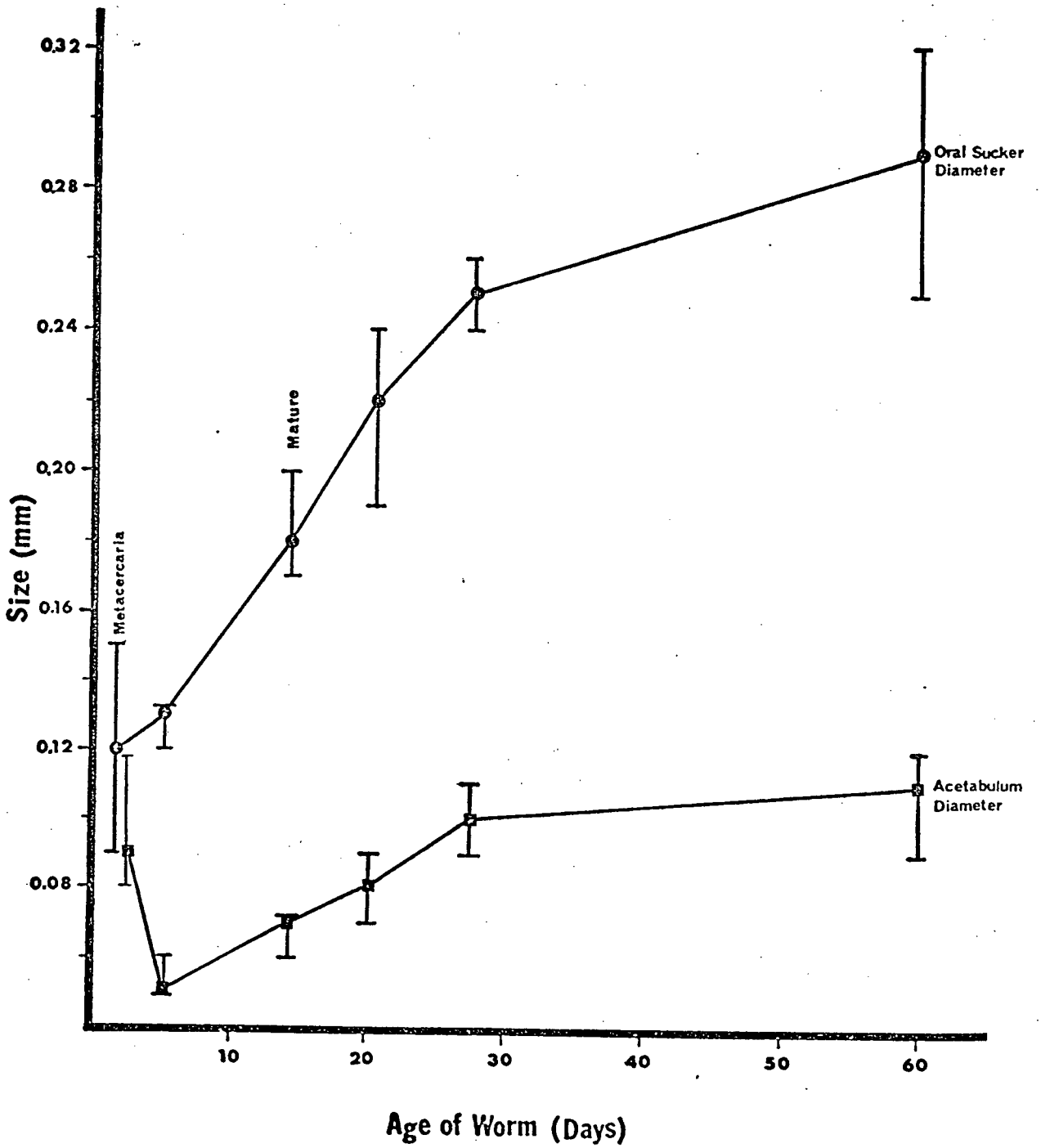

O/A Ratio
3.0
2.0
1.0
Mature
Metacercaria
10
20
30
40
50
60
Worm Age (Days)

Fig.20 Relationship between age of worms developed in Rana pretiosa and diameter of oral sucker and acetabulum. The position of the measurements for metacercariae has been staggerd for clarity. The mean and range is given for each age group.


0.32
0.28
0.24
0.20
0.16
0.12
0.08
Size (mm)
Metacercaria
Mature
Oral Sucker Diameter
Acetabulum Diameter
10
20
30
40
50
60
Age of Worm (Days)

3. VARIATION IN FLUKES DEVELOPED IN *RANA PRETIOSA* MAINTAINED AT DIFFERENT TEMPERATURES

INTRODUCTION

The temperature range at which larval and adult helminths live and develop varies considerably from species to species. A direct relationship exists between temperature and rate of growth and development to a certain point, after which various unfavourable effects may occur (Watertor, 1965).

This study examines the effect of temperature on the morphology of *H. buttensis* when reared in *R. pretiosa*, kept at 12°C, 20°C or 27°C.

Materials and Methods

Three groups of 15 frogs each were fed 10 metacercariae each. Each group was maintained in aquaria (50 cm by 25 cm by 30 cm) at either 12°C, 20°Cor 27°C. Wet paper towels were kept on the bottom of the tanks to provide moisture for the frogs. The tops of the tanks were covered with glass sheets to prevent excessive evaporation. Samples of three frogs were examined from each group at 5, 14, 21, 28 and 60 days post-infection. *Physa nuttalli* and *Ischnura perparva* served as the intermediate hosts.

Results

Growth and development of 204 experimentally-reared worms resulting from the feeding experiments with adult *R. pretiosa* were studied and measured. Measurements of taxonomically

significant characters are given in Appendices 7 to 9 inclusive.

Worms maintained at a higher temperature were longer and wider than worms of a comparable age but developed at a lower temperature. Worms reared at 27°C attained the largest sizes (length and width) of all age groups studied (Fig. 21). Worm length and width increased with age of worms maintained at 20°C and 27°C. Worms at 12°C did not show this age-related increase (Fig. 21).

Flukes reared at 27°C had the largest (length and width) oral suckers for each age group studied (Fig. 21). The only exception was in 14-day-old worms. Worms in this age group, developed at 20°C and 27°C, had similar oral sucker widths (Appendix 8 and 9). The length and width of the oral sucker in worms reared at 20°C and 27°C increased with age. Worms at 12°C did not show an age-related increase in these measurements (Fig. 21).

The diameter of the acetabulum did not increase in worms developed at 12°C. However, this measurement showed an increase with age in flukes reared at 20°C and 27°C (Fig. 21). Five- and 14-day-old worms, reared at 12°C had wider acetabula than did similarly aged worms developed at 20°C or 27°C. By 21 days, the acetabula of all flukes were the same size, but by 28 and 60 days worms developed at 20°C and 27°C had wider acetabula than did worms developed at the lowest temperature.

Older worms had larger testes at all temperatures studied. There was a marked increase in length of testes as well as rate of growth in worms reared at 20°C compared with those at

12°C. Worms at 27°C had shorter testes than those at 20°C (Fig. 22).

The ovary did not develop in worms maintained at 12°C. The ovary length in flukes developed at 20°C was greater than that develope 27°C (Fig. 22). All flukes reared at 20°C and 27°C were gravid by 14 days. Worms maintained at 12°C did not mature.

The increase in length of the posterior portion of the worm was greatest in worms developed at higher temperatures. This increase is expressed by comparing the distance of the posterior margin of the anterior as well as the posterior testis from the posterior end of the worm (Fig. 23). The greatest rate of increase occurred at 27°C between 14 and 21-day-old worms. This corresponds to the highest rate of increase in size of the ovary and testes, and the increase in egg production in worms at this time.

The O/A ratio of flukes did not differ with age of worm or when the host was maintained at 20°C or 27°C. Worms of all ages reared at 12°C had a significantly lower O/A ratio, essentially that of the metacercariae.

Fig. 21 The influence of temperature and age of worm on some characters of *H. buttensis* developed in *R. pretiosa*.

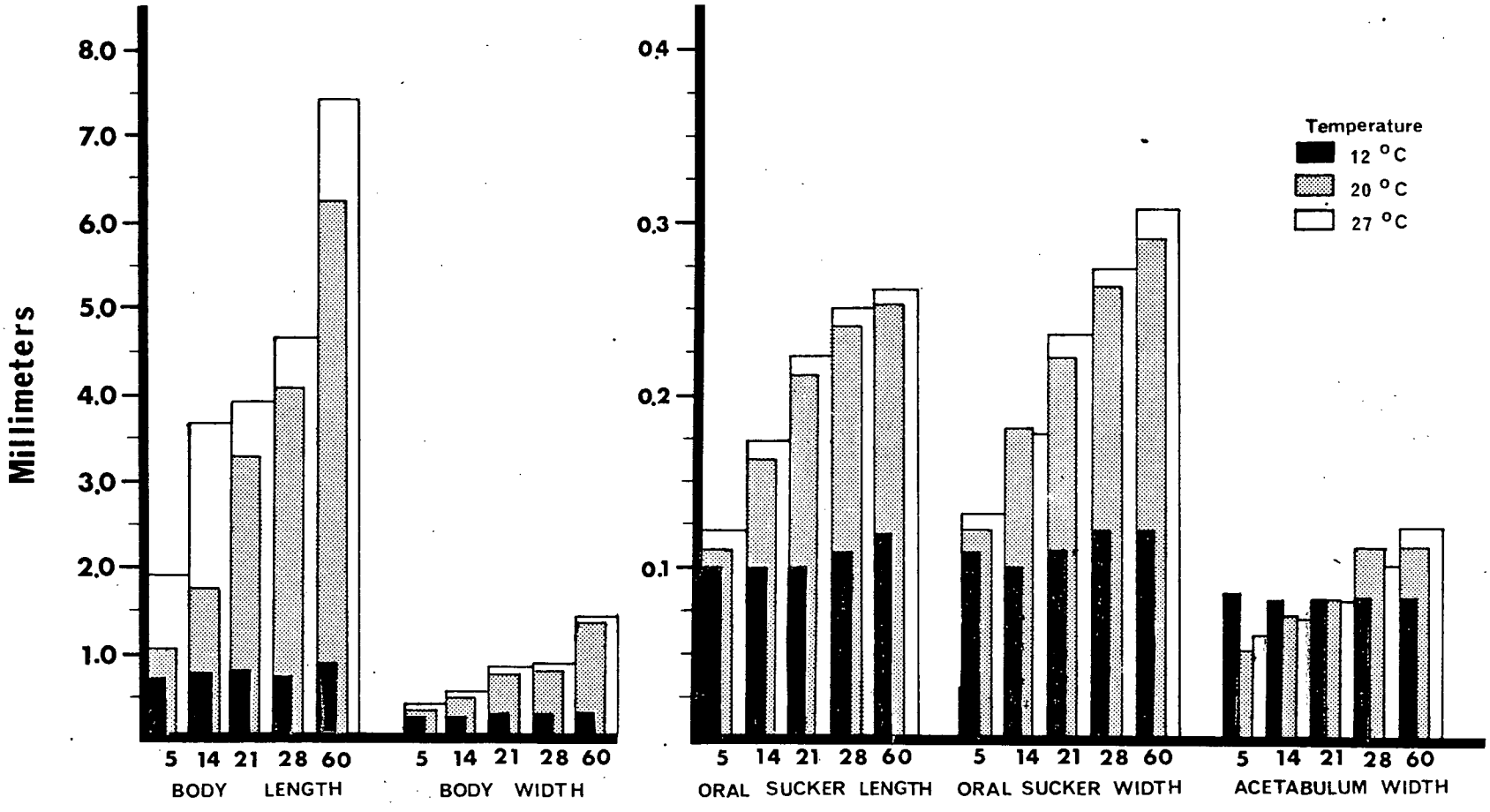

Millimeters
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.4
0.3
0.2
0.1
Temperature
12 °C
20 °C
27 °C
5 14 21 28 60
BODY LENGTH
5 14 21 28 60
BODY WIDTH
5 14 21 28 60
ORAL SUCKER LENGTH
5 14 21 28 60
ORAL SUCKER WIDTH
5 14 21 28 60
ACETABULUM WIDTH
Age of Worm (Days) and Character Measured

Fig. 22 The effect of temperature on the length of the ovary, anterior testis, and posterior testis of worms reared in R. pretiosa.

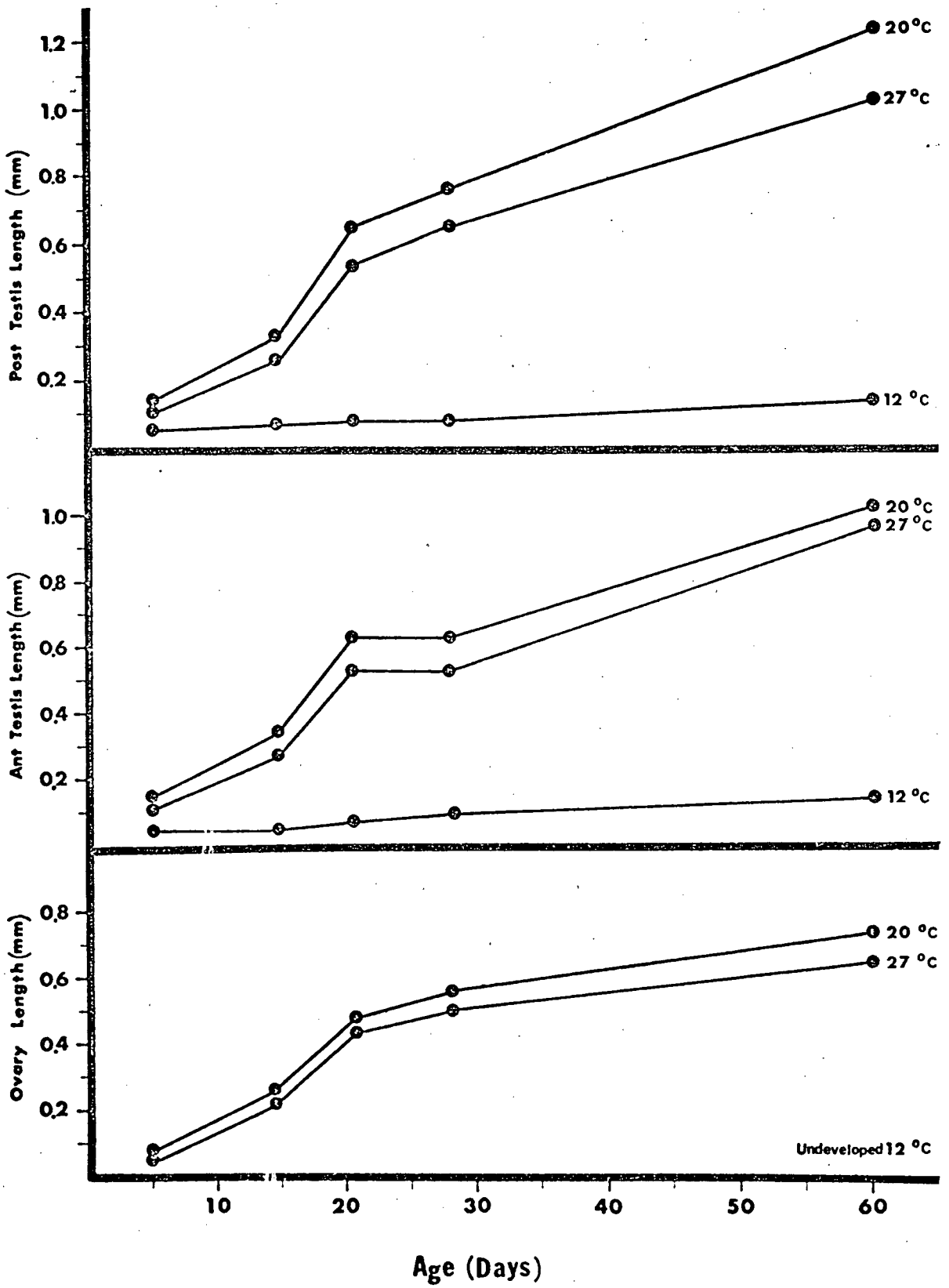
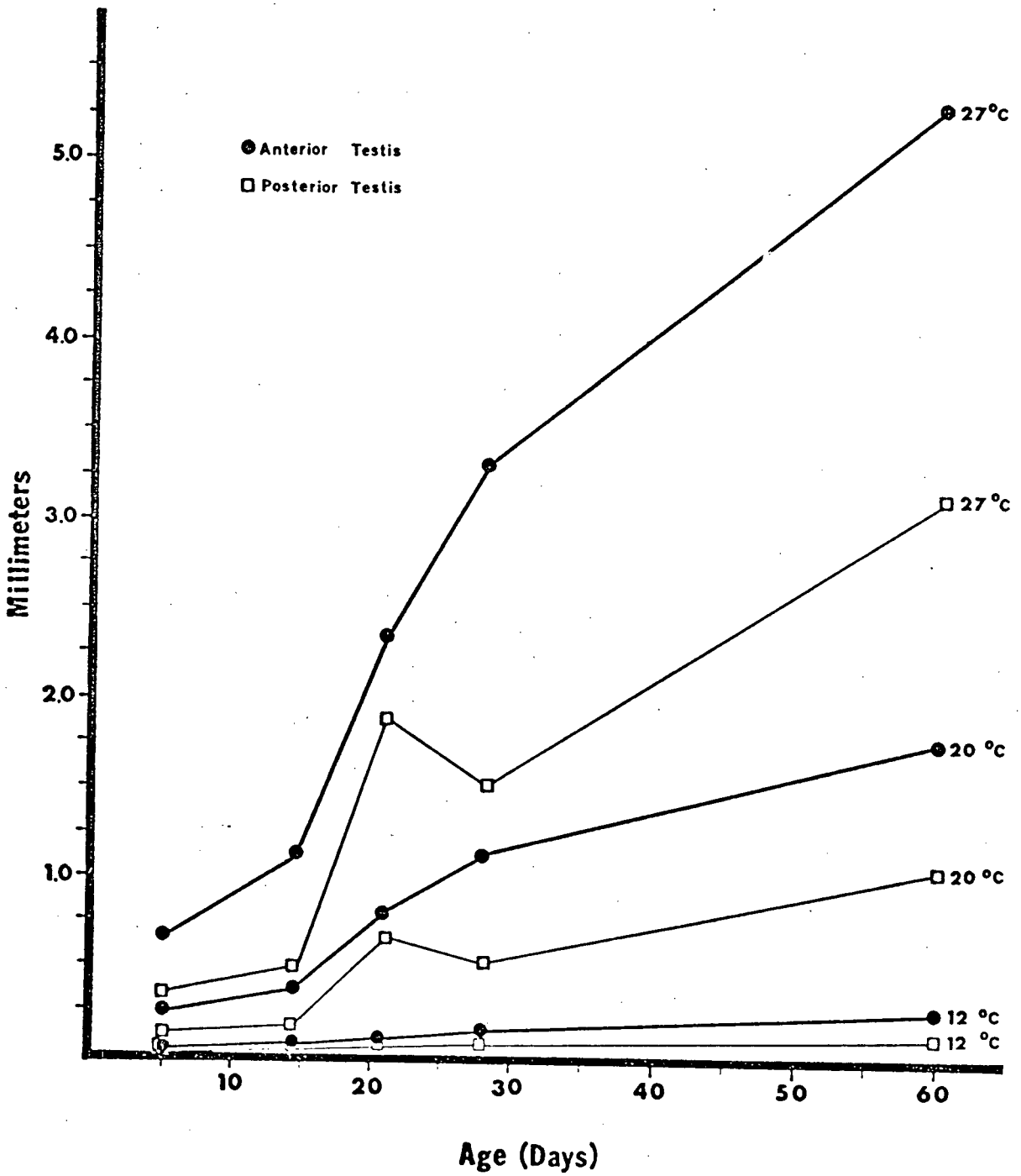

Post Testis Length (mm)
1.2
1.0
0.8
0.6
0.4
0.2
20°c
27°c
12 °c
Ant Testis Length (mm)
1.0
0.8
0.6
0.4
0.2
20 °c
27 °c
12 °c
Ovary Length (mm)
0.8
0.6
0.4
0.2
20 °c
27 °c
Undeveloped 12 °c
10
20
30
40
50
60
Age (Days)

Fig. 23 Relative growth of the hind body. Distance between the posterior margin of the anterior and posterior testis from the end of the worm.


Anterior Testis
Posterior Testis
27°c
27 °c
20 °c
20 °c
12 °c
12 °c
5.0
4.0
3.0
2.0
1.0
Millimeters
10
20
30
40
50
60
Age (Days)

4. EFFECT OF HOST SIZE ON DEVELOPMENT OF *H. BUTTENSIS*

Introduction

Relationships between size and form of parasites and size (age) of definitive hosts have been observed by many parasitologists, but few have examined these relationships in detail. Dogiel (1966) and others have summarized the literature on this relationship.

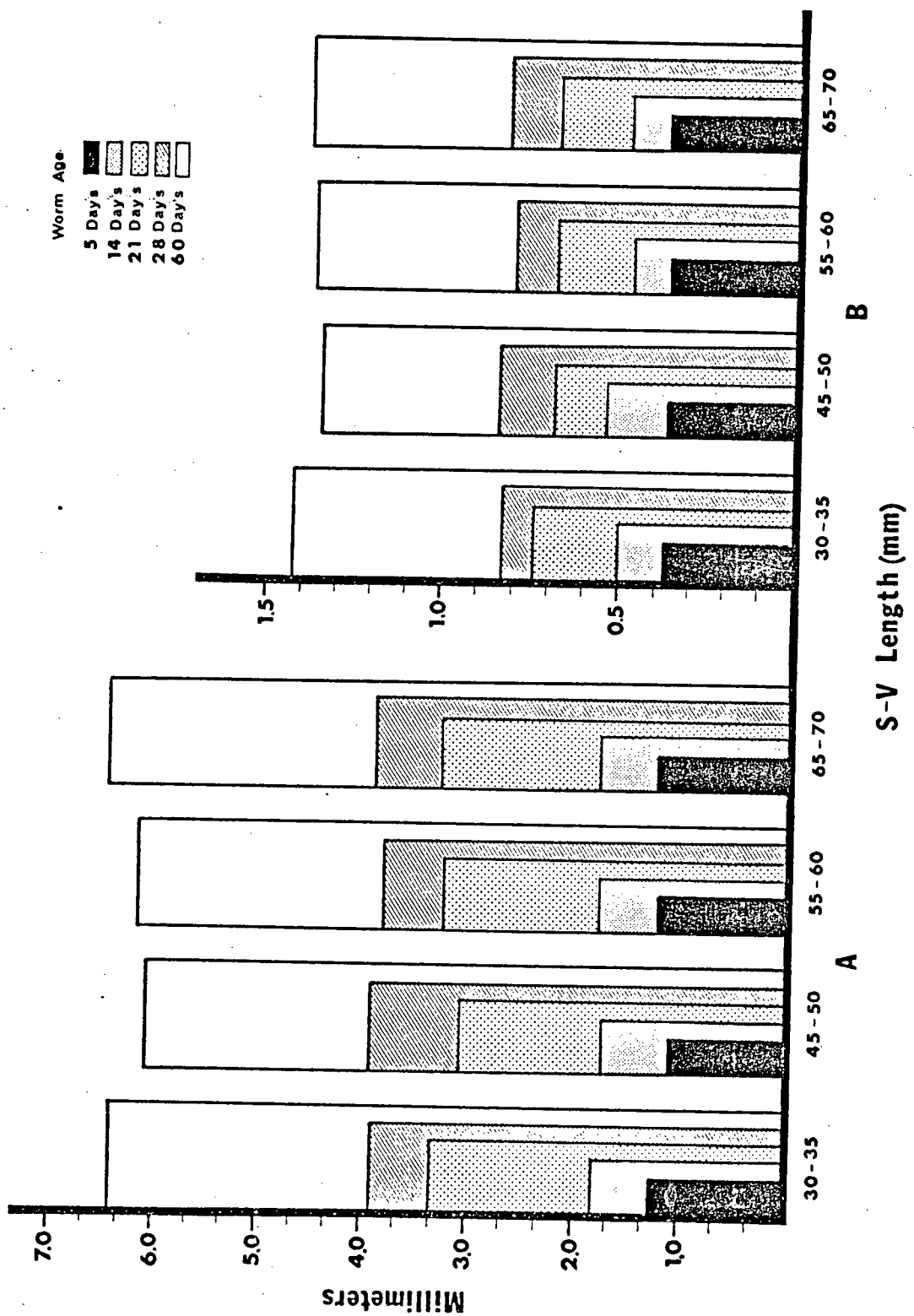
Materials and Methods

The experiments utilized 15 specimens of *R. pretiosa* in each of 4 size groups: 30–35 mm; 45–50 mm; 55–60 mm; and 65–70 mm. Ten metacercariae from *I. perparva* were fed to each frog in all groups. Frogs were maintained at 20°C for 60 days. Samples of three frogs were taken from each group at 5, 14, 21, 28 and 60 days post-infection and examined for lung flukes.

Results

Measurements of all specimens recovered from these experiments indicate that length (age) of *R. pretiosa* does not affect the size or shape of adult *H. buttensis* (Appendices 10 to 13). Worm length or width did not differ in samples from frogs of different size groups (Fig. 24). The increase in size of all characters measured did not differ in flukes developed in frogs of different sizes.

Fig. 24 The relationship between the length (A) and width (B) of H. buttensis developed in different size groups of R. pretiosa. S-V is the snout-vent length of the frog.


Worm Age
5 Day's
14 Day's
21 Day's
28 Day's
60 Day's
Millimeters
7.0
6.0
5.0
4.0
3.0
2.0
1.0
30-35
45-50
55-60
65-70
A
1.5
1.0
0.5
30-35
45-50
55-60
65-70
B
S-V Length (mm)

5. EFFECT OF HOST SEX ON MORPHOLOGY OF *H. BUTTENSIS*

Introduction

Another area of interest to parasitologists is the influence of the sex of the host on trematodes. Hollis (1972) found a significant difference in seasonal prevalence of *Haematoloechus medioplexus* between male and female *Rana pretiosa*. He concluded that female gonadotropins were involved in the seasonal prevalence. However, no mention was made of differences in morphology of flukes recovered from male or female frogs. Such differences as do exist between male and female frogs may alter the morphology of developing flukes.

The objective of this study was to investigate the effects of host sex on the morphology of *H. buttensis*.

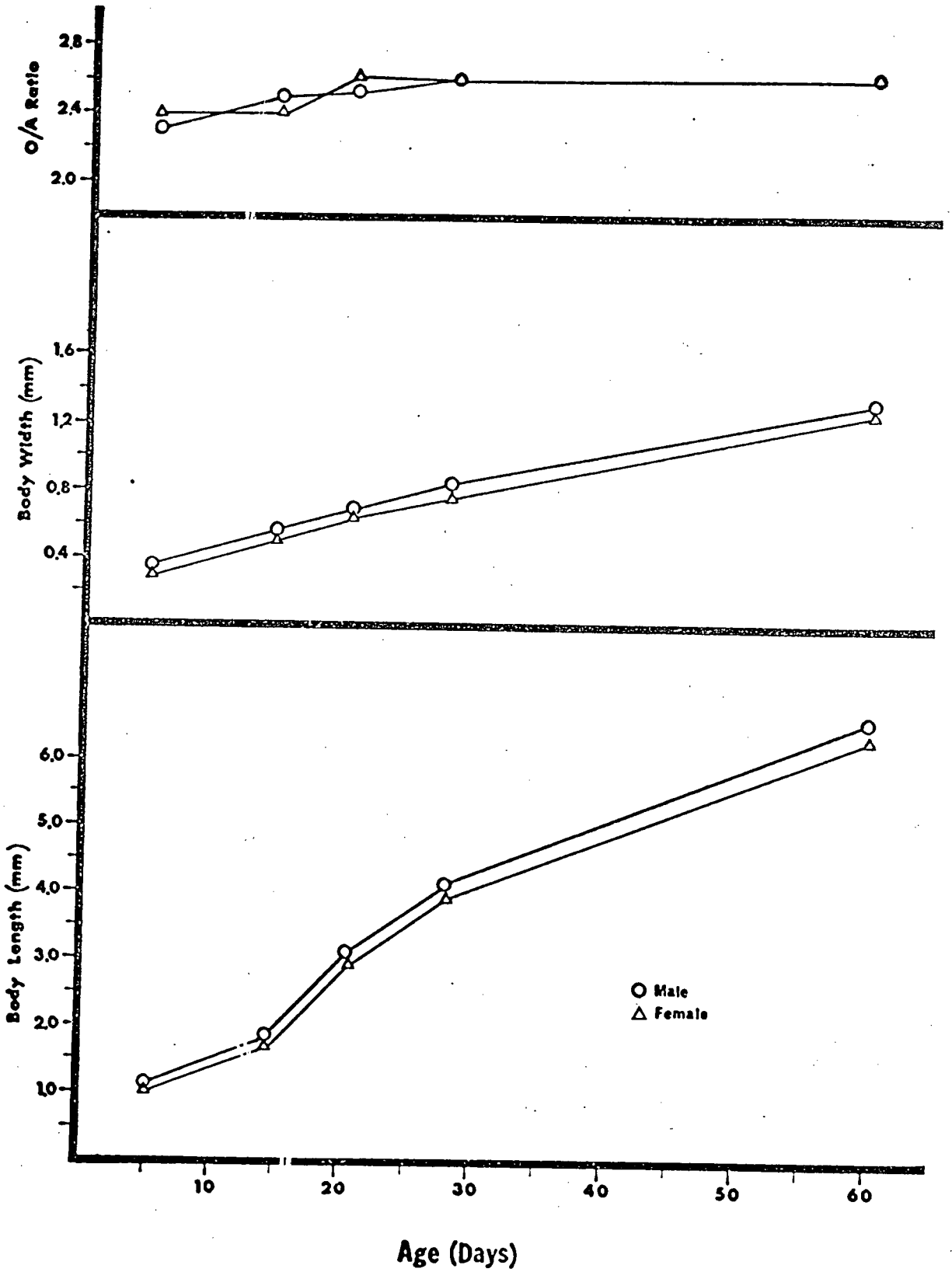
Materials and Methods

Fifteen male and fifteen female *R. pretiosa* were each infected with 10 metacercariae which had developed in *I. perparva*. Frogs were maintained at 20°C, and samples of three frogs were taken from each group at intervals of 5, 14, 21, 28 and 60 days.

Results

Adult *H. buttensis* developed in either male or female frogs show no significant differences in morphology. Figure 25 illustrates this for body length, body width, and O/A ratio. Values for other characters measured can be found in Appendices 14 and 15.

Fig. 25 The influence of host sex on body length, body width, and O/A ratio of flukes developed in either male or in female Rana pretiosa.


O/A Ratio
2.8
2.4
2.0
Body Width (mm)
1.6
1.2
0.8
0.4
Body Length (mm)
6.0
5.0
4.0
3.0
2.0
1.0
O Male
△ Female
10
20
30
40
50
60
Age (Days)

6. EFFECT OF WORM BURDEN[1] ON DEVELOPMENT OF *H. BUTTENSIS*

Introduction

Worm burden resulting in crowding can be an important factor in regulating the size of flukes (Blankespoor, 1970). This experiment was conducted to determine the effect of varying numbers of worms on size of adult *H. buttensis*.

Materials and Methods

Five groups of three male *R. pretiosa*, of approximately the same size (45-50), were fed either 10, 20, 40, 80 or 160 metacercariae. Infected frogs were maintained for 60 days at 20°C on a diet of beef heart and earthworms.

Results

The effect of degree of "crowding" on 930 experimental worms reared in *R. pretiosa* is summarized in Table 4 from data in Appendix 16. The number of worms recovered from frogs increased with increased number of metacercariae fed. However, the percentage of gravid worms decreased significantly. An analysis of the data, using linear regression, gave a coefficient, b=-0.19 which had a significance of 0.05 when a t-test was used. The percent survival of metacercariae decreased initially and then increased slightly (Table 4). The numbers of worms recovered from the left lung were the same as that for the right lung. The percent gravid flukes for left and right lung

---

[1]Worm burden is the number of worms recovered from the host

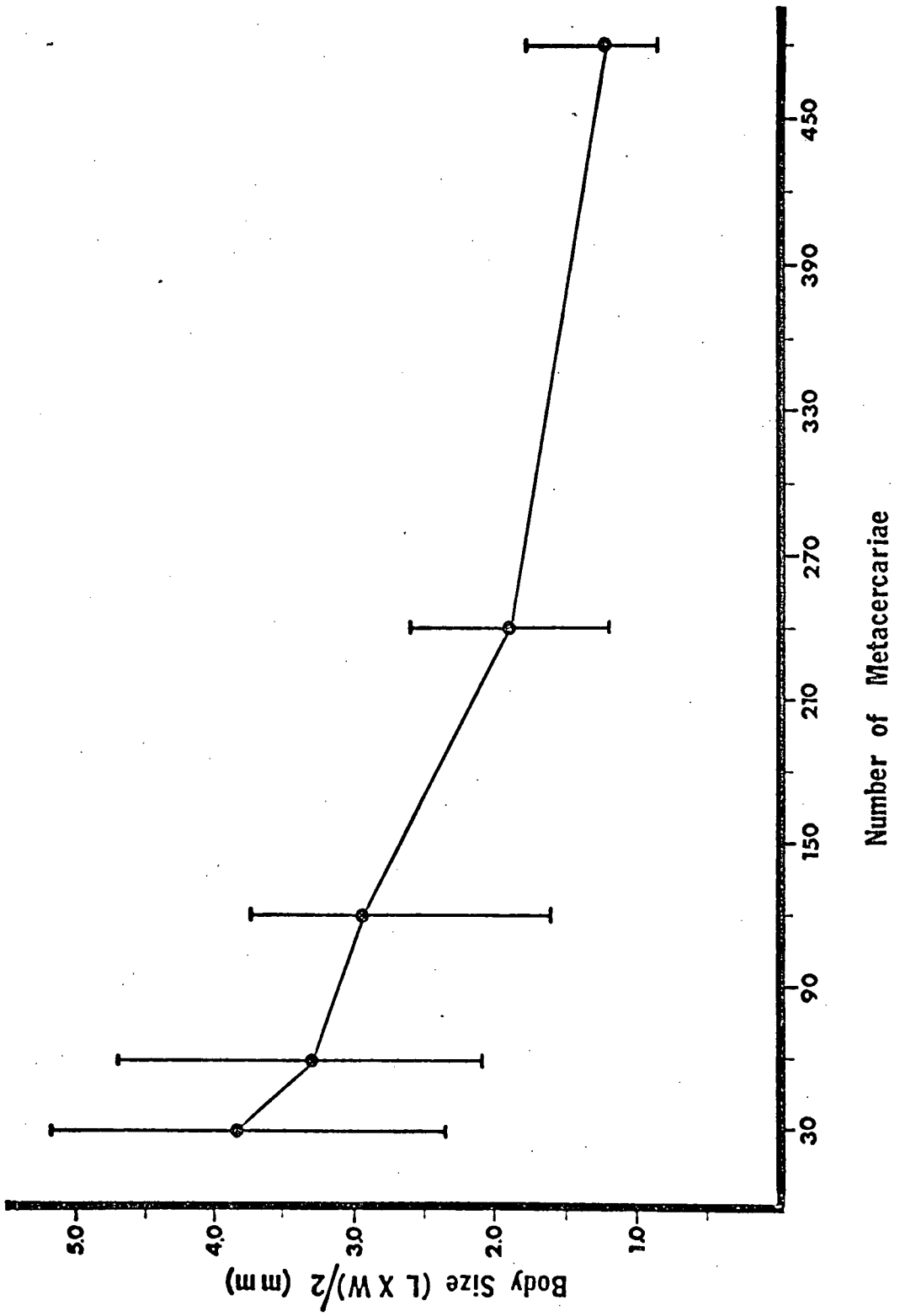
did not differ significantly (X^2, $P>0.05\%$). The average body size of flukes decreased with increased numbers of metacercariae fed (Fig. 26). Worms recovered from frogs fed 160 metacercariae were on an average 1/3 the size as those recovered from frogs fed only 10 metacercariae.

Table 4. Effects of crowding on 60-day-old H. buttensis in Rana pretiosa.

# metacercariae	# worms recovered	% survival	# worms Lung Left	# worms Lung Right	# gravid worms Lung Left	# gravid worms Lung Right	% gravid worms Lung Left	% gravid worms Lung Right	*average body size (LxW)/2
30	23	76.7	14	9	14	9	100	100	3.77(2.38-5.29)
60	46	76.7	25	21	21	21	84.0	100	3.36(2.13-4.73)
120	84	70.0	41	43	28	27	68.3	63.0	2.91(1.63-3.75)
240	122	50.8	62	60	32	34	51.6	56.7	1.95(1.31-2.63)
480	306	63.8	148	158	59	52	39.9	32.9	1.26(0.89-1.80)
		Total	290	291	154	143			

*measurements in square millimeters

Fig. 26 Decrease in body size of *H. buttensis* after 60 days of development in *R. pretiosa* which were fed either 30, 60, 120, 240 or 480 metacercariae.
The mean and ranges are graphed.


Body Size (L X W)/2 (mm)
5.0
4.0
3.0
2.0
1.0
30
90
150
210
270
330
390
450
Number of Metacercariae

7. VARIATION IN WORMS DEVELOPED IN DIFFERENT DEFINITIVE HOSTS

Introduction

Increasing attention has been focussed in recent years on the variations in a parasite's structure, physiology, and behaviour resulting from its development in different host species (Kinsella, 1971). The following experiments were conducted to determine if morphological changes in adult H. buttensis occur when they are reared in different amphibian hosts. Ninety amphibians representing six species of definitive hosts were used.

Materials and Methods

Metacercariae, obtained by the method described for "Development in Natural Hosts" (p.31), were fed to the following definitive hosts: Bufo boreas Baird and Girard, 1852; Hyla regilla Baird and Girard, 1852; Rana aurora Baird and Girard, 1852; Rana clamitans Latreille, 1802; Rana pipiens Schreber, 1782; and Rana pretiosa.

All experimental hosts were caught in the wild from non-infected populations; collecting localities for hosts are given in Table 5. Wild-trapped hosts were held in the laboratory for 21 days, and the feces were checked periodically to verify that natural infections of lung flukes were not present.

Fifteen frogs of each species were infected in the manner described on page 31. All frogs were maintained at 20°C on a diet of beef heart supplemented with earthworms during the developmental period of the parasites.

Samples of three frogs of each species were examined at 5, 14, 21, 28 and 60 days. Specimens of H. buttensis from the various hosts were then compared with specimens of similar age from R. pretiosa as well as with younger and older worms.

Table 5. Collecting sites for uninfected definitive hosts.

Host	Collecting locality
Bufo boreas	Haney, B.C.
Hyla regilla	U.B.C. Endowment Lands
Rana aurora	Chemainus Lake, near Duncan, B.C.
Rana clamitans	Blaine, Washington
Rana pipiens	Foxborough, Ontario
Rana pretiosa	15 miles east of Lumby, B.C.

Results

Comparative measurements of *H. buttensis* from experimental hosts are presented in Appendices 17 to 20. Most rapid development took place in the natural host *R. pretiosa* and in *B. boreas*, which is infrequently found naturally infected. Development in *R. aurora* was the same as that found for *R. clamitans*; in both, the flukes took about one week longer to produce eggs than did flukes developing in *B. boreas* or *R. pretiosa*. No worms were found in *R. pipiens* or *H. regilla* at any time during the sampling period (Table 6); they were judged to be unsuitable hosts.

Figures 29 to 34 and Figures 36 to 38 illustrate some variations occurring in 60-day old worms from *R. pretiosa*, *R. aurora*, *R. clamitans* and *B. boreas*.

Body size:

Adult *H. buttensis* recovered from *R. pretiosa* were usually larger than those obtained from *B. boreas*, *R. aurora* or *R. clamitans* for all ages sampled. Therefore, the former host, of those tested, appears to be the optimal one at sixty days. *H. buttensis* from *R. pretiosa* had an average length 6.41 (Fig. 27). Sixty-day old flukes developed in *R. aurora* or in *R. clamitans* were 4.95 and 4.88 long, respectively and in *B. boreas* 5.35 (Fig. 27).

Table 6. Variations in rate of development of H. buttensis maturing in various amphibian hosts.

Host	First appearance of gravid worms* (days)
Bufo boreas	21
Rana aurora	28
Rana clamitans	28
Rana pipiens	No worms present
Rana pretiosa	14
Hyla regilla	No worms present

*Determined by dissecting frogs, removing H. buttensis and preparing slides of the worms.

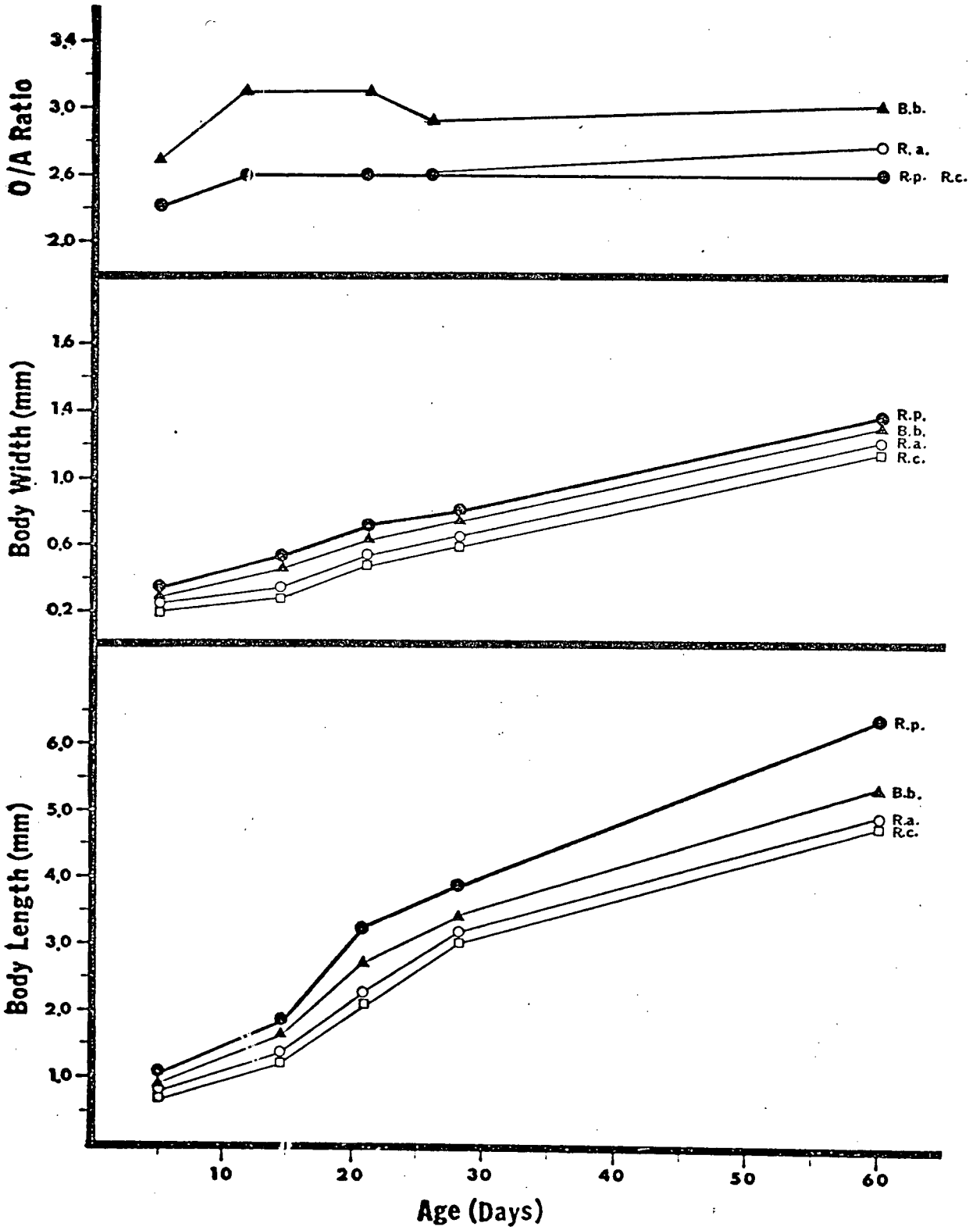
Fig. 27 The relationship between body length, body width, and O/A ratio of *H. buttensis* developed in different frog hosts.

R. a. *Rana aurora*

R. c. *Rana clamitans*

R. p. *Rana pretiosa*

B. b. *Bufo boreas*


O/A Ratio
3.4
3.0
2.6
2.0
B.b.
R. a.
R.p. R.c.
Body Width (mm)
1.6
1.4
1.0
0.6
0.2
R.p.
B.b.
R.a.
R.c.
Body Length (mm)
6.0
5.0
4.0
3.0
2.0
1.0
R.p.
B.b.
R.a.
R.c.
10
20
30
40
50
60
Age (Days)

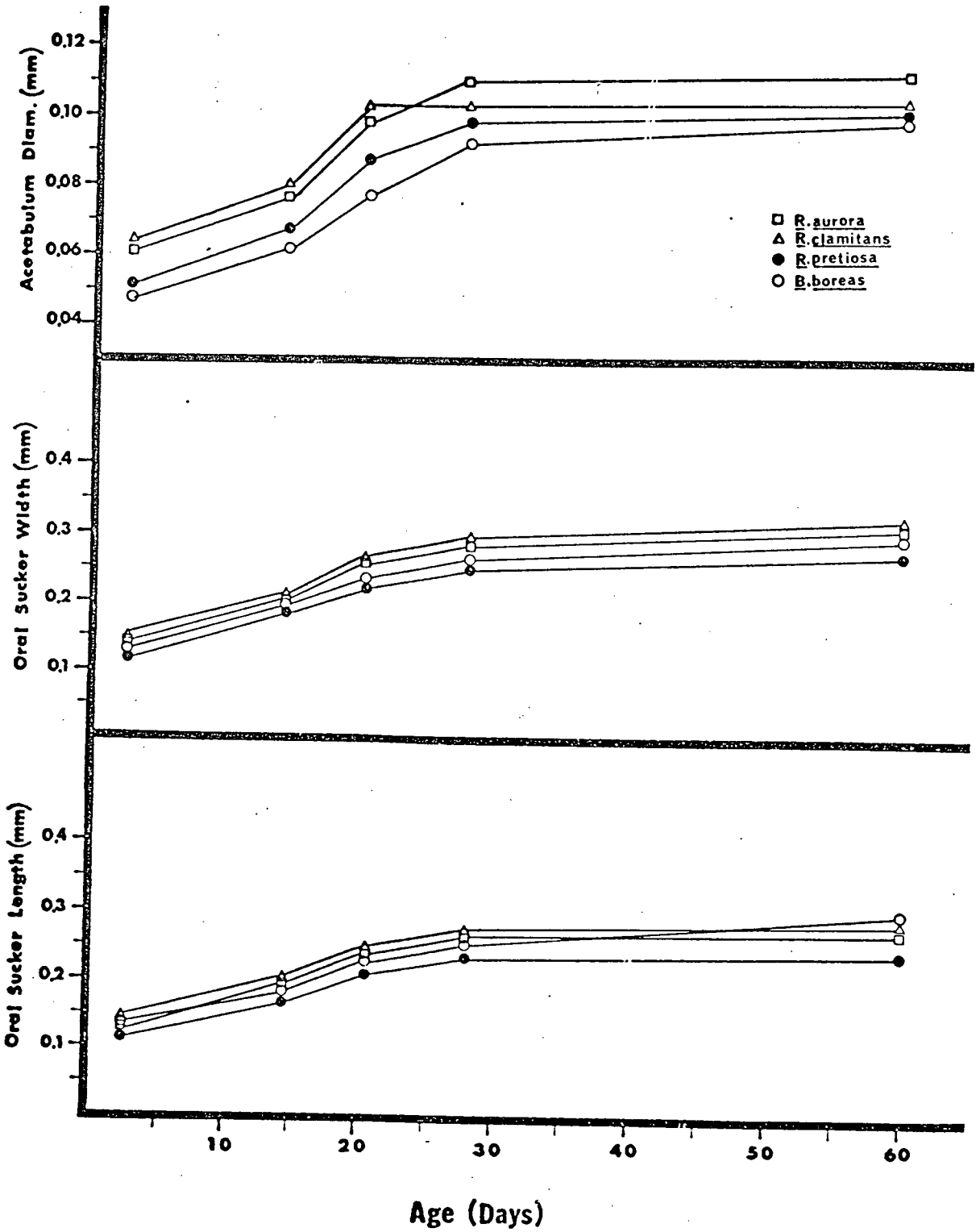
Flukes recovered from R. clamitans 28 and 60 days post-infection were the smallest of comparably aged worms recovered during this study (Fig. 27). Worms recovered from B. boreas were intermediate in size.

Average body widths were greater for flukes from R. pretiosa at all ages examined. Average body widths for 60-day-old flukes from R. pretiosa were 1.40; from B. boreas, 1.37, and from R. aurora and R. clamitans 1.20 and 1.17 respectively (Fig. 27). Worms, regardless of host, showed an increase in length and width with increased age of fluke.

Sucker size:

The oral sucker was consistently larger than the acetabulum in all flukes observed. Oral sucker width varied in flukes harboured by different host species. The maximum width (0.372) of the oral sucker was attained by a 60-day-old fluke developed in B. boreas. The maximum diameter of the acetabulum was 0.123 which occurred in a 60-day-old worm developed in R. clamitans. The smallest diameter in a 60-day-old worm occurred in B. boreas (0.082). The O/A ratio of mature worms developed in B. boreas was greater (2.9-3.1) than for flukes developed in the other host species (2.4-3.1). The larger O/A ratio in flukes from B. boreas is a result of smaller acetabulum diameter in those worms.

Fig. 28 The effect of different amphibian hosts on oral sucker length, oral sucker width, and acetabulum diameter of H. buttensis.


Acetabulum Diam. (mm)
0.12
0.10
0.08
0.06
0.04
R.aurora
R.clamitans
R.pretiosa
B.boreas
Oral Sucker Width (mm)
0.4
0.3
0.2
0.1
Oral Sucker Length (mm)
0.4
0.3
0.2
0.1
10
20
30
40
50
60
Age (Days)

Ovary size and shape:

Variations in gonad size in adult H. buttensis recovered from different host species are even more pronounced than variations in this organ in flukes obtained from a single host species.

Flukes possessing unusually large ovaries were obtained from experimental infections in R. clamitans (Figs. 31, 33 and 35). The ovary of one adult sixty-day-old H. buttensis from R. clamitans measured 0.85 long and 0.73 wide. The ovary did not become apparent until 21 days after infecting frogs. This was also true for flukes developed in R. aurora. Infections in R. aurora yielded H. buttensis adults with small ovaries. The smallest ovary in a 60-day-old fluke from R. aurora was 0.392 long by 0.113 wide.

The largest testes were found in one adult 60-day-old worm from R. clamitans, the anterior testis of which measured 1.32 long by 0.58 wide and the posterior testis measured 1.46 long by 0.65 wide. The smallest testis was found in a sixty-day-old worm from R. aurora; the anterior testis measured 0.62 long by 0.31 wide, and the posterior testis 1.25 long by 0.28 wide (Fig. 35).

Gonad size and uterine loop variation in flukes developed in different definitive hosts.

Fig. 29 Fluke developed in Rana pretiosa.

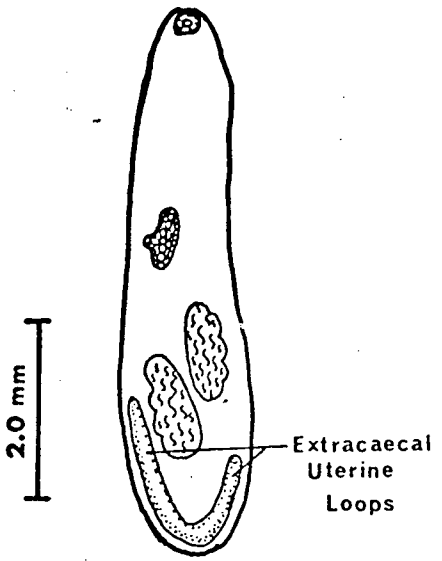
Fig. 30 Fluke developed in Rana aurora.

Fig. 31 Fluke developed in Rana clamitans.

Fig. 32 Fluke developed in Bufo boreas.

Fig. 33 Fluke developed in Rana clamitans.

Fig. 34 Fluke developed in Rana aurora.



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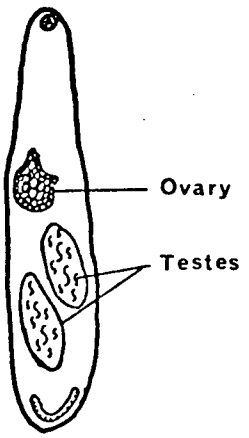
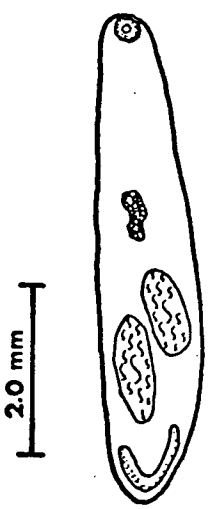


31

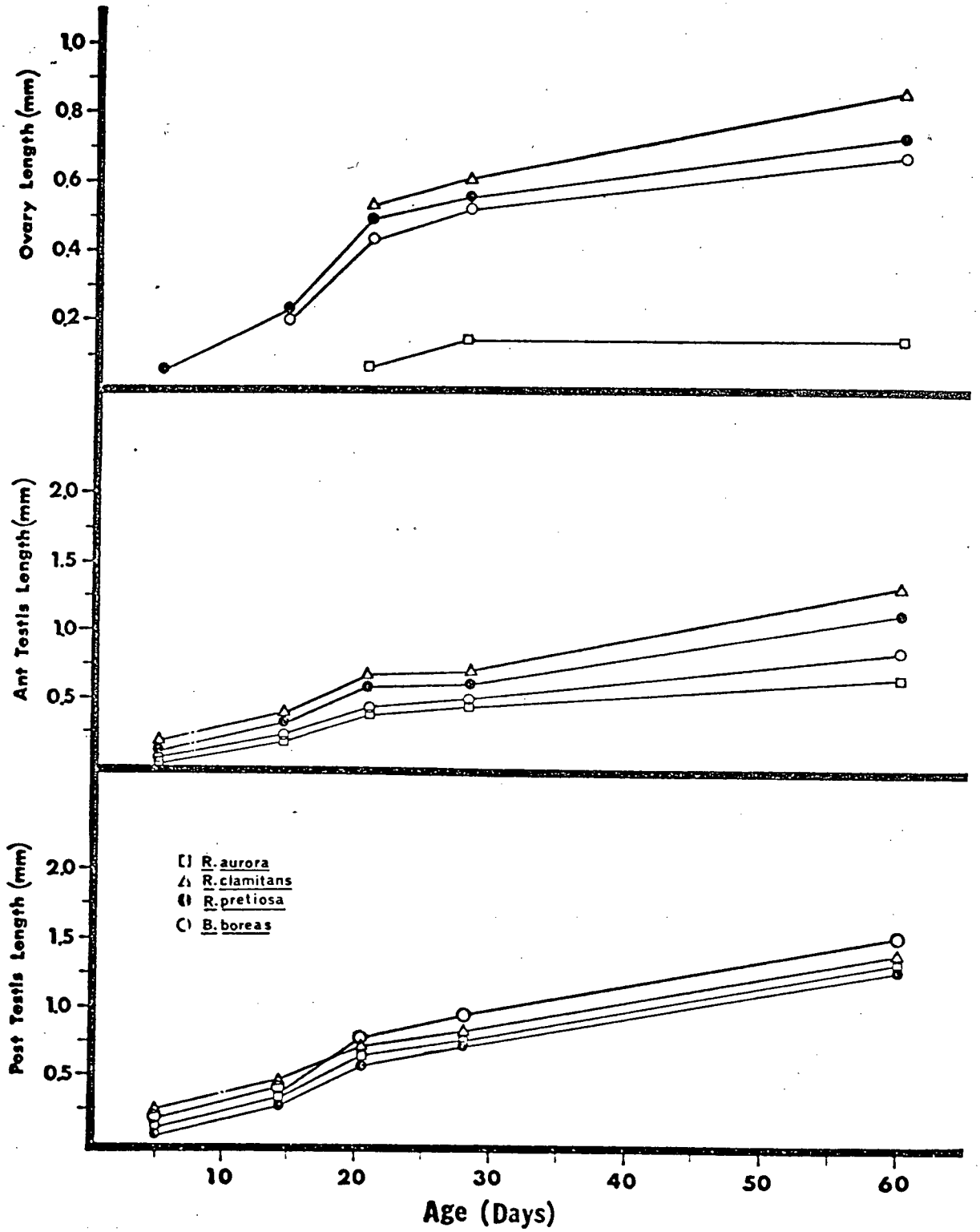


32

33

34

Fig. 35 The effect of different amphibian hosts on testes and ovary length of H. buttensis.


Ovary Length (mm)
1.0
0.8
0.6
0.4
0.2
Ant Testis Length (mm)
2.0
1.5
1.0
0.5
Post Testis Length (mm)
2.0
1.5
1.0
0.5
R. aurora
R. clamitans
R. pretiosa
B. boreas
10
20
30
40
50
60
Age (Days)

Vitellaria:

Vitellaria became evident 14 days post-infection in B. boreas and R. pretiosa but only after 21 days in R. clamitans and R. aurora. The length of the vitellaria on the right side of the worm was consistently shorter than that on the left (Figs. 36 to 38). The anterior limit of vitelline follicles occurred between the acetabulum and pharynx in 60-day-old worms recovered from all hosts. Vitelline follicles in flukes from R. aurora and R. clamitans reached a level just anterior to the acetabulum (Figs. 37 and 38). However, in worms from R. pretiosa and B. boreas, vitelline follicles extended further anterior to the region between the acetabulum and pharynx (Fig. 36). Specimens from R. pretiosa and B. boreas had a solid band of vitellaria across the body between the pharynx and acetabulum. In specimens from R. aurora and R. clamitans the vitelline band often lay beneath the acetabulum. Incomplete bands were sometimes observed, and in three cases a transverse band was absent (Fig. 38). Much greater variability characterizes the posterior limits of the vitelline follicles in specimens recovered from all amphibian hosts. A band of vitelline follicles usually extended medially half-way across the worm from the posterior part of the left vitelline band passing posteriad of the posterior testis.

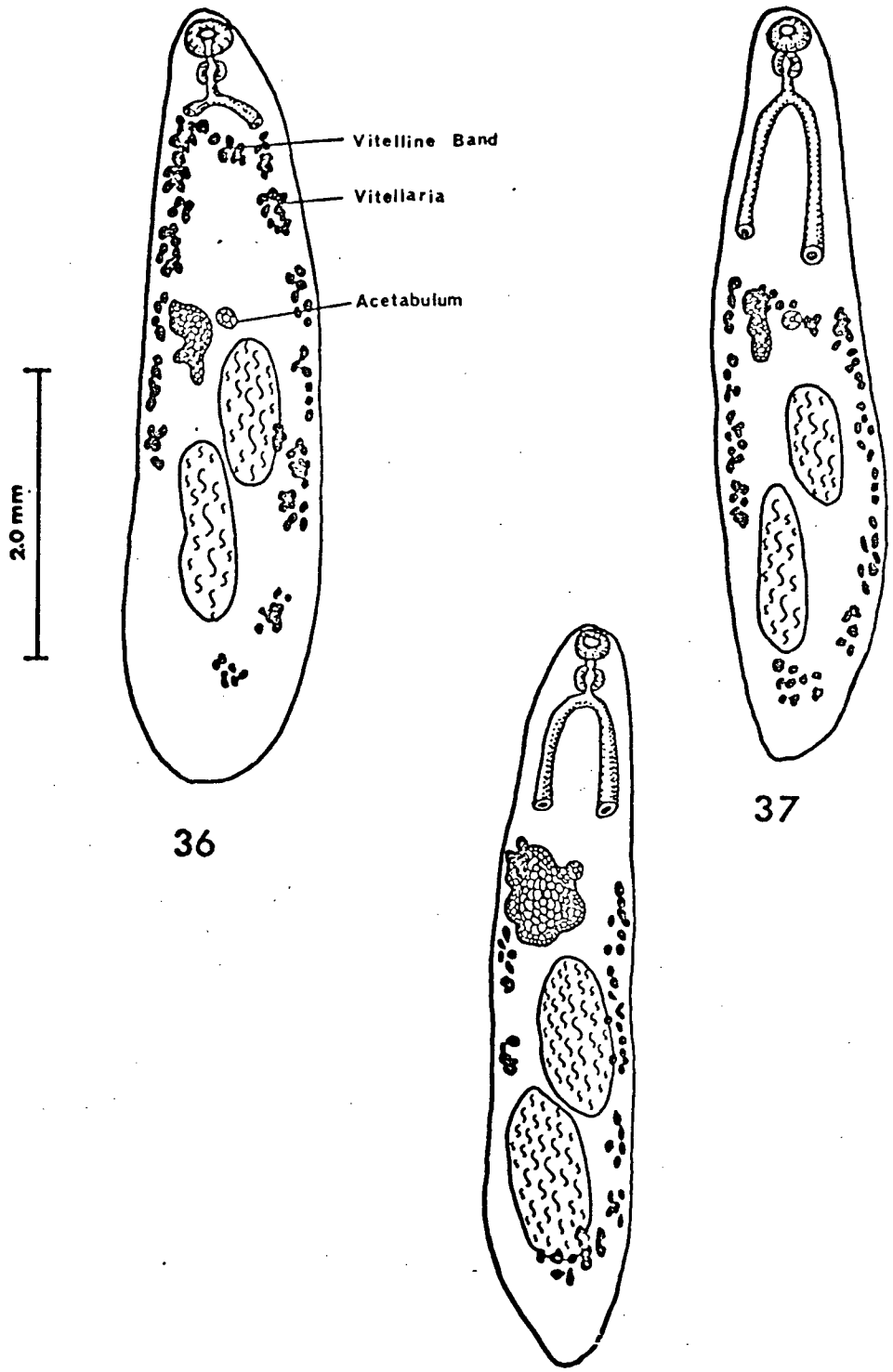
The experimental studies demonstrate that the presence or absence of a vitelline band, or the extent of the anterior or posterior vitellaria, are unreliable criteria for distinguishing species within this genus.

Fig. 36 Distribution of vitellaria in worms developed in Rana pretiosa.

Fig. 37 Distribution of vitellaria in worms developed in Rana aurora.

Fig. 38 Distribution of vitellaria in worms developed in Rana clamitans showing absence of vitelline band.

All flukes drawn in ventral view.



36

37

38

Extracaecal folds of the uterus:

Extracaecal longitudinal folds of the uterus were first evident at 14 days post-infection in R. pretiosa and B. boreas but did not become evident until the twenty-eighth day in R. clamitans or R. aurora. The right longitudinal fold was longer than the left in specimens developed in R. pretiosa. The reverse was true for specimens developed in B. boreas (compare Figs. 29 with 32). The right and left loops were of about the same length in specimens from R. aurora and R. clamitans.

The right uterine folds of 60-day old worms developed in R. pretiosa reached a distance half-way along the posterior testes. The left loop just reached the posterior part of the posterior testis (Fig. 29). Extracaecal loops reached about half-way to the posterior testis in worms developed in B. boreas. The left loop extended somewhat beyond the right (Fig. 32). These uterine loops extended only 1/3 the way to the posterior testis in 60-day-old worms developed in R. aurora and R. clamitans (Figs. 30,31,33 and 34).

Development in different definitive hosts altered the anterior extent of the uterine loops. The uterine loops extended the farthest in the natural host R. pretiosa, and the least in R. aurora and R. clamitans.

Spination:

Spines were uniformly distributed over the entire body surface of flukes of all ages from all hosts.

Egg size:

Eggs were not present in the uterus until 14 days post-infection in R. pretiosa; 21 days in B. boreas; and 28 days in R. aurora and R. clamitans. Eggs were of approximately the same length and width in 60-day old worms developed in R. pretiosa, R. clamitans and B. boreas. Eggs were smallest in worms from R. aurora (Appendices 17-20).

8. VARIATION DUE TO DEVELOPMENT IN DIFFERENT INSECT INTERMEDIATE HOSTS.

Introduction

Having demonstrated that some taxonomic characters are altered when flukes develop in different definitive hosts, we must now establish whether changes occur when worms develop in different intermediate hosts.

Materials and Methods

Infected snails, P. nuttalli, maintained at 20°C on a diet of boiled lettuce, shed xiphidiocercariae (of the "ornata group") of H. buttensis approximately 28 days after ingestion of the eggs.

Insect nymphs were collected from a small pond in Haney, B.C. This pond does not contain any frogs; naturally-occurring infections of Haematoloechus sp. are therefore absent.

Ten cercariae recovered from experimentally infected snails were placed in each of sixty 60-mm plastic petri dishes. Twenty dishes contained one naiad each of the damselfly, Ischnura perparva, twenty contained I. cervula and twenty contained one larva each of the dragonfly Aeschna palmata. All larvae were approximately 16 mm long. Cercariae and insects were left together for five hours, then naiads were removed and washed, and all naiads of a species were placed in a 100-mm plastic petri dish. Five days later, metacercariae from the two species of damselflies and the dragonfly were judged to be infective to frogs. Groups of fifteen R. pretiosa, 50-55 mm

long, were infected with metacercariae from one of the insect hosts. Frogs were maintained at 20°C. Samples of three frogs were examined from each group 5, 14, 21, 28 and 60 days post-infection.

Results

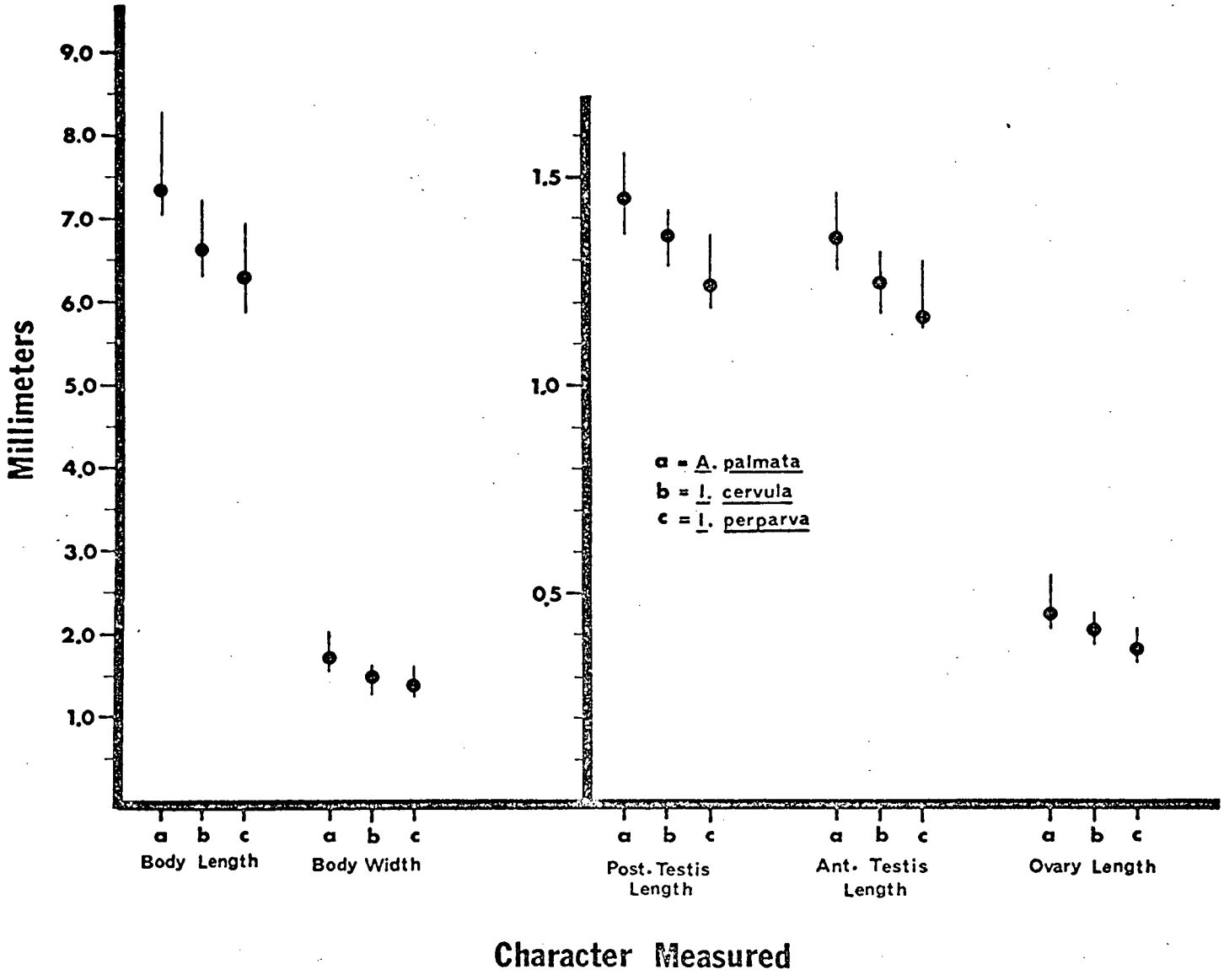
Flukes recovered from frogs fed infected damselflies differed from those from frogs fed infected dragonflies in having a smaller body size (length and width), smaller gonad size, and shorter extent of the extracaecal uterine loops (Table 7). The mean and range for body length and width, and gonad lengths are shown in Figure 39. The average length of worms with I. cervula or I. perparva intermediate hosts is 6.63 and 6.39, respectively. Worms averaged 7.33 long when Aeschna palmata was the insect host. Body width measured 1.52, 1.46 and 1.69, respectively. Larger average gonad size was attained in worms that had A. palmata as their insect host and in these worms both extracaecal loops extended the full length of the posterior testis. The left extracaecal loop extended 1/4 to 1/3 the length of the posterior testis and the right loop extended 1/2 to 3/4 the length of the posterior testis when the insect host is either I. cervula or I. perparva. The ratio of the oral sucker diameter to acetabulum diameter was the same (2.4 to 2.7) regardless of insect host.

Table 7. Morphological variation in 60-day old H. buttensis reared in Ischnura cervula, Ischnura perparva, or Aeschna palmata as the second intermediate host.

Character	Intermediate host I. cervula	I. perparva	A. palmata
Body length	6.63(6.27-7.24)	6.39(5.88-6.93)	7.33(7.08-8.35)
Body width	1.52(1.33-1.60)	1.46(1.37-1.68)	1.69(1.61-2.01)
O/A ratio	2.5 (2.4 -2.6)	2.5 (2.4 -2.7)	2.6 (2.4 - 2.7)
Ovary length	0.79(0.74-0.82)	0.73(0.69-0.79)	0.86(0.81-0.94)
Ovary width	0.41(0.37-0.45)	0.38(0.34-0.42)	0.45(0.42-0.53)
Ant. testis length	1.25(1.18-1.32)	1.16(1.14-1.30)	1.36(1.28-1.47)
Ant. testis width	0.52(0.49-0.61)	0.46(0.40-0.57)	0.54(0.47-0.69)
Post. testis length	1.33(1.29-1.42)	1.24(1.19-1.36)	1.45(1.33-1.56)
Post. testis width	0.57(0.50-0.65)	0.52(0.49-0.67)	0.61(0.53-0.76)
Extracaecal loops L.[1]	1/4 to 1/3 PT.	1/4 to 1/3 P.T.	full length
Extracaecal loops R.	1/2 to 3/4 P.T.	1/2 to 3/4 P.T.	full length

[1]Extent of extracaecal uterine loop along the posterior testis.

Fig. 39 Variations in some body measurements of H. buttensis reared in different insect intermediate hosts.


Millimeters
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
1.5
1.0
0.5
a = A. palmata
b = I. cervula
c = I. perparva
a b c
Body Length
Body Width
Post. Testis Length
Ant. Testis Length
Ovary Length
Character Measured

9. MORPHOLOGICAL VARIATION IN H. BUTTENSIS WHEN CERCARIAE ARE DEVELOPED IN VARIOUS SNAIL HOSTS.

Introduction

Underwood and Dronen (1977) noted that measurements of cercariae shed from ancylid snails (Ferrissia sp.) experimentally infected with H. breviplexus, differed from those of the same lung fluke given by Schell (1965), who used Gyraulus sp. as molluscan host. Underwood and Dronen did not compare the adult stages. However, they noted that the differences may indicate that these flukes, identified as H. breviplexus in Idaho and Texas, are not the same species. They stated that cross-infection studies, utilizing hosts and parasites from Idaho and Texas, are necessary to determine that the differences are not a result of host-induced variation.

Materials and Methods

To determine the possible effects of various first intermediate hosts on the morphology of adult H. buttensis, experimental infections of Physa nuttalli, Stagnicola elodes and Helisoma trivolvis were attempted.

Eggs of H. buttensis used for experimental feedings were originally obtained from worms in naturally infected R. pretiosa collected in Manning Park. Flukes removed from the lungs were placed in dechlorinated tapwater. After one hour, mature eggs released by the worms were fed to 30 each of the three species of snails.

Cercariae which developed in and were shed from snails were

placed in 100-mm plastic petri dishes. Each dish contained ten cercariae and one I. perparva. Twenty naiads in all were infected in this way. Five days later the metacercariae were fed to R. pretiosa 50-55 mm in snout-vent length. Fifteen frogs were exposed and maintained at 20°C. Samples of three frogs were examined at 5, 14, 21, 28 and 60 days.

Results

Only Physa nuttalli became infected. Infected snails shed cercariae approximately 28 days after ingestion of the eggs. Flukes recovered from R. pretiosa which were previously developed in I. perparva and P. nuttalli showed no morphological variations different from those in experiments on "Development in Rana pretiosa" (Appendix 21).

10. DISCUSSION AND CONCLUSIONS FROM EXPERIMENTAL RESULTS

Body Size:

The length and width of the body of H. buttensis increased by as much as 78% and 140% respectively when weight was added to the coverslip during the drying process of slide preparation.

In this study, reduced growth rate in H. buttensis occurred in flukes maintained at 12°C. Worms maintained in hosts at higher temperatures (20°C and 27°C) were larger than flukes of a comparable age maintained in hosts kept at lower temperatures.

Studies on the effects of temperature on trematode development are limited. Willey (1941) observed that the trematode Zygocotyle lunata (Diesing, 1836) matured more rapidly in ducks than in rats. He attributed this to the higher body temperature of birds.

Wohlgemuth (1920) and later Izyumova (1956) reported that variations in water temperature affect development of the monogenetic trematode, Dactylogyrus vastator Nybelin, 1924, a parasite on the gills of fish.

Watertor (1965) found that the growth and development of adult Telorchis bonnerensis Waitz, 1960 were greatly altered when experimentally infected definitive hosts (adult Ambystoma tigrinum Green, 1825) were maintained at various constant temperatures of 10°, 22°, 30° and 34°C. Studies on other parasitic organisms have demonstrated similar responses to temperature.

Smaller H. buttensis were recovered from R. pretiosa experimentally infected with larger numbers of metacercariae.

The body size of flukes decreased as much as two-thirds in heavily infected frogs (Table 4). The number of worms present in a host has been shown to be an important size-controlling factor in trematodes (Lyubinski and Kulakovskaya, 1940; Willey, 1941; Dawes, 1962; Watertor, 1965, and others). This has also been demonstrated for nematodes (Chitwood, 1957; Haley and Parker, 1961) and tapeworms (Pavlovski and Gnezdilov, 1949, 1953; Brooks and Mayes, 1976; and others). Rankin (1937) observed that specimens were unusually small when large numbers of worms were present in natural infections of the salamander trematodes, Brachycoelium, Plagitura, and Megalodiscus sp.

Body lengths were on an average 18%–30% greater in 60-day-old H. buttensis reared in R. pretiosa than in those reared in amphibian hosts which are seldom naturally infected. Similarly, 60-day-old flukes were as much as 15% longer when the dragonfly naiad was used as second intermediate host than when a damselfly naiad was used.

Experimental studies by Beaver (1937) on Echinostomum revolutum Froelich, 1802 developing in avian and mammalian hosts, the work of Rankin (1937) on Brachycoelium sp. collected from various species of naturally infected salamanders, and studies by Wharton (1940) and Watertor (1967, 1968) on several species of Telorchis sp. from chelonian and amphibian hosts, present further evidence that morphological changes may occur when adult trematodes mature in different species of definitive hosts. Boddeke (1960b) studied experimental infections of Prosthogonimus ovatus and observed great morphological variations among these flukes from different

avian hosts. Blankespoor's 1974 studies on Plagiorchis noblei in avian hosts demonstrate th flukes recovered from passerine birds were usually larger (in length and width) than those obtained from gallinaceous birds.

Development of H. buttensis in four size classes of R. pretiosa did not greatly affect the morphology of this fluke. As well, fluke morphology was not affected by the sex of the host.

Herrick (1925) and Ackert (1935) observed marked differences in length of Ascaridia galli (Shrank, 1788) from chickens of varying ages. Worms from older birds were significantly smaller than those from younger birds. Bouchard (1951) found the parasites in larger hosts (frogs of various species) to be larger regardless of the parasite species. According to Read and Rothman (1958), the size of Hymenolepis diminuta (Rudolphi, 1819) was a function of the age of the rat host. Bourns (1966), working with natural infections of Plagiorchis noblei in nestling red-winged blackbirds, found a higher percentage of gravid adults in older hosts. A longer time was required to become gravid in domestic chickens.

Blankespoor (1974) infected 1-, 7- and 20-day old laboratory-reared house sparrows and noted that age of sparrows did not greatly affect the body size of adult P. noblei.

Gonad Size and Shape:

The length and width of ovary increased as much as 27% and 64% respectively when weight was added to the cover slip during slide preparation. Similarly, anterior and posterior testes

increased as much as 32% and 50% in length and width, respectively.

A major problem in identifying species of the genus Haematoloechus has been the lack of experimental data on character changes with increasing size of worms. My experimental studies on H. buttensis reared in adult R. pretiosa provided evidence of pronounced age-related variation in gonad size and shape. Both the ovary and testes continued to increase in length and width during the study period. Ovaries changed from smooth to highly lobed, and testes either remained smooth or became highly lobed.

Several authors have used the degree of lobing in ovaries and in testes as a species-specific character. Ingles (1936) used it to help him distinguish H. buttensis (ovary never lobed) from H. parviplexus (ovary deeply lobed). Ingles (1932) also used it to separate H. confusus (ovary and testes lobed) from H. coloradensis (ovary and testes unlobed).

Manter (1938) in his key to North American species of frog lung flukes made extensive use of degree of lobation of testes and ovary to separate species.

The ovary and testes were smaller in worms maintained in hosts at 27°C compared with worms developed at 20°C. This response to temperature produced longer and wider worms with smaller gonads at 27°C than at 20°C. This is an important observation because frogs are often collected from different areas with vastly different environmental conditions

The size of the ovary and testes and degree of lobation of these organs were also affected when worms were reared in

different definitive hosts. Flukes possessing unusually large ovaries were obtained from experimental infection in R. clamitans. Infections in R. aurora yielded adult worms with small ovaries. The longest anterior testes were found in worms reared in R. clamitans, the smallest in worms from R. aurora. However, the longest posterior testes were found in worms reared in B. boreas and the smallest in R. pretiosa.

Sucker Size and O/A Ratio:

The length and width of the oral sucker and acetabulum of H. buttensis increased when weight was added to the coverslips during slide preparation. Sucker size also varies with age of worm and host species. An increase in size of the suckers occurred during the 60-day study period. However, the O/A ratio remained fairly constant regardless of age of worm or method of PREPARATION.

Blankespoor (1970) determined the effects of flattening and fixation on morphology of adult Plagiorchis noblei Park, 1936 developed in a five-day old chick. He found that various degrees of flattening had little effect on the size-ratio of muscular structures such as the oral sucker and acetabulum. Similar results were found by Boddeke (1960a) in his studies with the fluke Prosthogonimus ovatus Rudolphi, 1803. He concluded that the only taxonomic character useful in distinguishing species within that genus is the ratio between the diameter of the oral and ventral suckers.

Blankespoor (1974) reported the unreliability of sucker size in Plagiorchis noblei reared in a variety of avian and

mammalian hosts. However, he noted that the O/A ratio was a stable taxonomic character in adults of this species. Berrie (1960). Boddeke (1960a) and Watertor (1967) have also found significant differences in sucker size between adult flukes recovered from different host species.

Spination:

My experiments demonstrated that distilled water caused live H. buttensis to lose spines; the warmer the water the more rapid the loss. This finding is important because some workers (Cort, 1915a) assembled their specimens in water before fixation. The presence or absence of spines has been used to separate the following species:

H. coloradensis (Cort, 1915a) (spined) from H. complexus (Seely, 1906) (no spines)

H. confusus Ingles, 1932 (spines) from H. oxyorchis Ingles 1932 (no spines)

H. tumidus Ingles, 1932 (spined) from H. kernensis Ingles, 1932 (no spines)

Spination was not affected by: fluke age; species, sex or size of host; or temperature at which the infected host was maintained.

Egg Size:

Egg size did not differ in flukes older than 21 days. Eggs averaging 0.024 by 0.016 in size were found in the anterior portion of the uterus in 21- to 60-day old worms. Egg size was not affected by any of the other variables investigated.

Extracaecal Loops:

The lengths of the extracaecal loops differed in flukes from different frog hosts as well as from different insect hosts (Figs. 29-35, Table 7). The variations found experimentally for H. buttensis overlap those reported as being a distinguishing feature for H. floedae and H. parviplexus . Measurements from three other described species that have extracaecal uterine loops (H. similiplexus, H. uniplexus and H. varioplexus) also fall within the range of those described here. This suggests that a single highly variable species may be present and not six distinct species.

Distribution of the Vitellaria:

This thesis has demonstrated that vitelline distribution was affected by developing in different definitive hosts. Vitelline follicles extended further anterior in worms reared in R. pretiosa or B. boreas than in worms reared in R. aurora or R. clamitans. The band of vitellaria extending across the worms developed in R. pretiosa and B. boreas was incomplete or absent in worms developed in R. aurora and R. clamitans.

Rankin (1937), Wharton (1940), Watertor (1967) and Blankespoor (1974) have also shown the unreliability of vitelline distribution as a taxonomic character.

Summary:

Several trematode species have been shown to undergo morphological changes when subjected to various methods of fixation or flattening. These changes make it very difficult to

compare the absolute size and shape of various flukes when different handling procedures are used. Thus, statements such as "It [H. varioplexus Stafford, 1902] rivals No.2 [H. breviplexus] in size" (Stafford, 1902, p.906); "the pharynx was somewhat smaller than is usual" [H. floedae Harwood, 1932] (Manter, 1938, p. 31) and "The specimens of H. longiplexus from R. pipiens are on the average smaller than those described by Stafford from the bullfrog" (Cort, 1915a, p. 213), are difficult to interpret.

Changes in fluke morphology resulting from different methods of fixation and flattening have also been demonstrated by Ulmer (1950), working with the trematode Postharmostomum helicis (Leidy, 1847); Gilford (1955), working with Allassogonoporus vespertilionis Macy, 1940 from bats; and by Angel (1959) working with Plagiorchis maculosus (Rudolphi, 1802).

Kavelaars and Bourns (1968) indicated that many of the differences seen in adult Plagiorchis peterborensis Kavelaars and Bourns, 1968 reared in a laboratory mouse (Mus musculus Linnaeus, 1758), resulted from various killing methods and degrees of flattening.

The validity of a species, in a group, should be carefully considered when characters used for species determination are altered by changing handling techniques. The degree of variation caused by various procedures should be studied for each group, and an effort be made to standardize these procedures.

A major problem in identifying species of the genus Haematoloechus has been the lack of experimental data on

host-induced morphological modifications.

In the present study I have demonstrated that adult H. buttensis can be experimentally established in four species of definitive hosts and three species of insect second intermediate hosts. H. buttensis has previously been described from two other frog hosts (R. boylei, and R. catesbeiana Shaw, 1802) (Appendix 23). The apparent lack of host specificity in laboratory infections, as well as the morphological variations which occurred in flukes developed in different frog and insect hosts, casts doubt on the use of host specificity alone for delineating species within the genus Haematoloechus.

Host specificity of adult trematodes has been used in separating one species from another; yet morphological differences that constitute the criteria for the definition of new species may be the result of the influence of the particular host species (Stunkard, 1957).

Rankin (1938) noted considerable variation in characters in species of Brachycoelium collected from twenty different species of salamanders. This variation rendered identification of the flukes with described species impossible. As a result of his study, Rankin (1938) reduced twelve species to synonymy. Similar conclusions from studies on other flukes have been arrived at by Beaver (1937), Boddeke (1960a), Kinsella (1971) and others.

The characters which appear to be the most stable in H. buttensis and consequently may have the greatest value for separating species of Haematoloechus are: the O/A ratio, egg size of 21-day old and older worms, position of the extracaecal loops relative the the testes and ovary, spination of the

tegument and position of the testes.

PART II. TAXONOMY AND MORPHOLOGICAL VARIATION

Introduction

Our present knowledge of species of Heamatoloechus from Canada and the United States is spotty. Most published reports of new species deal only with one or two localities which are usually close together (Stafford, 1902; Cort, 1915a; Ingles, 1932,1936; and others). Brooks (1976) has noted that a large number of amphibian parasite surveys have been published in North America, but in most cases, unless new taxa were described, no morphological data have been presented or specimens made available by deposition in a museum collection. To date no attempt has been made to collect over a broad geographical area and to make intraspecific and interspecific comparisons of characters regarded as important in separating species of this group.

A study was undertaken to assess the variation in certain characters from flukes collected from several localities in Canada and the United States. Emphasis is placed on those characters previously used to delineate species in this group.

Materials and Methods

Collections of trematodes made from British Columbia, Alberta, Saskatchewan, Washington and Oregon by the author, were examined for variation.

Shipments of live frogs were sent by air by several Canadian and American collectors to the University of British Columbia. Fresh material was dissected on arrival and flukes

recovered from the lungs were fixed in hot 70% alcohol and stained with Harris's Haematoxylin (see Appendix 3 for procedure). Host species, sex, size and presence of other worm parasites were also recorded. Two thousand one hundred and sixty-two lung flukes collected in this manner were examined. Slides of specimens borrowed from private and institutional collections were also examined for variation. The total number of specimens examined from these sources was 422.

Results

In the present study, 2851 amphibians, representing 14 species and five families, were collected and examined for natural infections of Haematoloechus by the author (Table 8). Natural infections were found in seven species representing two families. Within the family Ranidae, frog species R. catesbeiana, R. clamitans, R. pipiens, R. pretiosa and R. sylvatica LeConte, 1825 contained natural infections of Haematoloechus, as did toads Bufo americanus Holbrook, 1836 and B. boreas of the family Bufonidae. The highest percentage of natural infections was found in members of the Ranidae.

Other known definitive hosts of Haematoloechus in Canada and the United States are given in Appendix 23.

Of the 2851 frogs examined in this investigation, 1090 (38.2%) harbored infections of Haematoloechus. Prevalence[2] of natural infections among these hosts ranged from 2.9 to 64.0 percent. The highest prevalence of infection was in Rana pipiens

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[2]Prevalence is defined here as the percent of animals in a population which are infected.

(64.0%) and in R. pretiosa (46.5%).

Table 8. Summary of amphibian hosts examined for natural infections of Haematoloechus spp.

Host	# adults examined	# infected	*Lung fluke present
Ranidae			
Rana aurora	65	0	
R. catesbeiana	81	14	H. longiplexus
R. clamitans	112	11	H. longiplexus
			H. breviplexus
R. pipiens	602	385	H. medioplexus
R. pretiosa	1405	653	H. medioplexus
			H. buttensis
R. sylvatica	77	18	H. parviplexus
R. grylio Stejneger, 1901	32	0	
Hylidae			
Hyla regilla	196	0	
Pseudacris triseriata	12	0	
Bufonidae			
Bufo americanus	8	2	H. medioplexus
B. boreas	243	7	H. buttensis
B. marinus	3	0	
Scaphiopodidae			
Scaphiopus hammondi	5	0	
Ascaphidae			
Ascaphus truei	10	0	
Total	2851	1090	

*Identifications are based on type descriptions and type specimens, when available.

The maximum number of flukes recovered from a single frog (R. clamitans) was 358. The mean[3] number of flukes per infected R. pretiosa was 3.8 and for R. clamitans it was 64. The high mean found in R. clamitans was a result of large numbers of flukes in two of the infected frogs. The specimens of R. clamitans collected from Maple Ridge, B.C. contained both H. longiplexus and H. breviplexus. The remaining nine infected R. clamitans contained only H. breviplexus. All other frogs collected from any one locality contained only a single species of Haematoloechus.

Examination of prepared slides of Haematoloechus and worm specimens fixed and sent in vials supplied an additional source of flukes for examination. This material represents collections by many people from numerous locations throughout Canada and the United States. A total of 2584 specimens was examined.

Variations in characters previously considered as important for separating species in this genus will be discussed for the above material under each species heading.

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[3]The mean number of parasites per infected host is called the intensity of infection by some authors.

Generic Diagnosis

Haematoloechus Looss, 1899

syn. *Pneumonoeces* Looss, 1902

Ostiolum Pratt, 1903

Pneumobites Ward, 1917

The genus *Haematoloechus* Looss, 1899 is here defined as follows: (modified after Yamaguti, 1971)

Haematoloechidae. Body elongate, spinose or not. Acetabulum in anterior or middle third of body, and usually smaller than the oral sucker. Testes oblique or symmetrical, in posterior half of body. Cirrus and cirrus pouch present, containing seminal vesicle. Genital pore ventral to pharynx. Ovary lobed or not, close to acetabulum. Seminal receptacle large, often larger than ovary. Laurer's canal absent. Vitellaria follicular, dorsal and lateral, extending for variable length. Uterus coiled, occupying most of hindbody, with or without extracaecal uterine loops. The ascending limb passes intercaecally through the forebody. Eggs, numerous, embryonated, do not hatch until ingested by appropriate snail. Excretory vesicle Y-shaped. Parasitic in the lungs of Anura. Type species *Haematoloechus variegatus* (Rudolphi, 1819) Looss, 1899.

Species of this genus considered valid from North America may be divided into two groups:

Group I: Extracaecal uterine loops are present and extend anteriad for varying lengths. This group includes *H. longiplexus*, *H. breviplexus*, *H. varioplexus*, and *H. kernensis*.

Group II: extracaecal uterine loops are absent. This group

includes H. medioplexus and H. complexus.

Valid Species

Group I

Haematoloechus longiplexus Stafford

Haematoloechus longiplexus Stafford, 1902: 901.

(Fig. 42)

Pneumonoeces longiplexus Stafford, 1905: 687.

Pneumobites longiplexus Ward, 1917: 5.

Re-description (measurements based on 20 specimens):

Body elongate, 4.90–8.64 (5.86) long by 1.50–2.27 (1.77) wide. Tegument spined or not. Oral sucker terminal, 0.286–0.469 (0.380) long by 0.330–0.512 (0.149) wide. Acetabulum medial, slightly posterior of ovary, 0.18–0.260 (0.197) wide. O/A ratio 1.6:1.0 to 2.6:1.0 (2.2:1.0). Pharynx muscular, 0.165–0.336 (0.243) long by 0.154–0.276 (0.26) wide. O/P ratio 1.9:1.0 to 2.5:1.0 (2.2:1.0). Intestinal caeca narrow tubes, extending to near posterior extremity. Testes parallel, elongate, lobed or not. Ovary lobed or not, anterior to acetabulum, pretesticular. Extracaecal uterine loops present, extending anterior beyond the ovary, often extending to the level of the pharynx. Genital pore ventral to pharynx. Vitellaria follicular, symmetrically placed on each side of the body. Extent ranging from about level of intestinal bifurcation, and terminating posterior to the testes. Eggs operculate, 0.022–0.025 (0.023) long by 0.015–0.018 (0.016) wide.

Host: *Rana catesbeiana*.

Site of infection: lungs.

Specimens examined:

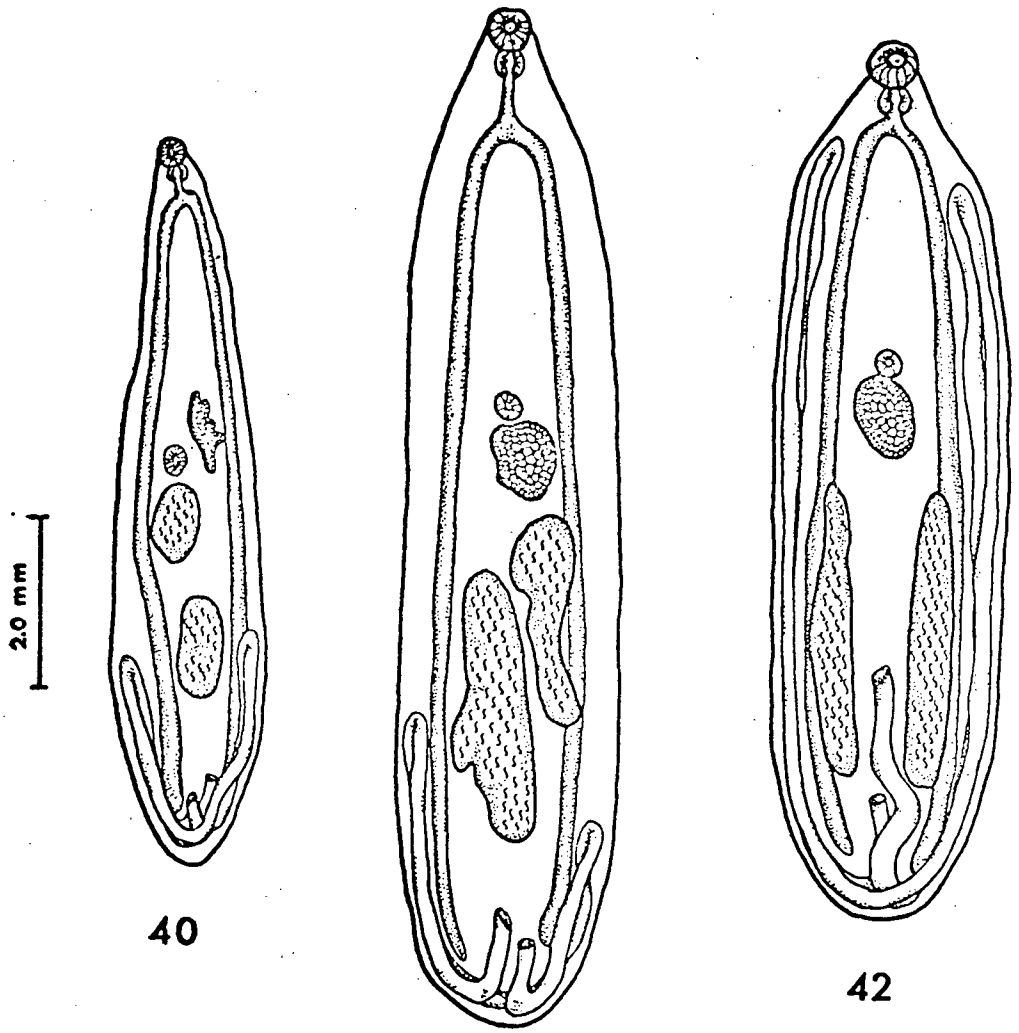
Thirty-one specimens, Nova Scotia, Halifax ex R. catesbeiana, (Mr. and Mrs. Bonnyman), author's coll.; 2 specimens, Iowa, ex R. catesbeiana, Dr. R. Campbell coll,; 110 specimens, Nebraska, ex R. catesbeiana, H.W. Manter Laboratories coll.; 23 specimens, New York, ex R. catesbeiana, author's coll.; 17 specimens, Ontario, Belleville, ex R. catesbeiana, author's coll.; 20 specimens, British Columbia, Maple Ridge, ex R. catesbeiana, author's coll. Type specimens were not available to the author.

Fig.40 *Haematoloechus parviplexus* (*Pneumonoeces parviplexus* of Irwin, 1929) redrawn from the type specimen.

Fig.41 *H. breviplexus* redrawn from Stafford (1902).

Fig.42 *H. longiplexus* redrawn from Stafford (1902).

Several structures have been omitted for clarity. All specimens are depicted in ventral view. Stafford (1902) did not give a scale to his drawings and so absolute size is unknown for his type drawings.



40

41

42

Discussion:

Stafford (1902) described H. longiplexus as being spineless. His type material for this species was not available for examination. However, of the 203 specimens examined by me, from six localities (Map 1), 170 had spines. All flukes from Iowa; Belleville, Ontario; Maple Ridge, B.C.; and New York had spines; 12.9% (4/31) of the worms from Halifax and 26.4% (29/110) from Nebraska had none.

The extracaecal loops in H. longiplexus commonly lie between the posterior border of the pharynx at their greatest extent and 1/2 the distance between the ovary and pharynx at their least extent (Figs. 43 and 44). The position of these loops did not vary in worms from different collection sites. The following results are based on 203 worms examined: 69% of the worms had uterine loops of equal or near equal length. Of these, 55% had both loops reaching the level of the pharynx or 90% of the distance from the ovary to the pharynx. Twenty-eight percent had the loops extending 75-85% of this distance and sixteen percent had loops extending about one-half the distance. Thirty-one percent of the specimens had uterine loops of unequal length. Of these, 72.7% had shorter left uterine loops. Extreme variation in extracaecal loop extent occurred in 2% of the specimens examined from Nebraska (Fig. 45 and 47).

Elongate, parallel or near parallel testes (Fig. 44) occurred in 98% of the specimens. Abnormal testes position involved either the left or right testis (Fig. 45 and 47). These specimens were also from Nebraska.

Eighty percent of the anterior and posterior testes were

unlobed. Twenty percent of the anterior and posterior testes were lobed and 51.5% of the ovaries were lobed, 48.5% unlobed.

The O/A ratio ranged from 1.5:1.0 to 2.8:1.0 with a mean of 2.1:1.0. Variations in the O/A ratios between populations of H. longiplexus are given in Map 1. There was no significant difference in the O/A ratio when the means of five localities were compared using a one-way analysis of variance. The Iowa sample was too small to be compared with those from other localities.

The ratio between the transverse diameters of the oral sucker and pharynx (O/P ratio) did not differ in flukes from the five different localities. The O/P ratio had a range of 1.9:1.0 to 2.8:1.0 and a mean of 2.2:1.0.

Egg lengths and widths did not differ in flukes from different localities (Fig. 48).

Map 1. Variation in O/A ratio between populations of H. longiplexus. Each locality shows the mean (horizontal line), standard deviation (open rectangle), and range (vertical line). The samples size is given below each range.

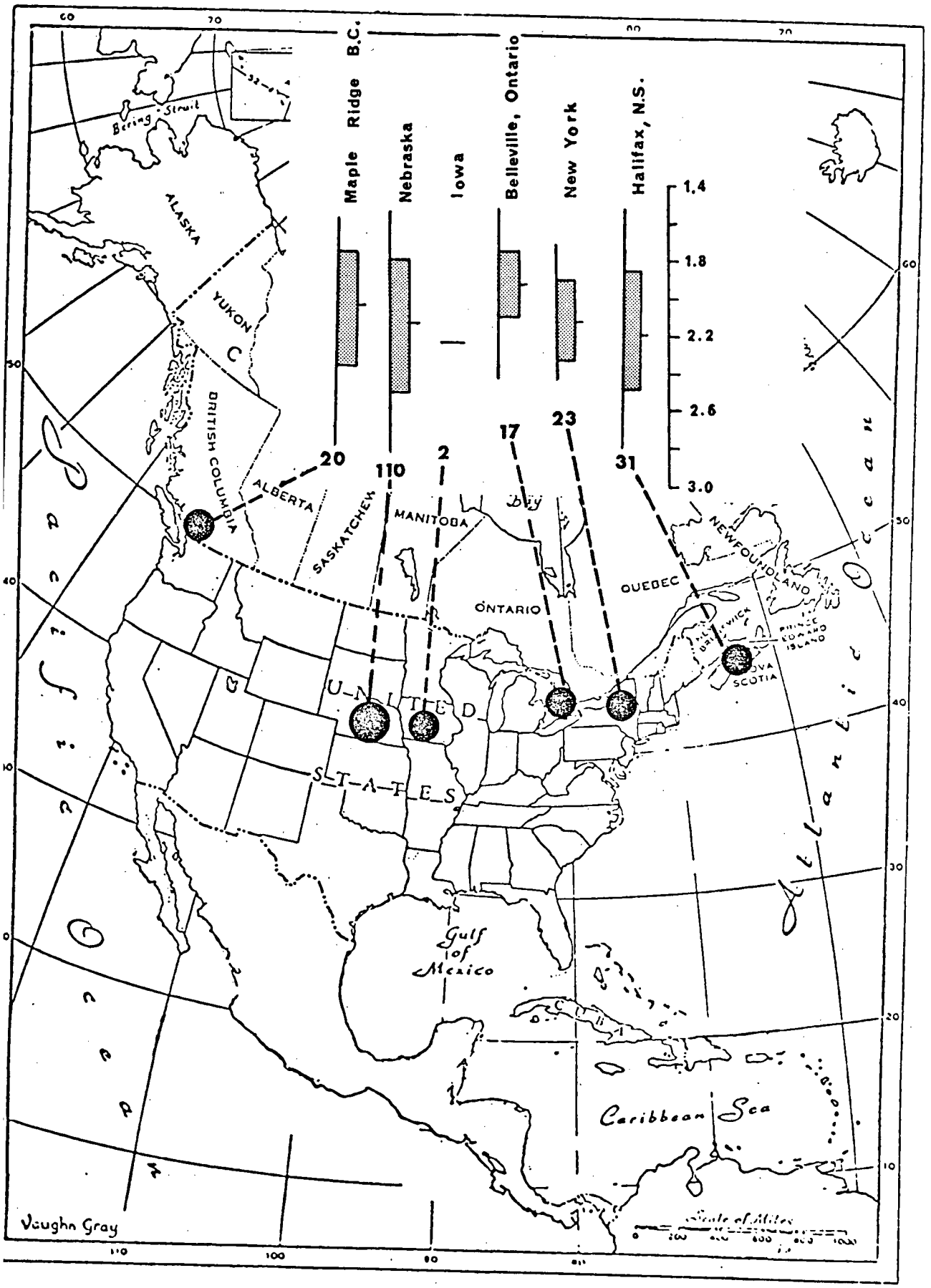

Maple Ridge B.C.
Nebraska
Iowa
Belleville, Ontario
New York
Halifax, N.S.
1.4
1.8
2.2
2.6
3.0
20
110
2
17
23
31
ALASKA
YUKON
BRITISH COLUMBIA
ALBERTA
SASKATCHEWAN
MANITOBA
ONTARIO
QUEBEC
NEWFOUNDLAND
NOVA SCOTIA
UNITED STATES
Gulf of Mexico
Caribbean Sea
Vaughn Gray

Fig.43 H. longiplexus showing variation in extracaecal uterine loops.

Fig.44 H. longiplexus showing variation in extracaecal uterine loops.

Fig.45 Abnormal extent of extracaecal uterine loops in H. longiplexus.

Fig.46 Abnormal extent of extracaecal uterine loops in H. longiplexus.

Fig.47 Abnormal testes position in H. longiplexus.

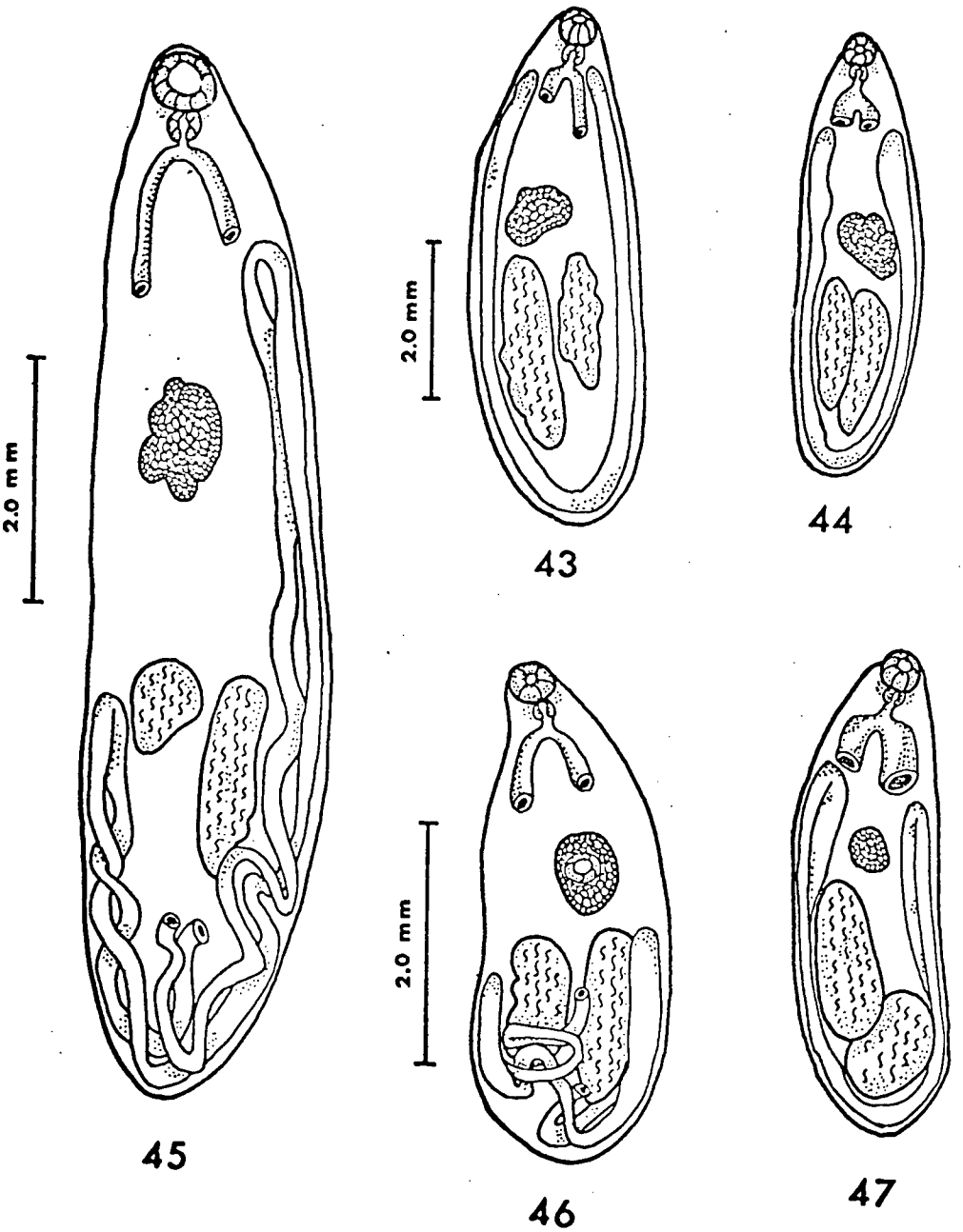
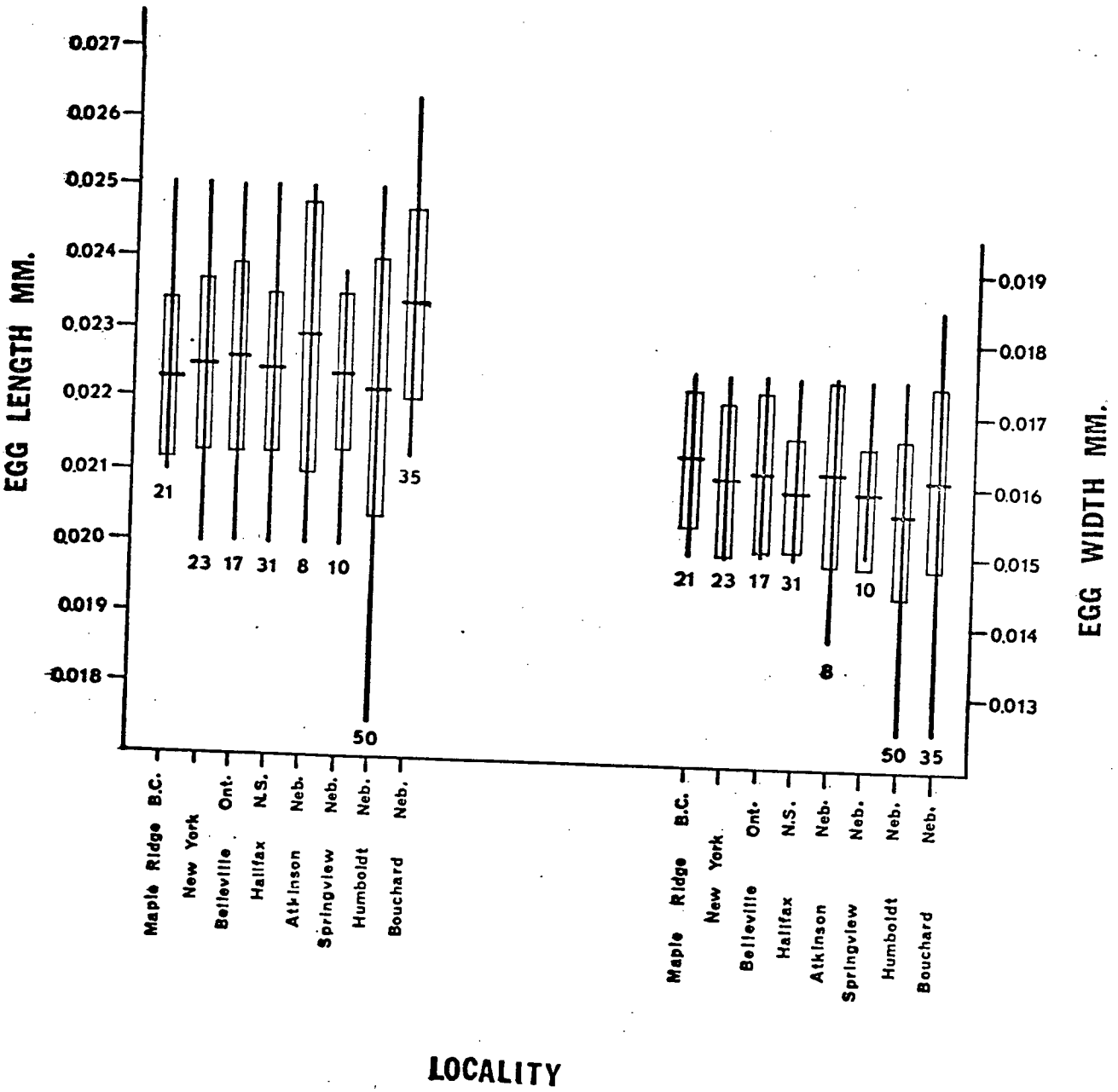

2.0 mm
2.0 mm
2.0 mm
43
44
45
46
47

Fig. 48 Variation in egg measurements of H. longiplexus from different localities, indicating the mean, range, and standard deviation (open rectangle), with the sample size indicated for each locality.


EGG LENGTH MM.
0.027
0.026
0.025
0.024
0.023
0.022
0.021
0.020
0.019
0.018
21
23
17
31
8
10
50
35
EGG WIDTH MM.
0.019
0.018
0.017
0.016
0.015
0.014
0.013
21
23
17
31
8
10
50
35
Maple Ridge B.C.
New York
Belleville Ont.
Halifax N.S.
Atkinson Neb.
Springview Neb.
Humboldt Neb.
Bouchard Neb.
LOCALITY

Haematoloechus breviplexus Stafford

Haematoloechus breviplexus Stafford, 1902: 904.

(Fig. 41)

Pneumonoecus breviplexus Stafford, 1905: 687.

Pneumobites breviplexus Ward, 1917: 5.

Re-description (based on 20 specimens):

Body elongate 7.13–10.99 (8.86) long by 1.80–2.27 (2.06) wide. Tegument spined or not. Oral sucker terminal, 0.465–0.573 (0.526) 40 long by 0.462–0.558 (0.489) wide. Acetabulum medial, may overlie the ovary or be anterior to it, 0.165–0.198 (0.176) wide. O/A ratio 2.3:1.0 to 3.4:1.0 (2.8:1.0). Pharynx muscular, 0.198–0.264 (0.225) long by 0.209–0.252 (0.236) wide. O/P ratio 1.8:1.0 to 2.4:1.0 (2.1:1.0). Intestinal caeca narrow tubes, extending to near posterior extremity. Testes, elongate, overlap from 35% to 60% of their lengths, lobed or not. Ovary lobed or not, pretesticular. Extracaecal uterine loops present, extend from the anterior border of the posterior testis, to the posterior border of the ovary. Genital pore ventral to pharynx. Vitellaria, follicular, symmetrically placed on each side of the body. Extent ranging from about level of intestinal bifurcation, and terminating posterior to the testes. Eggs operculate, 0.019–0.025 (0.023) long by 0.015–0.019 (0.017), wide.

Host: *R. catesbeiana*, *R. clamitans*, *R. grylio*, *R. utricularia*.

Site of infection: Lungs

Specimens examined:

Seventeen specimens, British Columbia, Maple Ridge, ex. R. catesbeiana, author's coll.; 1 specimen, Florida, Jacksonville, ex R. utricularia, Dr. G.J. Bowers coll.; 3 specimens, Louisiana, Avery Island, ex R. grylio, Dr. M.L. Eberhard coll.; 3 specimens, Massachusetts, North Dartmouth, ex R. catesbeiana, Dr. R.A. Campbell coll.; 1 specimen, Mississippi, ex R. catesbeiana, Dr. J.D. Lynch coll.; 10 specimens, Nevada, LasVegas, ex R. catesbeiana, Dr. B.B. Babero coll.; 10 specimens, Nova Scotia, Halifax, ex R. catesbeiana, (Mr. and Mrs. Bonnyman), author's coll.; 12 specimens, Ontario, Belleville, ex R. catesbeiana, (Dr. R.J.G. Lester), author's coll.; 5 specimens, Virginia, Richmond, ex R. clamitans, Dr. R.A. Campbell coll. Type specimens were not available to the author.

Discussion:

Sixty-six H. breviplexus from ten localities in Canada and the United States were examined for variation in taxonomically important characters (Map 2).

No significant difference in characters of flukes from the different localities occurred. Too few samples of R. grylio, R. utricularia Harlan, 1826 and R. clamitans were obtained to test for differences in flukes from different hosts. The following results represent pooled data from all localities.

Spines were located on the anterior one-third of the tegument of flukes from Nevada and Louisiana. Two specimens from

Virginia did not have spines. All other specimens examined had spines over their entire surface.

The ovary was lobed in 81.5% of the flukes and smooth in 18.5%. Testes were lobed in 76.9% of the worms and unlobed in 23.1%. Neither testis was lobed more frequently than the other. Both testes were elongate and overlapped for at least 1/3 their length.

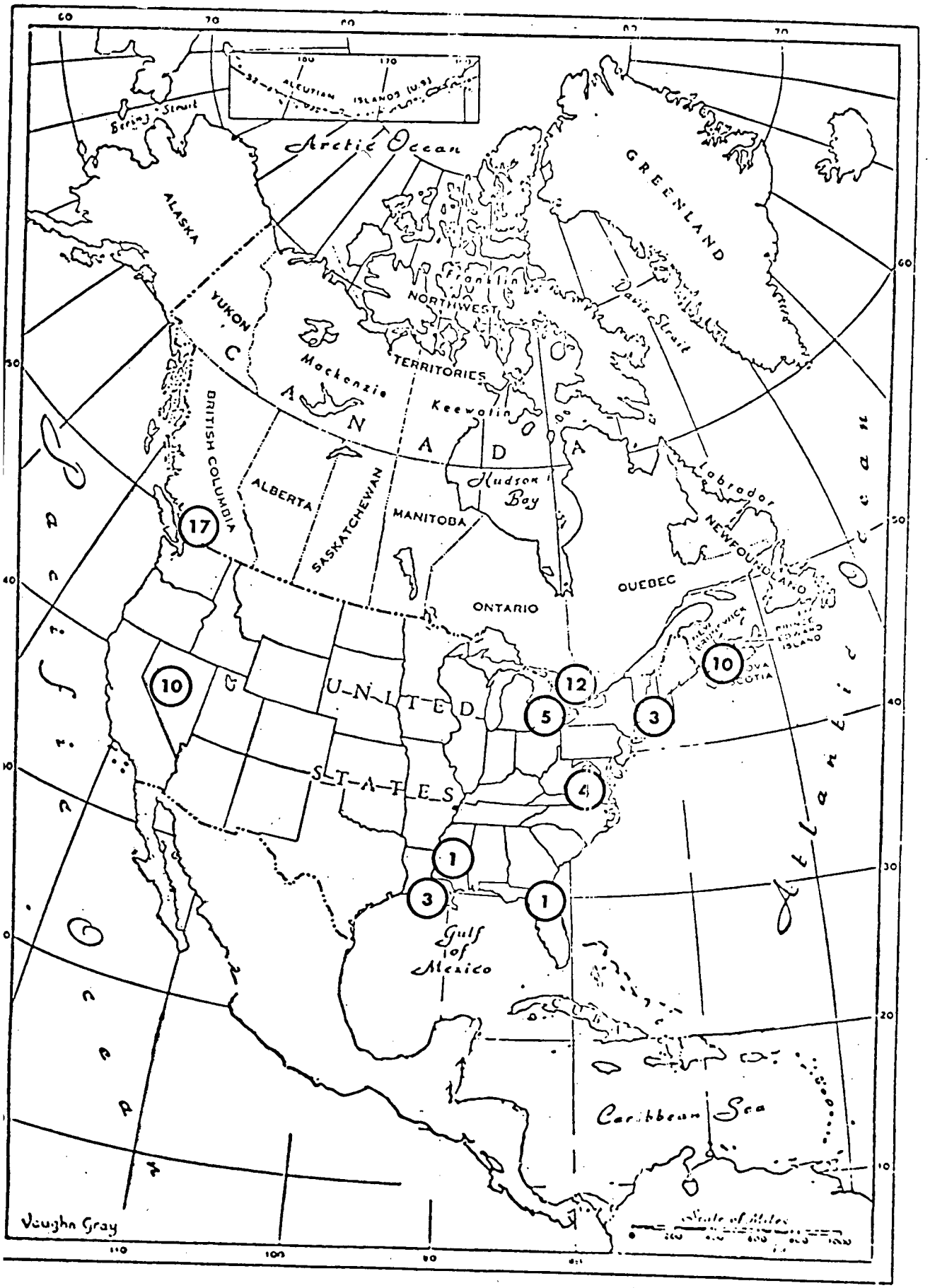
Stafford, 1902 used the arrangement of the uterus as a useful character for separating H. longiplexus from H. breviplexus. He noted that the extracaecal uterine loops in H. breviplexus do not extend beyond the posterior testis, whereas, in H. longiplexus they extend nearly to the pharynx.

I found the extracaecal loops in H. breviplexus to extend to the level of the ovary. The right loop is most frequently the longer (71.4% of the cases) and ranged from 1/2 the distance along the length of the ovary to the level of the ovary. The left loop ranged from the level of the posterior testis to 1/2 the distance along the length of the posterior testis. Thus despite the close morphological resemblance to H. longiplexus, the two species do differ in the extent of their extracaecal uterine loops.

The O/A ratio ranged from 2.1:1.0 to 3.4:1.0 with a mean of 2.8:1.0. The O/P ratio did not differ in flukes from different localities. This ratio ranged from 1.4:1.0 to 2.4:1.0 (2.0:1.0).

Egg lengths ranged from 0.019 to 0.025 (0.021) and egg width from 0.015 to 0.018 (0.016).

Map 2. Areas providing samples of H. breviplexus. Sample size is represented in the circles.


Arctic Ocean
Bering Strait
ALEUTIAN ISLANDS (U.S.)
ALASKA
GREENLAND
Davis Strait
YUKON
NORTHWEST TERRITORIES
Mackenzie
Keewatin
Franklin
BRITISH COLUMBIA
ALBERTA
SASKATCHEWAN
MANITOBA
Hudson Bay
Labrador
NEWFOUNDLAND
ONTARIO
QUEBEC
PRINCE EDWARD ISLAND
NOVA SCOTIA
CANADA
UNITED STATES
Gulf of Mexico
Caribbean Sea
Atlantic Ocean
Pacific Ocean
17
10
12
5
3
10
4
1
3
1
Vaughn Gray

Haematoloechus varioplexus Stafford

Haematoloechus varioplexus Stafford, 1902: 906.

(Fig. 65)

Haematoloechus similiplexus Stafford, 1902: 907

(Fig. 64)

Haematoloechus parviplexus (Irwin, 1929): 74. syn. nov.

(Fig. 40)

Haematoloechus floedae Harwood, 1932: 16. syn. nov.

(Fig. 66)

Haematoloechus uniplexus Harwood, 1932: 18. syn. nov.

(Fig. 67)

Haematoloechus buttensis Ingles, 1936: 78. syn. nov.

(Fig. 63)

Re-description (based on 78 specimens):

Body elongate, 3.57-11.75 (6.19) long by 0.73- 4.02 (1.31) wide. Tegument spined. Oral sucker terminal, 0.039-0.589 (0.340)long by 0.187-0.589 (0.317) wide. Acetabulum medial, overlies the ovary, 0.066-0.363 (0.156) wide. O/A ratio 1.1:1.0 to 3.4:1.0 (2.3:1.0:. Pharynx muscular, 0.110-0.297 (0.175) long by 0.110-0.314 (0.165) wide. O/P ratio 1.2:1.0 to 2.3:1.0 (1.7:1.0). Intestinal caeca narrow tubes, extending to near the posterior extremity. Testes tandem or oblique, round to elliptical, smooth or lobed. Ovary lobed or not, pretesticular. Extracaecal uterine loops present, extending anterior to anterior border of the posterior testis, occasionally extending to mid-region of anterior testis. Genital pore ventral to pharynx. Vitellaria follicular, variable in distribution .

Extent ranging from midway between ovary and pharynx to posterior to the posterior testis on the side opposite the ovary or to the middle of the posterior testis on the ovarian side. Eggs operculate, 0.018–0.043 (0.028) long by 0.013–0.031 (0.017) wide.

Hosts: R. catesbeiana; R. clamitans; R. pipiens; R blairi Mecham, Littlejohn, Oldham, Brown and Brown, 1973; R. pretiosa; R. sylvatica; R. sphenocephala; B. woodhousei Girard, 1854.

Site of infection:lungs.

Specimens examined:

A. H. parviplexus:

71 specimens, British Columbia, ex R. sylvatica, author's coll.; 83 specimens, Nebraska, ex R. catesbeiana, H.W. Manter Laboratories coll.; 1 specimen U.S.N.M. Helm. Coll. No.8085.

B. H. varioplexus / H. similiplexus :

12 specimens, Ontario, ex R. clamitans, Mr. M. Baker's coll. ; 10 specimens, Virginia, ex R. catesbeiana, Dr. R.A. Campbell coll.; 23 specimens, Nebraska, ex R. pipiens, R. blairi, B. woodhousei, H.W. Manter Laboratories coll. Type specimens were not available to the author.

C. H. buttensis:

One thousand five hundred sixty-two specimens, British Columbia, ex R. pretiosa, author's coll.; 1 type specimen, U.S.N.M. Helm. Coll. No. 8926.

D. H. floedae:

1 type specimen, U.S.N.M. Helm. Coll. No. 30879.

E. H. uniplexus:

1 type specimen, U.S.N.M. Helm. Coll. No. 30880.

Discussion:

H. parviplexus

One hundred and fifty-four specimens of H. parviplexus, from five localities, were examined for variation in characters used to distinguish this species from other North American members of Haematoloechus.

The ovaries were lobed in 87.1% and unlobed in 12.9% of the specimens examined. The anterior testis was lobed in 17.9% and unlobed in 82.1% while the posterior testis was lobed in 14.3% and unlobed in 85.7% of the specimens.

The O/A ratio could be determined for 139 of the flukes. The acetabulum could not be located in the other 15 flukes so a ratio could not be calculated. A one-way analysis of variance followed by a Newman-Kuls Multiple Range Test was used to test for significance between the means of the five localities sampled. There was no significant difference between the means of flukes from Vanderhoof, Slim Lake, or McBride (Fig. 49). All of these flukes occurred in Rana sylvatica. The Atkinson, Nebraska, sample had a mean significantly different from that of the British Columbia samples ($\alpha \leq 0.001$). The Humboldt sample was also significantly different from the British Columbia sample ($\alpha \leq 0.001$) and from the Atkinson sample ($\alpha < 0.001$). The samples from Nebraska were from R. catesbeiana.

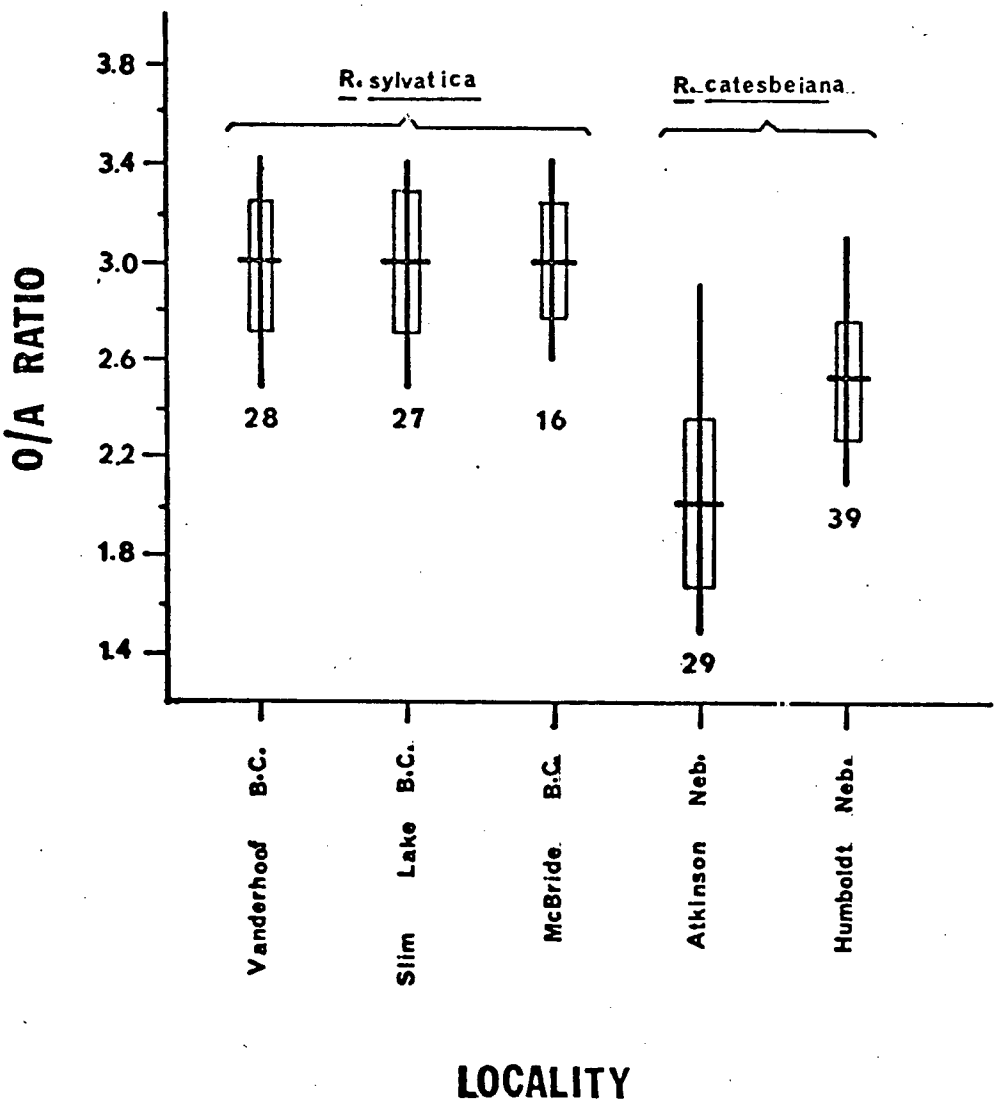
The O/P ratio ranged from 1.2:1.0 to 2.1:1.0 (1.5:1.0). This ratio did not differ significantly in flukes from different

localities or hosts.

Variations in the extent of the extracaecal loop, between localities sampled, did not differ. A description of the variation in extracaecal loops is given from pooled results. The tegument of all specimens had spines.

Fig. 49 O/A ratios of H. parviplexus from collections made in British Columbia and Nebraska.

The range, mean and standard deviation (open rectangle) are given. Sample size is illustrated at the lower limit of each range.


O/A RATIO
3.8
3.4
3.0
2.6
2.2
1.8
1.4
R. sylvatica
R. catesbeiana
28
27
16
29
39
Vanderhoof B.C.
Slim Lake B.C.
McBride B.C.
Atkinson Neb.
Humboldt Neb.
LOCALITY

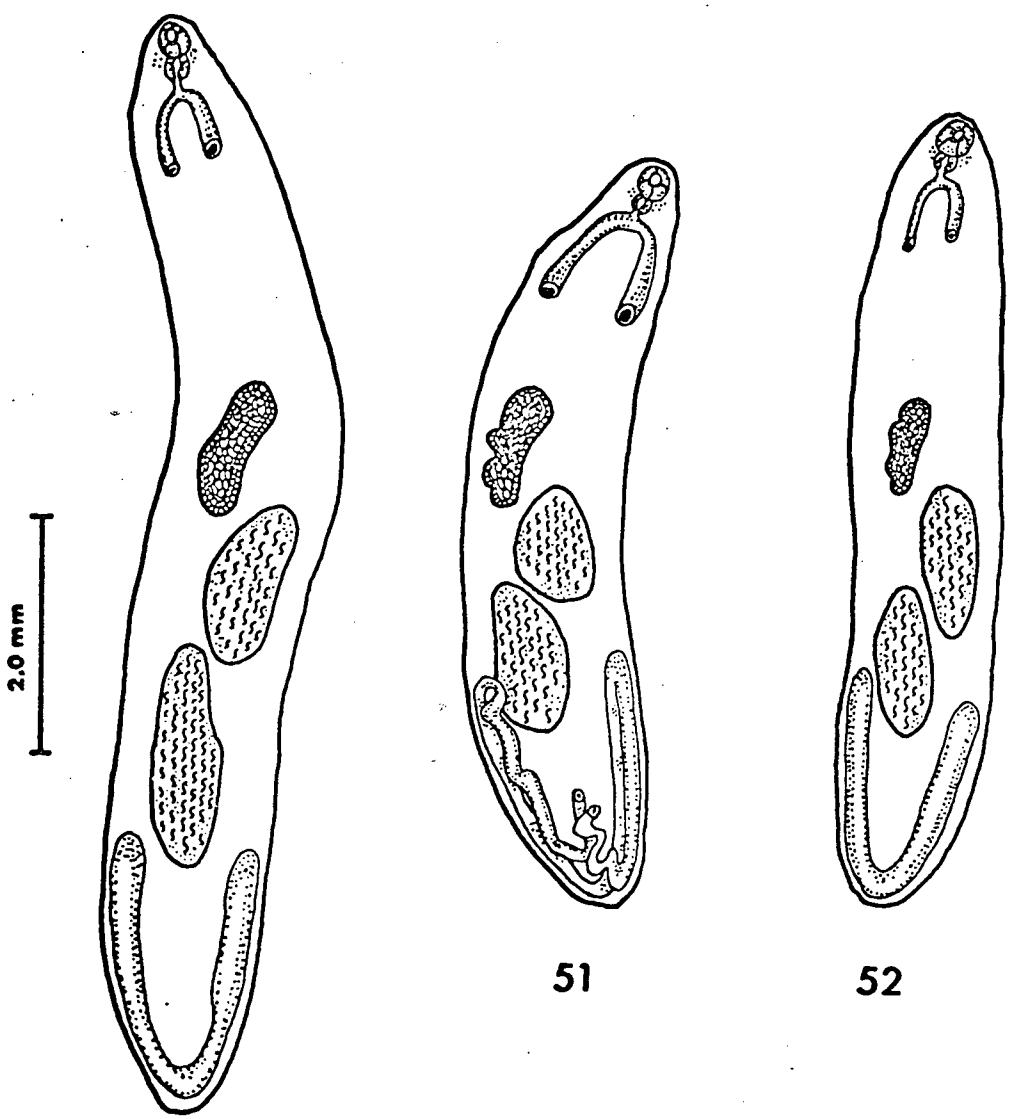
Extracaecal uterine loops were equal or near equal in length in 31.9% of the flukes examined; the right loop longer than the left in 41.7% and the left longer than the right in 26.4% of the flukes. The left extracaecal loop was absent in 4.2% of the cases. When both loops are present and of equal or near equal length, the variation in extent is from 1/2 the distance from the posterior end to the posterior border of the posterior testis to 3/4 the distance along the length of the posterior testis. The average extent is 1/3 the distance along the length of the posterior testis. When the right loop is longer than the left it extends between 1/5 and 9/10 the distance along the length of the posterior testis, with a mean of 52.6%. The left loop ranges from just reaching the posterior border of the posterior testis to 25.7% the distance along the posterior testis. When the left loop is longest it extends from 20% to 80% this distance, with a mean of 54.5%. The right loop extends from 18% to 50%, with a mean of 30.4%. Some variations in ovary and testis shape and in uterine loops are given in Figures 50 to 52.

There is no significant difference in widths of eggs from different localities or in the length of eggs from Vanderhoof, Slim Lake, McBride and Humboldt (Fig. 53). Flukes from Vanderhoof, Slim Lake and McBride had R. sylvatica as their definitive host.

Flukes from Nevada had as their definitive host R. catesbeiana. The egg lengths in flukes from R. sylvatica differed significantly from flukes from R. catesbeiana from Atkinson ($\alpha < 0.005$). Egg lengths of flukes from Humboldt

differed significantly from those of worms from Atkinson ($\alpha \leq 0.001$). The tegument of all specimens had spines.

Fig. 50 to 52. Some variations in shape of ovaries and testes and in extent of extracaecal uterine loops in H. parviplexus.

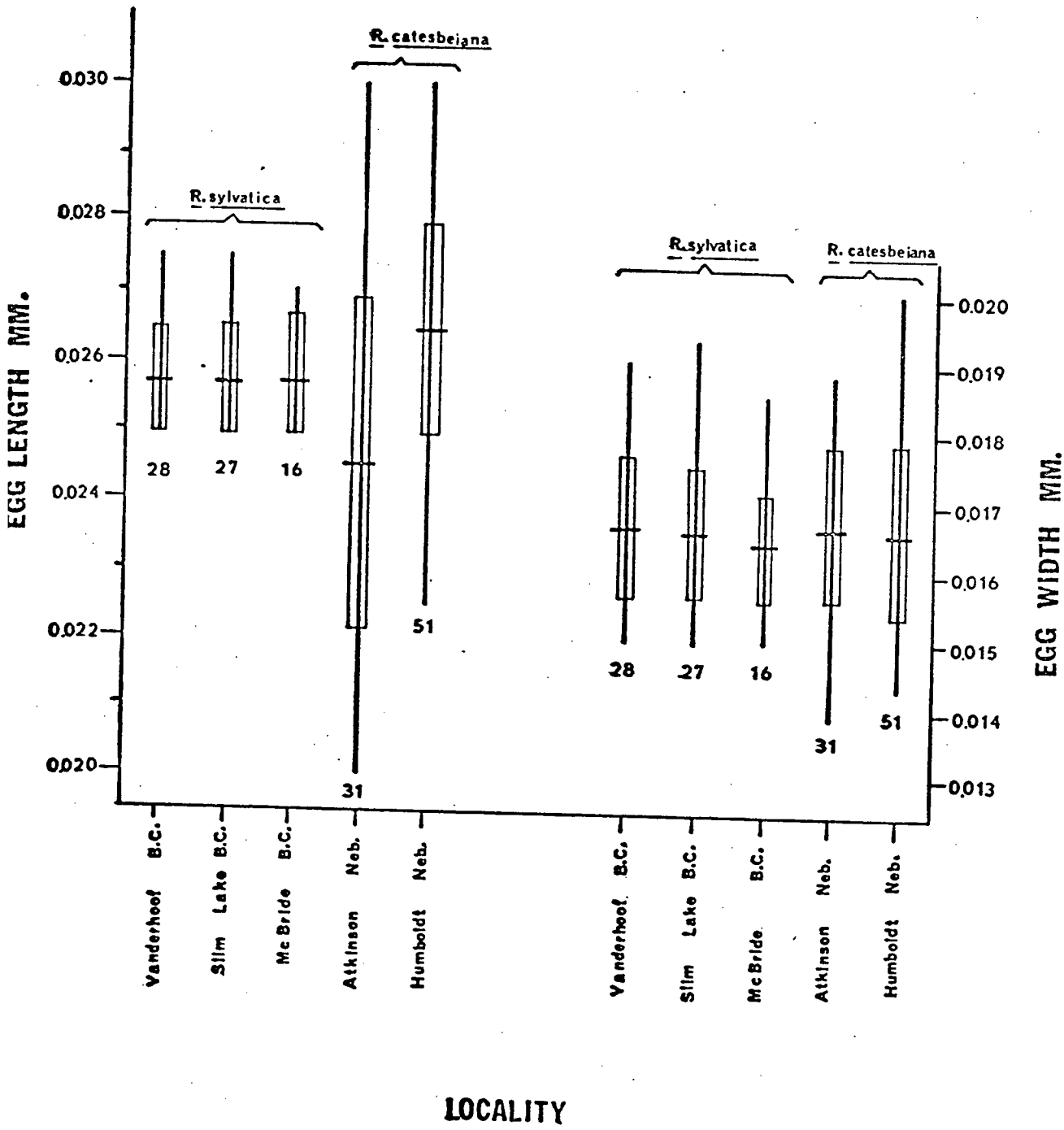


50

51

52

Fig. 53 Length and width of eggs of H. parviplexus from different localities, indicating mean, standard deviation (vertical rectangle), and the range (vertical line), with the sample size and frog host indicated for each location.


R. catesbeiana
R. sylvatica
R. sylvatica
R. catesbeiana
0.030
0.028
0.026
0.024
0.022
0.020
EGG LENGTH MM.
0.020
0.019
0.018
0.017
0.016
0.015
0.014
0.013
EGG WIDTH MM.
28
27
16
31
51
28
27
16
31
51
Vanderhoof B.C.
Slim Lake B.C.
McBride B.C.
Atkinson Neb.
Humboldt Neb.
Vanderhoof B.C.
Slim Lake B.C.
McBride B.C.
Atkinson Neb.
Humboldt Neb.
LOCALITY

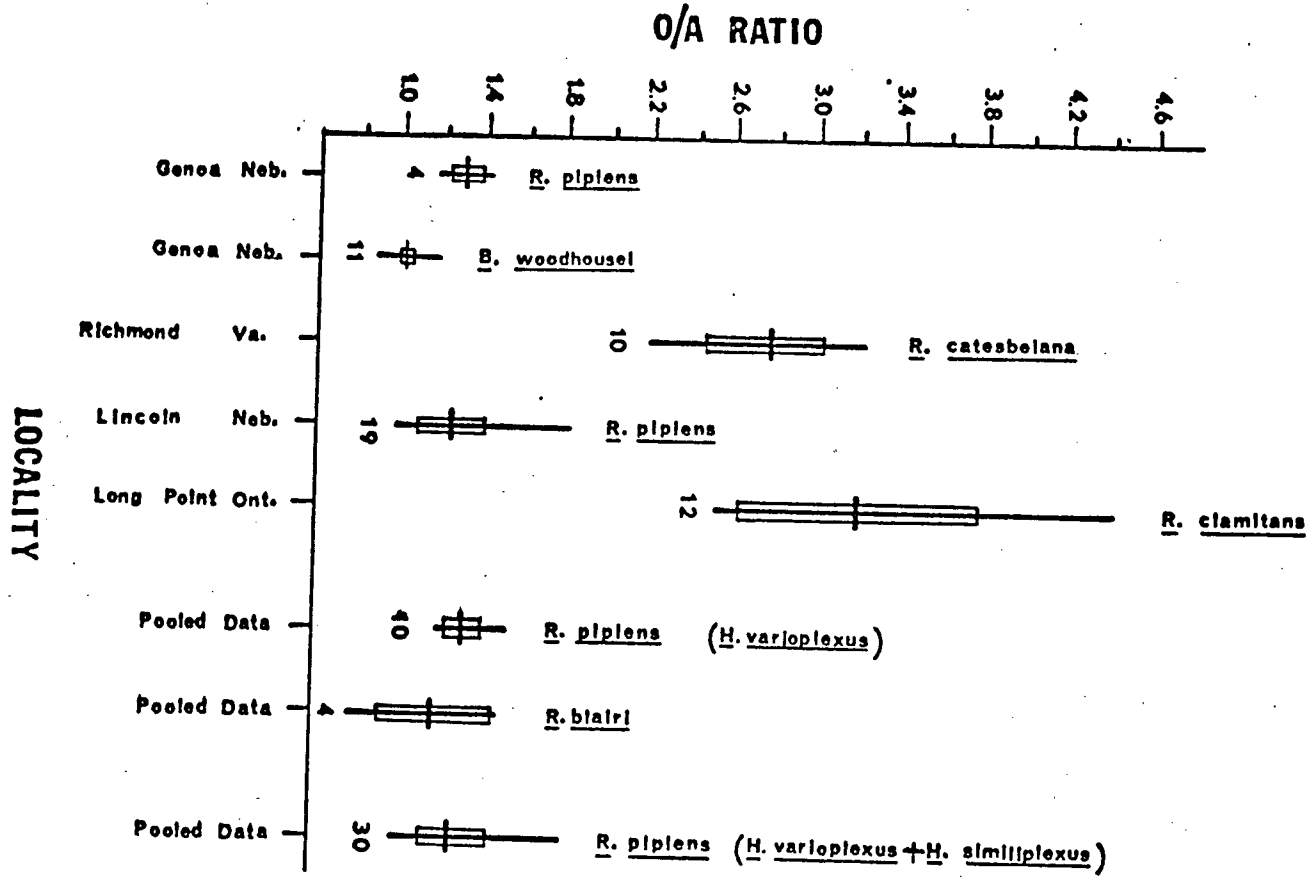
H. varioplexus and H. similiplexus

Seventy-five specimens of flukes labelled either H. varioplexus or H. similiplexus were examined. The range in variation of characters used to separate H. similiplexus from H. varioplexus was too great to be certain of positive identification. Therefore specimens labelled H. varioplexus and H. similiplexus were treated according to host.

The O/A ratio of flukes from different localities and different hosts were calculated and are presented in Figure 54. A Newman-Keuls Multiple Range Test was used to test for significance of means between samples from different localities. There is no significant difference between flukes collected from Genoa and Lincoln, Nebraska when the host is R. pipiens. However, there is a significant difference between these flukes and worms from Genoa recovered from B. woodhousei ($\alpha = 0.02$). A significant difference between flukes from B. woodhousei and R. pipiens also occurs when the data for R. pipiens are pooled ($\alpha \leq 0.001$). The O/A ratio of flukes recovered from R. catesbeiana from Richmond, Virginia differed from those in R. clamitans from Long Point, Ontario ($\alpha < 0.05$). The means, standard deviations and ranges are given for samples equal to or greater than four and for pooled data (Fig. 54).

The O/P ratio did not differ significantly in flukes from different localities. The ratio ranged from 1.3:1.0 to 2.3:1.0 (1.7:1.0).

Fig. 54 O/A ratios of H. varioplexus and H. similiplexus from different localities, indicating the mean , standard deviation (vertical rectangle) and the range (vertical line), with sample size and frog host indicated for each location.


O/A RATIO
1.0
1.4
1.8
2.2
2.6
3.0
3.4
3.8
4.2
4.6
LOCALITY
Genoa Neb.
4
R. pipiens
Genoa Neb.
11
B. woodhousei
Richmond Va.
10
R. catesbeiana
Lincoln Neb.
19
R. pipiens
Long Point Ont.
12
R. clamitans
Pooled Data
40
R. pipiens (H. variolexus)
Pooled Data
4
R. blairi
Pooled Data
30
R. pipiens (H. variolexus + H. simillimus)

Egg measurements from flukes from Nebraska did not differ significantly. Egg measurements of flukes from Long Point, Ontario and Richmond, Virginia differed significantly from each other as well as from Nebraska ($\alpha < 0.001$) (Fig. 55).

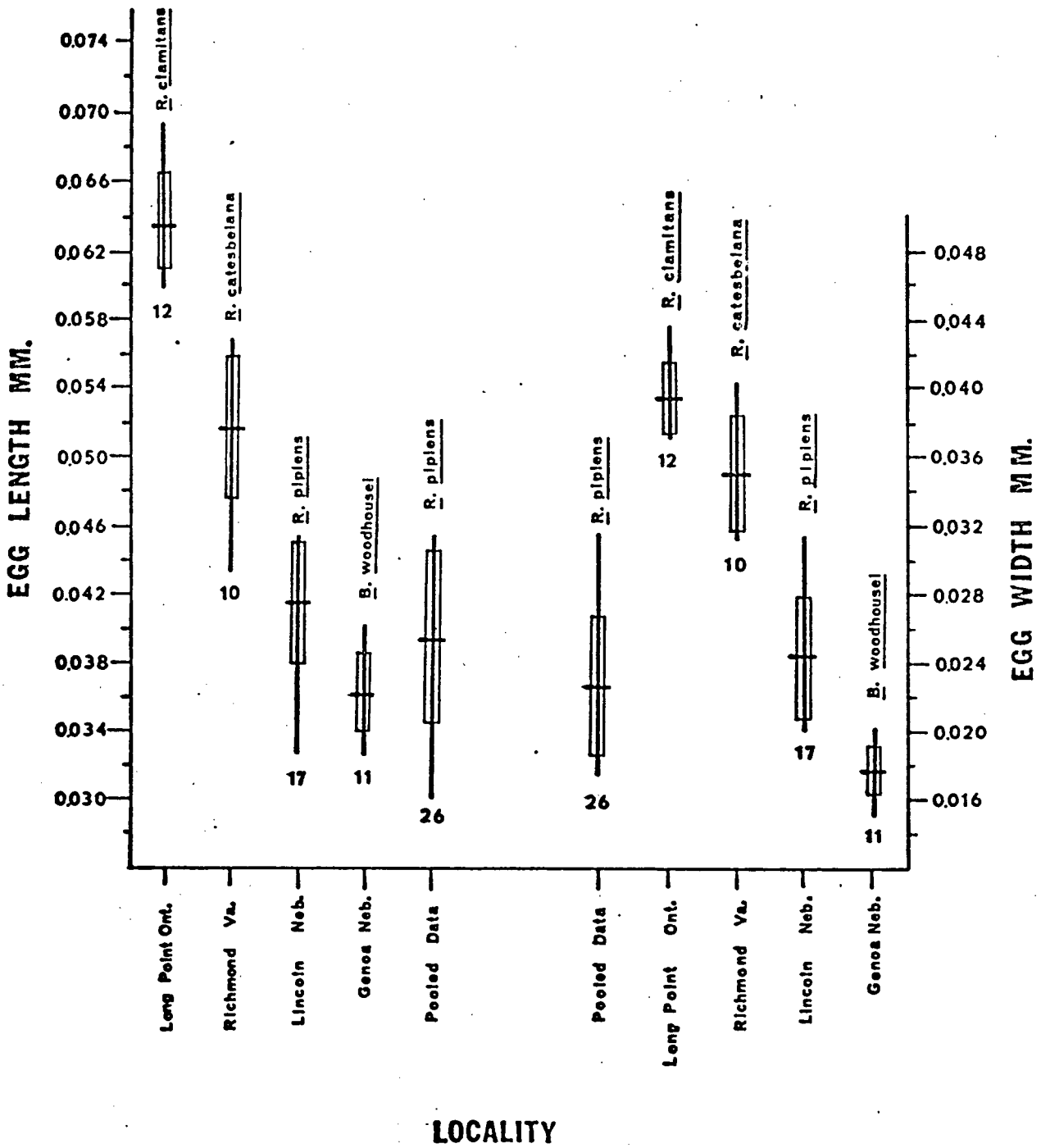
No difference in extracaecal loops in flukes from different hosts or localities occurred. A description of these loops is given to indicate their variability.

When both extracaecal loops reached the posterior testis (88.9% of the time) the loops were of approximately the same length in 43.3% of the flukes. In 35.1% of the flukes the right loop was longest and in 21.6% the left. When uterine loops were of near-equal length they extend 40-65% the distance along the posterior testis. When the right loop was longest it reached at least 50% the distance along the posterior testis. When the left loop was longest it also reached at least 50% the distance along the posterior testis.

In 11.1% of the cases examined one or both uterine loops failed to reach the posterior testis. Of these cases, 16.7% had loops the same length and 66.7% had the right loop longer than the left, 8.3% had the left loop longest and 8.3% had the left loop missing. Some of the variations in extent of extracaecal loops are given in Figures 56 to 59.

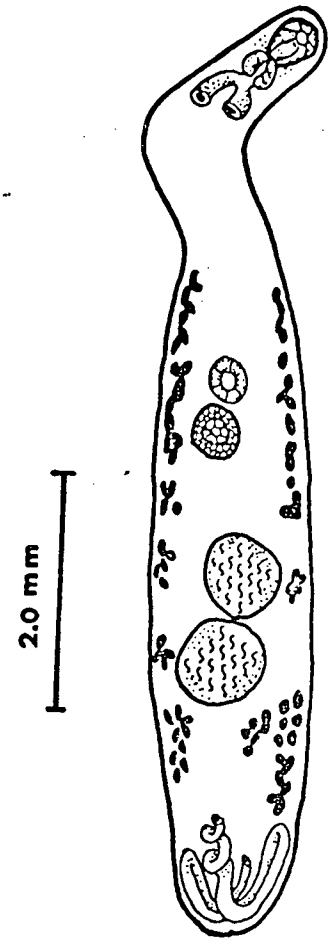
Anterior and posterior testes and ovaries are commonly unlobed. Only 2.6% of the anterior testis examined and 5.7% of the posterior testis examined were lobed, and 5.6% of all ovaries examined were lobed. All specimens had spines distributed over the tegument.

Fig. 55 Length and width of eggs of H. varioplexus and H. similiplexus from different localities, indicating the mean, standard deviation (vertical rectangle) and the range (vertical line), with the sample size and amphibian host indicated for each locality.

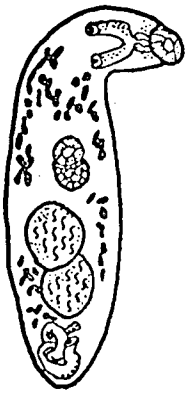

EGG LENGTH MM.
0.074
0.070
0.066
0.062
0.058
0.054
0.050
0.046
0.042
0.038
0.034
0.030
EGG WIDTH MM.
0.048
0.044
0.040
0.036
0.032
0.028
0.024
0.020
0.016
R. clamitans
R. catesbeiana
R. pipiens
B. woodhousei
12
10
17
11
26
Long Point Ont.
Richmond Va.
Lincoln Neb.
Genoa Neb.
Pooled Data
LOCALITY

Figures 56 to 59.

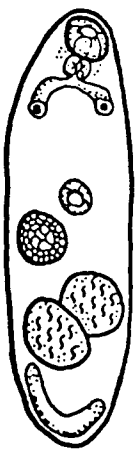
Variations in extent of extracaecal uterine loops in H. varioplexus.



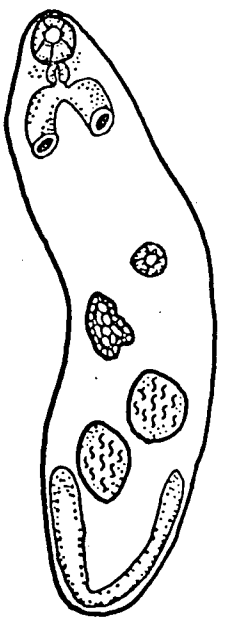
56



57



58



59

H. buttensis

One thousand five hundred sixty-two H. buttensis collected from seventy-four locations in British Columbia were examined. All specimens were from R. pretiosa and were prepared by the author.

Lobed ovaries were found in 93.8% of the specimens examined (Fig. 60). Anterior and posterior testes were lobed in 18.7% of the flukes examined. Testes overlapped 15% to 50% of their length, averaging 32.5% (Fig. 61).

Extracaecal uterine loops are equal or near-equal in length 72.7% of the time (Fig. 62). In these cases the right loop ranged a distance of 1/4 the length of the posterior testis to 3/4 the distance. At the same time the left loop extends 1/2 the distance to the posterior testis from the end of the worm. When the right loop is longest the anterior extent of the right loop reached a distance 1/3 the way along the posterior testis. When the left extracaecal loop is longest the left loop extends, at most, to the posterior margin of the posterior testis. In 9% of the flukes examined the right loop was absent.

The O/A ratio ranged from 2.2:1.0 to 2.9:1.0 with an average of 2.6:1.0. The O/P ratio did not differ in flukes collected from different localities. This ratio had a range of 0.9:1.0 to 1.9:1.0 and a mean of 1.4:1.0.

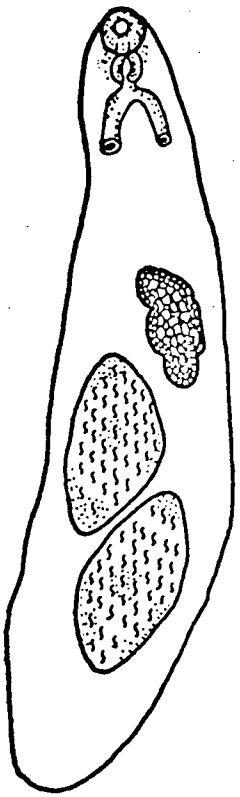
No significant difference occurred in egg lengths or in egg widths when fukes from different localities were compared. Eggs measured 0.020 to 0.025 (0.023) long by 0.016 to 0.018 (0.017) wide.

Spines covered the entire body surface on all flukes.

Fig.60 Lobed ovaries and overlap of testes in H. buttensis.

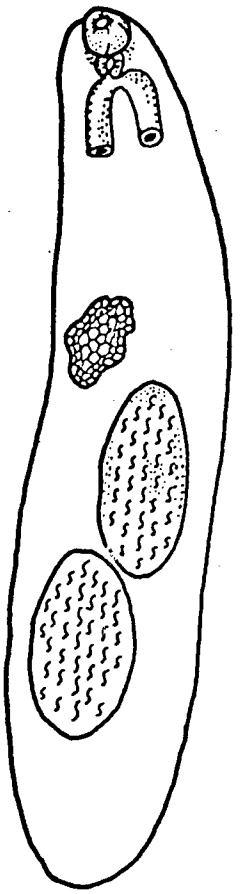
Fig.61 Lobed ovaries and overlap of testes in H. buttensis.

Fig.62 Extent of extracaecal uterine loops in H. buttensis.

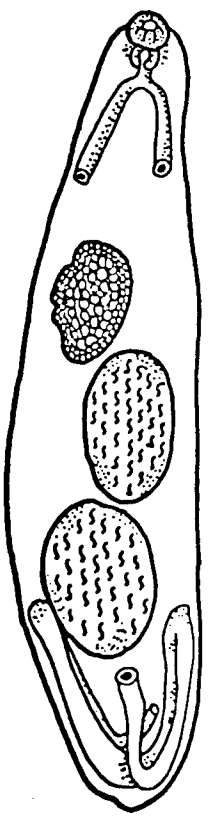


2.0 mm

60



61



62

Examination of the type specimen of H. buttensis (U.S.N.M. Helm. Coll. No. 8926) showed certain inconsistencies between description and observed morphology.

Ingles' drawing of the type specimen is in dorsal view, not ventral view as stated by him in the caption to his Figure 3. This view depicted the left extracaecal loop extending 1/3 along the length of the posterior testis, and the right loop did not reach the posterior testis. Examination of the type specimen indicated that both extracaecal loops just reach the level of the posterior testis (Fig. 63).

"Cuticle armed with spines posteriorly to the acetabulum" (Ingles, 1936). There are spines over the entire body, but they become smaller and less numerous posterior of the acetabulum.

H. floedae and H. uniplexus

Only the type specimens of H. floedae and H. uniplexus (Figs. 66 and 67) were available for examination. These specimens showed some inconsistencies between description and observed morphology.

H. floedae (U.S.N.M. Helm. Coll. No. 30879):

Harwood described H. floedae as being "entirely without spines" and that "the oral sucker measured 3.6 to 4.4 mm in diameter," p. 16.

My examination of the type specimen showed spines to be present. There are large spines on the oral sucker and anterior 20% of the worm. Spines become smaller posteriorly.

The oral sucker of the type specimen has a transverse diameter of 0.432mm. Therefore I feel Harwood's description

should be amended to read: oral sucker measures 0.36 to 0.44 mm in diameter.

The absence of spines in H. floedae was one character used by Harwood to distinguish this species from H. parviplexus.

H. uniplexus (U.S.N.M. Helm. Coll. No. 30880):

Harwood (1932: 18) reported that the "cuticula is smooth and without spines." However, I found small spines over most of the body surface and larger spines in the region of the oral sucker.

No emphasis is placed on the supposed smooth nature of the tegument for species separation. The main feature used for recognizing this species is the single uterine loop. The validity of this character for identifying species in this genus will be discussed in the next section.

Fig. 63 H. buttensis redrawn from the type material.

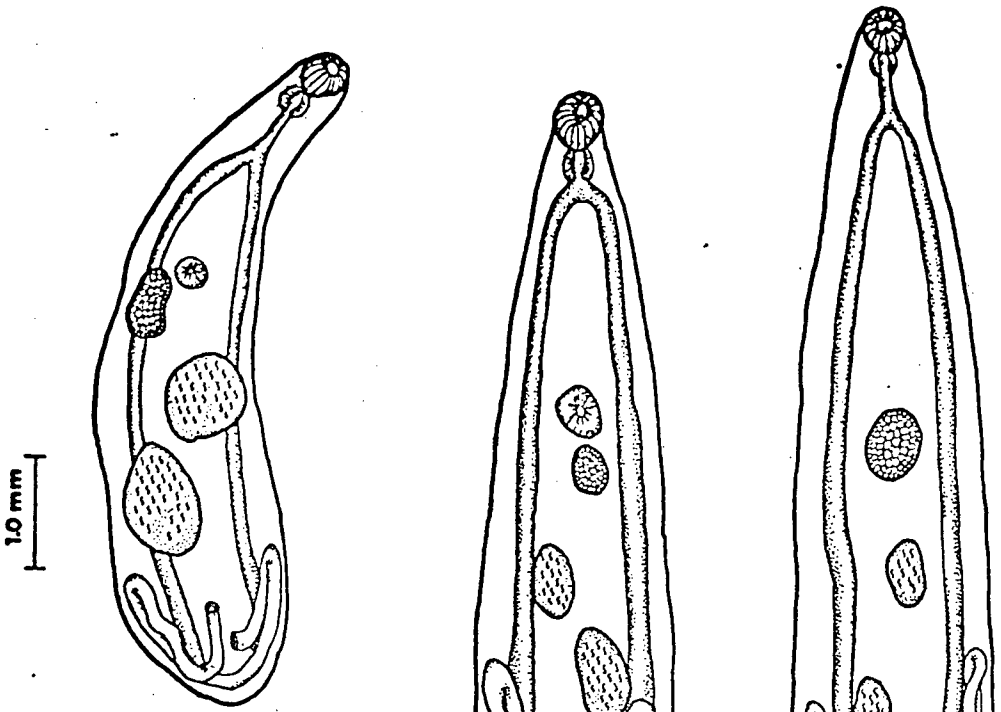
Fig. 64 H. similiplexus redrawn from Stafford (1902).

Fig. 65 H. varioplexus redrawn from Stafford (1902).

Fig. 66 H. floedae redrawn from the type specimen.

Fig. 67 H. uniplexus redrawn from the type specimen.

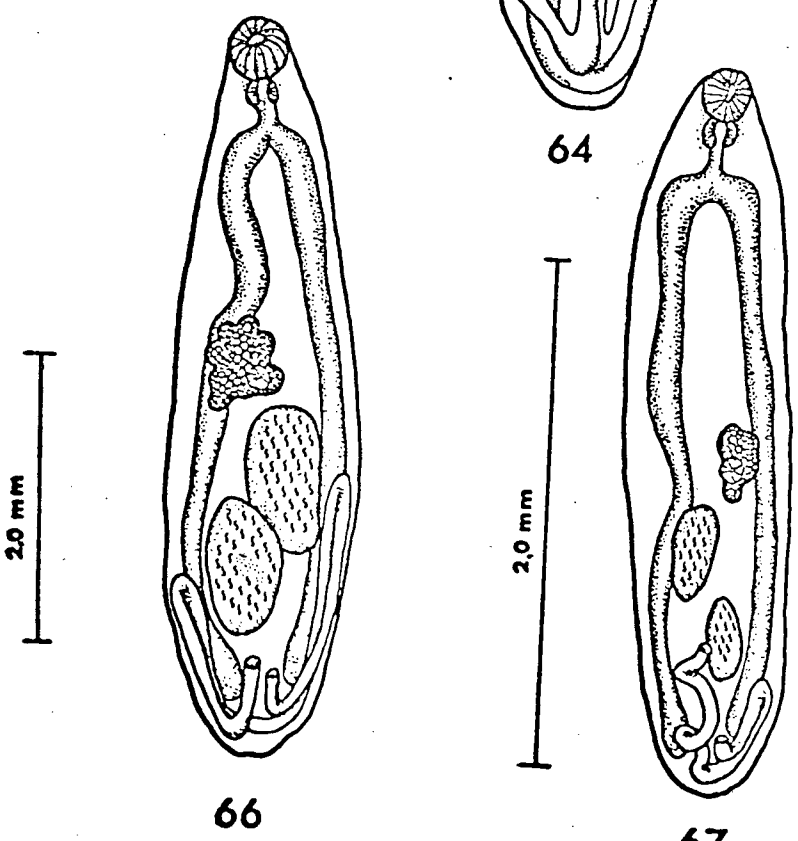
Several structures have been omitted for clarity. All specimens are depicted in the ventral view. Stafford (1902) did not give a scale to his drawings and so absolute size is unknown for his type drawings.



63

64

65



66

67

Haematoloechus kernensis Ingles

Haematoloechus kernensis Ingles, 1932: 191.

(Fig. 69)

Haematoloechus tumidus Ingles, 1932: 199. *syn. nov.*

(Fig. 70)

Description (based on 2 specimens):

Body elongate, 5.51–8.57 (7.04) long by 0.98–2.93 (1.95) wide. Tegument spined. Oral sucker terminal, 0.369–0.614 (0.492) long by 0.351–0.693 (0.522) wide. Acetabulum medial, overlies the ovary or anterior to it, 0.292–0.759 (0.526) wide. O/A ratio 0.9:1.0 to 1.2:1.0 (1.1:1.0). Pharynx muscular, 0.27–0.498 (0.353) long by 0.243–0.473 (0.358) wide. O/P ratio 1.4:1.0 to 1.5:1.0 (1.5:1.0). Intestinal caeca narrow tubes, extending to near posterior extremity. Testes oblique, round, lobed or not. Ovary not lobed, pretesticular. Extracaecal uterine loops present, extending anterior to the posterior border of the anterior testis. Genital pore ventral to pharynx. Vitellaria, follicular, symmetrically placed on each side of the body. Extent ranging from midway between acetabulum and pharynx and terminating posterior to the posterior testis on the side opposite to the ovary and extending midway along the posterior testis on the side of the ovary. Eggs operculate, 0.023–0.036 (0.030) long by 0.016–0.020 (0.018) wide.

Host: *Rana aurora draytoni* Camp, 1917

Site of infection: Lungs.

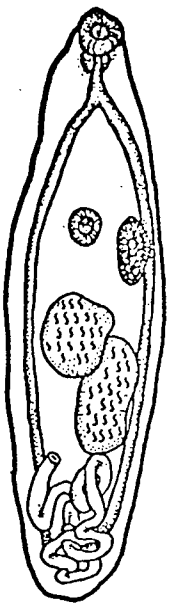
Specimens examined:

One type specimen, U.S.N.M. Helm. Coll. No. 8654; 1 type specimen, U.S.N.M. Helm. Coll. No. 8657.

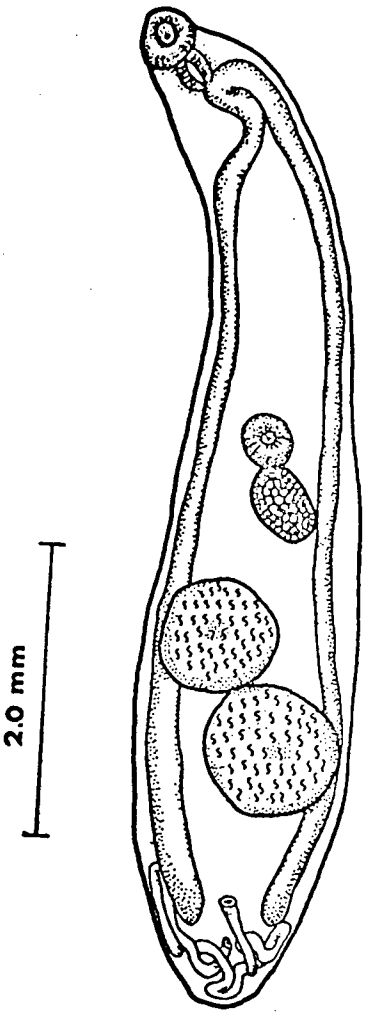
Fig. 68 *Haematoloechus complexus* (*Pneumonoeces complexus* of Seely, 1906) redrawn from Seely (1906). No scale given.

Fig. 69 *H. kernensis* redrawn from the type specimen.

Fig. 70 *H. tumidus* redrawn from the type specimen.



68



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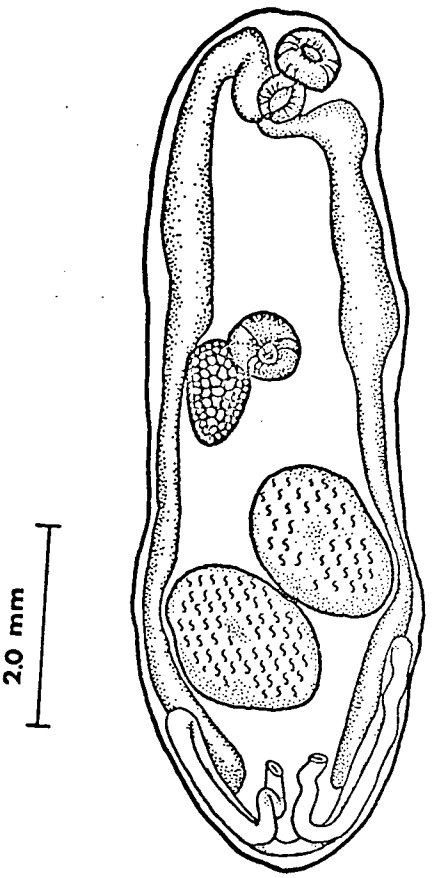


70

Discussion:

Examination of the type specimen of H. kernensis (U.S.N.M. Helm. Coll. No. 8654) and H. tumidus (U.S.N.M. Helm. Coll. No. 8657), showed certain inconsistencies between description and observed morphology.

Ingles (1932) states that "The cuticula of Haematoloechus kernensis is entirely smooth, there being no indication of spines at any place," p. 191. The absence of spines is one of the main features distinguishing this fluke from H. tumidus, which is spined.

Examination of the type material revealed the presence of spines. Spines are sparsely distributed anterior of the acetabulum, more concentrated at the level of the acetabulum and posterior to it. The tegument appears to be sloughed off in several places anterior to the acetabulum. Spines are present around the middle portion of the worm where the tegument is intact.

Ingles (1932) states that " Haematoloechus tumidus differs from all the previously described species of this genus in being larger, and in having the acetabulum larger than the oral sucker," p.200. However, Ingles measured the length and width of living animals. When he measured mounted specimens he found them to be smaller, but even then he felt their size to be generally larger than that of other species of Haematoloechus previously described. Size alone is a poor criterion to use in species separation of lung flukes.

H. tumidus differs from H. kernensis mainly in having spines. However, I have demonstrated that H. kernensis is

spined. Therefore I feel that the two species should be synonymized and that H. kernensis has page priority and is the valid name. The above two species have been reported only one other time, that by Ingles (1936).

More specimens are needed to assess the relationship of the above two species to other Haematoloechus sp. which also contain extracaecal loops.

Group II

Haematoloechus medioplexus Stafford

Haematoloechus medioplexus Stafford, 1902: 908.

(Fig. 75)

Pneumonoeces medioplexus Stafford, 1905: 5.

Ostiolum formosum Pratt, 1903; Stafford, 1905: 5. syn. nov.

Re-description (based on 25 specimens):

Body elongate, 8.43–9.15 (8.71) long by 0.93–1.53 (1.05) wide. Tegument spined. Oral sucker terminal, 0.275–0.365 (0.327) long by 0.275–0.352 (0.314) wide. Acetabulum medial, overlies ovary, 0.083–0.099 (0.083) wide. O/A ratio 3.0:1.0 to 3.9:1.0 (3.4(1.0). Pharynx muscular, 0.176–0.231 (0.204) long by 0.192–0.242 (0.203) wide. O/P ratio 1.3:1.0 to 1.6:1.0 (1.5:1.0). Intestinal caecae narrow tubes, extending to near posterior extremity. Testes tandem, round to elliptical, lobed or not. Ovary lobed or not, pretesticular. Extracaecal uterine loops absent. Genital pore ventral to pharynx. Vitellaria follicular, variable in y distribution. Extent ranging from midway between ovary and pharynx to posterior to the posterior testis on the side opposite the ovary or half-way along the posterior testis on the ovarian side. Eggs operculate, 0.025–0.030 (0.026) long by 0.015–0.021 (0.018) wide.

Hosts: R. pipiens, R. pretiosa, B. americanus.

Site of infection: Lungs.

Specimens examined:

19 specimens, Alberta, ex R. pipiens and B. americanus,

author's coll.; 54 specimens, British Columbia, ex R. pipiens and R. pretiosa, author's coll.; 1 specimen, Iowa, ex R. pipiens, Dr. R.A. Campbell coll.; 87 specimens, Ontario, ex R. pipiens (from Dr. R.J.G. Lester and Mr. M. Baker), author's coll.; 14 specimens, Nebraska, ex R. pipiens, H.W. Manter Laboratories coll.; 18 specimens, Saskatchewan, ex R. pipiens, author's coll.; 1 specimen, Wisconsin, ex R. pipiens, Dr. J.D. Lynch coll.; 232 specimens, Wisconsin, ex R. pipiens (Biological Supply), author's coll.

Discussion:

H. medioplexus

Four hundred and twenty-six specimens of H. medioplexus from 27 collecting loclities in Canada and the United States were examined. The following variations in characters of major taxonomic importance are presented below. The results given for ovaries, testes and uterine loops are for pooled data from all localities.

Extracaecal uterine loops were absent in all specimens examined. Seventy-seven point eight percent of the ovaries were unlobed and 22.2% lobed. The anterior testis were lobed in 82.5% of the specimens, unlobed in 17.5%. Posterior testes were lobed in 88.9% of the specimens and unlobed in 11.1% of the specimens.

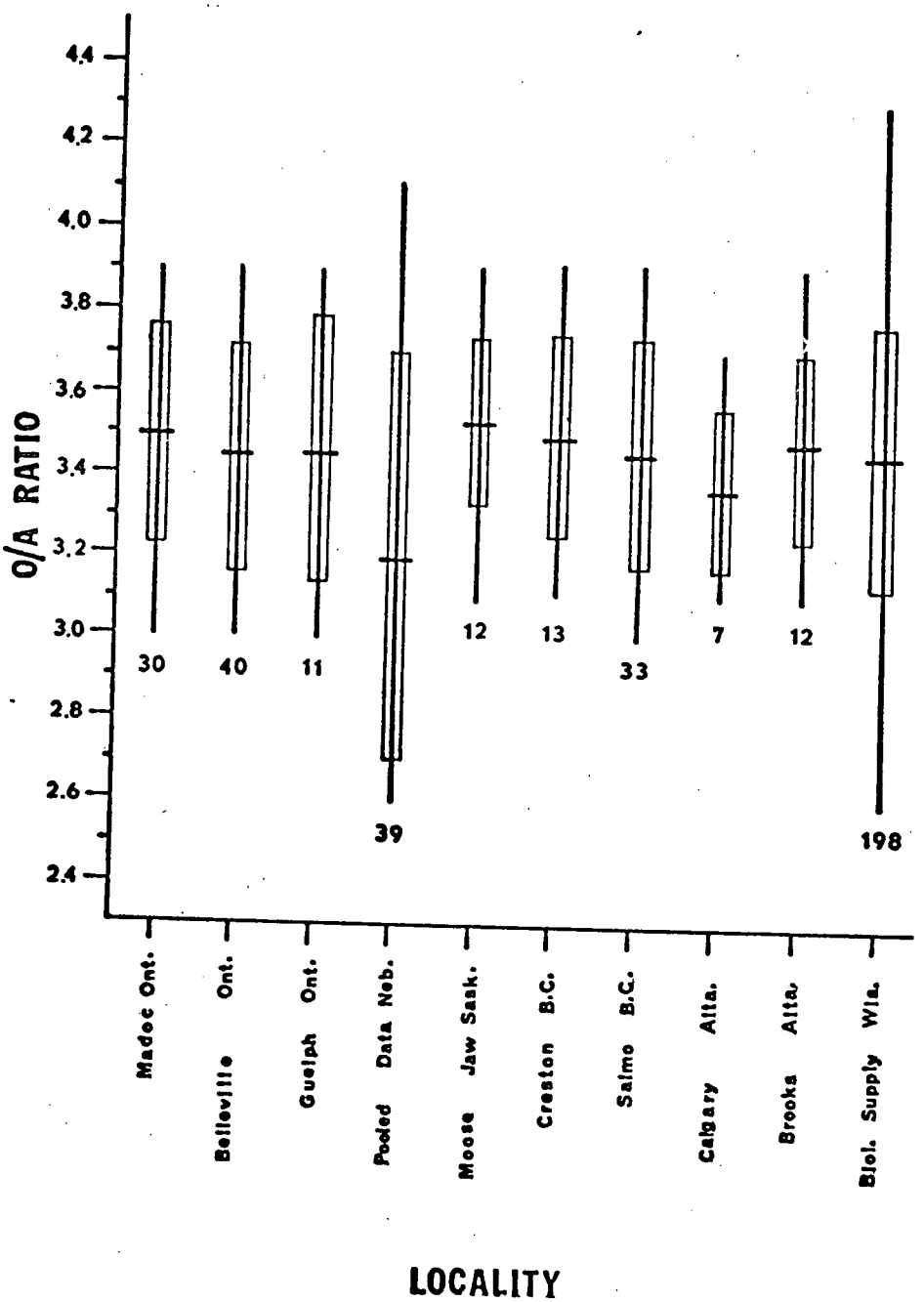
The O/A ratio could be determined for only 371 flukes. The range, mean and standard deviation for ten collecting sites are given in Figure 71. Nebraska represents pooled data from six localities in the state. No significant difference in O/A ratio occurred among the means of the ten localities (Analysis of

Variance $p > 0.05$). R. pipiens, B. boreas, and R. pretiosa were the definitive hosts for all flukes. When all data are pooled, the ratio ranged from 2.6:1.0 to 4.3:1.0 (3.5:1.0). The O/P ratio did not differ in flukes from different localities. The range of the O/P ratio was from 1.1 to 1.6:1.0 (1.4:1.0).

No significant difference occurred in egg length or in width when worms from different localities were compared. Egg length ranged from 0.024 to 0.035 (0.028) and width from 0.015 to 0.021 (0.018).

All specimens were spined.

Fig. 71 O/A ratio of H. medioplexus from different localities, indicating the mean, standard deviation (vertical rectangle) and the range (vertical line), with the sample size indicated for each location. Nebraska represents pooled data from six localities in that State.


O/A RATIO
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
30
40
11
39
12
13
33
7
12
198
Madoc Ont.
Belleville Ont.
Guelph Ont.
Pooled Data Neb.
Moose Jaw Sask.
Creston B.C.
Salmo B.C.
Calgary Alta.
Brooks Alta.
Biol. Supply Wis.
LOCALITY

Haematoloechus complexus (Seely)

Haematoloechus complexus (Seely, 1906): 249.

(Fig. 68)

Haematoloechus coloradensis Cort, 1915a: 213. *syn. nov.*

(Fig. 74)

Haematoloechus oxyorchis Ingles, 1932: 193. *syn. nov.*

(Fig. 72)

Haematoloechus confusus Ingles, 1932: 195. *syn. nov.*

(Fig. 73)

Re-description (based on 50 specimens):

Body elongate, 2.73–7.72 (5.82) long by 0.35–1.95 (1.23) wide. Tegument spined or not. Oral sucker terminal, 0.253–0.513 (0.379) long by 0.242–0.492 (0.375) wide. Acetabulum medial, overlies the ovary, or anterior to it, 0.120–0.450 (0.271) wide. O/A ratio 1.0:1.0 to 3.5:1.0 (1.5:1.0). Pharynx muscular, 0.127–0.432 (0.228) long by 0.122–0.369 (0.208) wide. O/P ratio 1.2:1.0 to 2.4:1.0 (1.9:1.0). Intestinal caeca narrow tubes extending to near posterior extremity. Testes tandem, round, lobed or not. Ovary lobed or not, pretesticular. Extracaecal uterine loops absent. Vitellaria follicular, symmetrically placed on each side of the body, extent ranging from midway between ovary and pharynx to posterior to posterior testis on the side opposite the ovary, or half-way along the posterior testis on the ovarian side. Eggs operculate, 0.023–0.043 (0.034) long by 0.014–0.024 (0.019) wide.

Host: *R. blairi*, *R. pipiens*, *R. catesbeiana*.

Site of infection: Lungs.

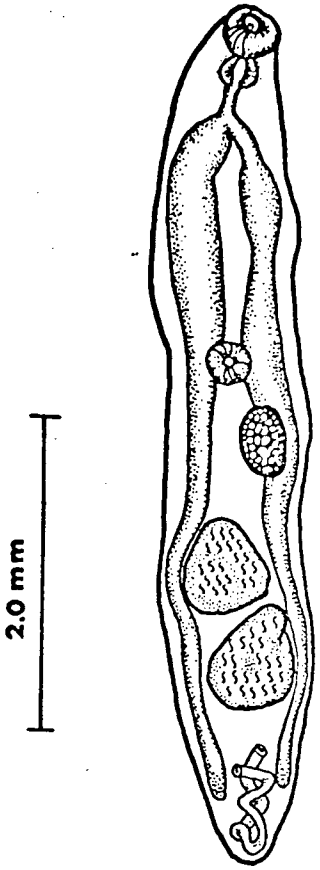
Fig. 72 H. oxyorchis redrawn from the type material.

Fig. 73 H. confusus redrawn from the type specimen.

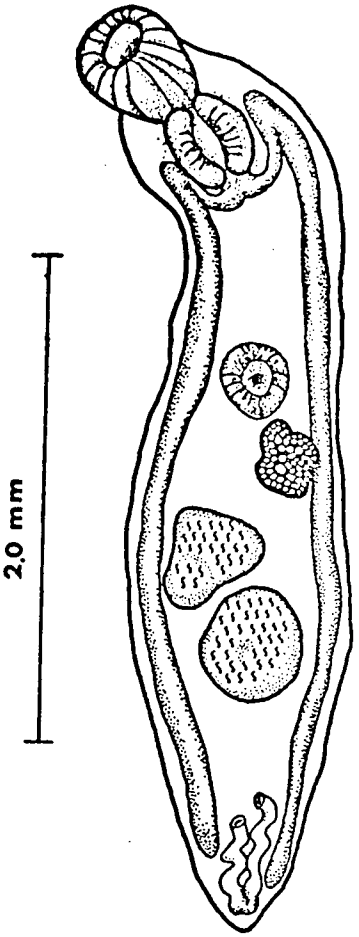
Fig. 74 H. coloradensis (Pneumonoeces coloradensis of Cort, 1915a) redrawn from Cort (1915a).

Fig. 75 H. medioplexus redrawn from Stafford (1902).

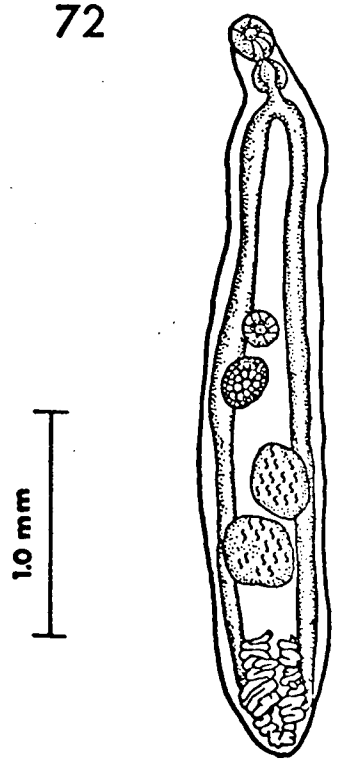
Several structures have been omitted for clarity. All specimens are depicted in the ventral view. Stafford (1902)) did not give a scale to his drawings and so absolute size is unknown for Figs 75. type drawings.



72



73



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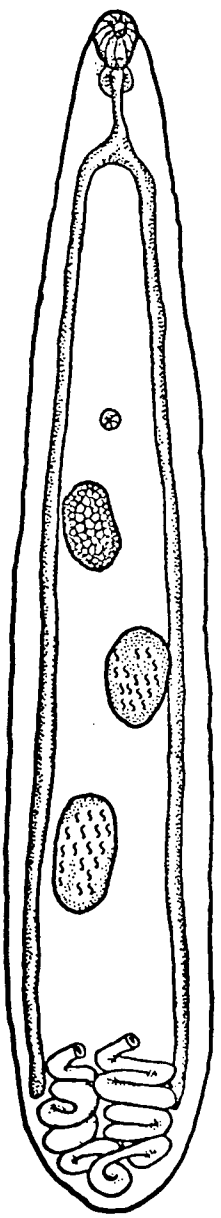


75

Specimens examined:

A. H. complexus:

105 specimens, Nebraska, ex R. blairi, H.W. Manter Laboratories coll.; 5 specimens, Nevada, ex R. pipiens, Dr.B.B. Babero coll.

The type specimen was not available to the author.

B. H. coloradensis:

10 specimens, Nebraska, ex R. pipiens, H.W. Manter laboratories coll.; 5 specimens, Florida, ex R. catesbeiana, Dr. G.J. Bowers coll.

The type specimen was not available to the author.

C. H. oxyorchis:

1 type specimen, U.S.N.M. Helm. Coll. No. 8655, ex Rana aurora draytoni.

D. H. confusus:

1 type specimen, U.S.N.M. Helm. Coll no. 8656, ex Rana aurora draytoni,

Discussion:

H. complexus

H. complexus (Pneumonoeces complexus of Seely, 1906) was described by Seely (1906) as without spines. However, he states that this may have been due to maceration of the worm. Only patches of intact tegument remained and these did not have spines. Unfortunately Seely did not mention the position of these patches. Type material was not available for examination.

Of the 105 H. complexus examined from Nebraska and five from Nevada, 90 had spines over the entire body surface. Spines are smaller and less numerous posterior of the acetabulum.

Extracaecal loops are absent in this species. The anterior testis was lobed in 6% of the specimens, the posterior testis in 11%. All ovaries were unlobed.

The O/A ratio ranged from 1.0:1.0 to 2.4:1.0 with a mean of 1.4:1.0. The worm with the 2.4:1.0 ratio had a very small acetabulum. The O/P ratio of flukes from different localities did not differ significantly. The mean O/P was 1.9:1.0 with a range of 1.6:1.0 to 2.0:1.0.

The widths of eggs did not differ significantly in flukes from different localities (Fig. 76).

Egg lengths from the four localities in Nebraska did not differ significantly (Fig. 76). The egg lengths of flukes from Nevada differed significantly (Newman-Keuls, $\alpha > 0.05$) from the egg lengths of flukes of pooled data from Prague, Davey and Mead (Nebraska) ($\alpha << 0.05$), however, the sample size from Nevada is very small (n=5). More samples from this locality are needed.

Egg length had a range of 0.030 to 0.043 (0.036). Egg width had a range of 0.017 to 0.025 (0.020).

H. coloradensis

When all anterior testes were compared. 86.7% were observed to be lobed. This also holds for the posterior testes. All ovaries examined were lobed.

Only 15 specimens from the United States and Canada were examined. Extracaecal loops were absent in all of these

specimens. However, short loops do occur occasionally (21.0% of the time) but do not extend extracaecally (Figs. 79 and 80). The form of coiling of the posterior uterus depended on the host. When the host was R. pipiens, the coils formed tight spring-like loops (Fig. 77). When the host was R. blairi, the coils formed loose twists (Fig. 78).

The O/A ratio ranged from 1.0:1.0 to 1.4:1.0 with a mean of 1.2:1.0. The O/P ratio did not differ significantly in flukes from different localities or hosts. This ratio ranged from 1.2:1.0 to 2.0:1.0 (1.4:1.0).

No difference occurred in egg length or width in flukes from different hosts or localities. Egg length ranged from 0.031 to 0.038 (0.035) and width from 0.017 to 0.024 (0.019).

All specimens were spined.

Fig. 76 Length and width of eggs of H. complexus from different localities, indicating the mean, standard deviation (vertical rectangle) and range (vertical line), with the samples size indicated for each locality. Data for Verdon and Humboldt were pooled as are data for Prague, Davey and Mead. R. blairi was the host for all flukes from Nebraska and R. pipiens for flukes from Nevada.

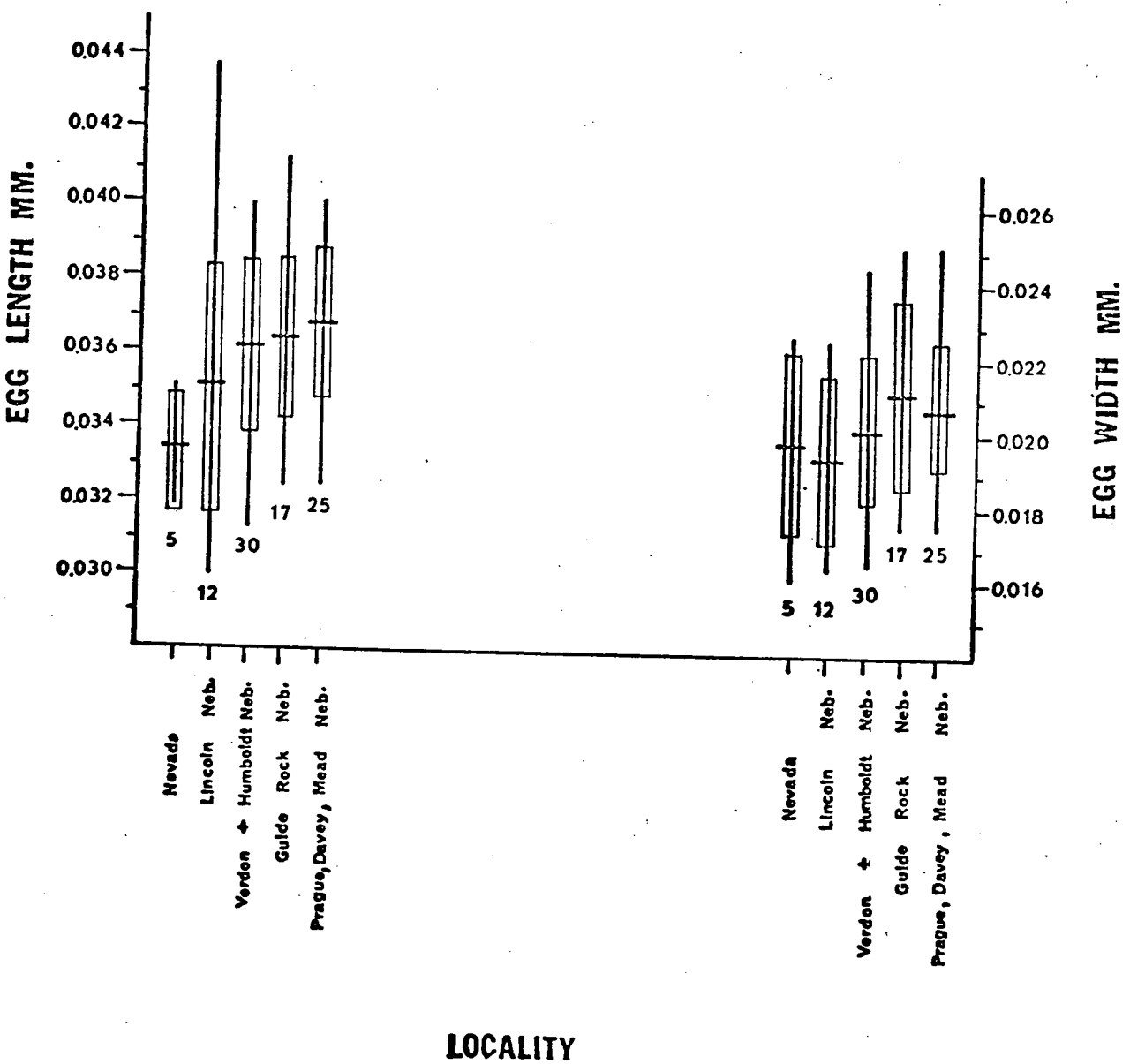
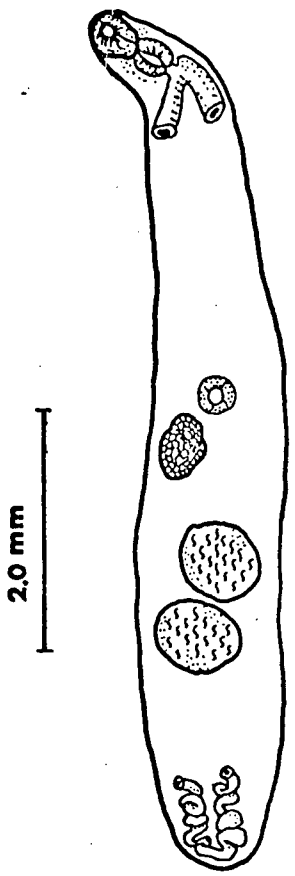

EGG LENGTH MM.
0.044
0.042
0.040
0.038
0.036
0.034
0.032
0.030
5
12
30
17
25
EGG WIDTH MM.
0.026
0.024
0.022
0.020
0.018
0.016
5
12
30
17
25
Nevada
Lincoln Neb.
Verdon + Humboldt Neb.
Guide Rock Neb.
Prague, Davey, Mead Neb.
LOCALITY

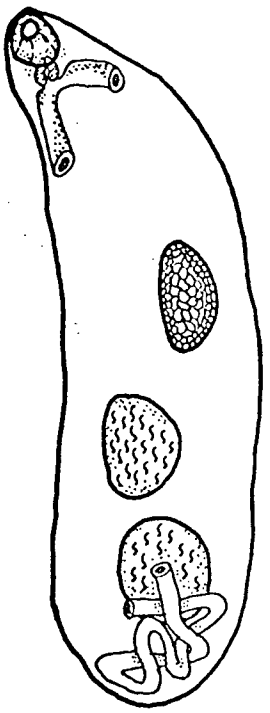
Fig. 77 The shape of posterior uterine coils of H. coloradensis when the fluke is recovered from R. pipiens.

Fig. 78 The shape of posterior uterine coils of H. coloradensis when the fluke is recovered from R. blairi.

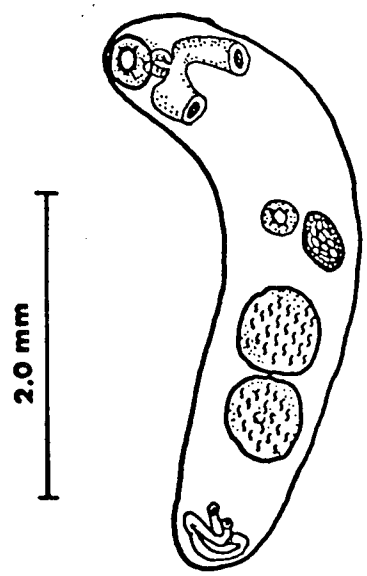
Fig. 79-80 Some variations in uterine loops of H. coloradensis.



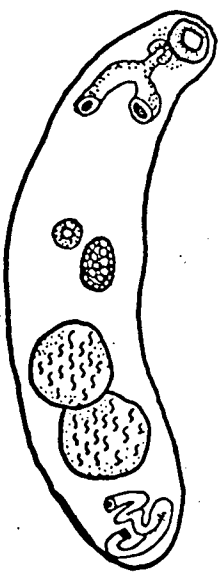
77



78



79



80

H. oxyorchis and H. confusus

Examination of the type specimens of H. oxyorchis (U.S.N.M. Helm. Coll. No. 8655) and H. confusus (U.S.N.M. Helm. Coll. No. 8656) revealed some discrepancies between description and observed morphology.

H. oxyorchis:

Ingles (1932: 193) described H. oxyorchis as having "cuticula free from spines." I found that the tegument appeared to be lacking anterior of the acetabulum. Small spines are present on the anterior and mid-body where the tegument is intact. These spines are very small, barely protruding through the tegument. Spines are absent posterior of the posterior testes.

The main feature used to distinguish H. oxyorchis from H. confusus has been the supposed absence of spines from the former species. The presence of spines in the type specimen of H. oxyorchis suggests that these two species should be synonymized.

H. confusus:

"eggs average 26 microns in length and 15 microns in width" (Ingles, 1932). The average measurement of 25 eggs measured from location A (see Appendix 22, Fig. 81) was, 0.031 mm long by 0.023 mm wide. The discrepancy between Ingles' measurements and mine may be due to measuring eggs from a different part of the uterus.

Egg size and the presence of spines were used by Ingles to

distinguish this species from H. complexus, described by Seely (1906). The type specimens of H. ccmplexus is not available for examination. The difference in egg size between H. complexus (0.029 mm by 0.014 mm) and H. confusus is not sufficient for species separation.

DISCUSSION AND CONCLUSIONS

A. Differentiation of Species

H. longiplexus and *H. breviplexus*:

H. longiplexus can only be confused with one other North American haematoloechid: *H. breviplexus*. They are similar in their O/A ratios, 2.0:1.0 for both species (Stafford, 1902; Cort, 1915a). My observations show a range of 2.1:1.0 to 3.4:1.0 (2.8:1.0) for *H. breviplexus* and 1.5:1.0 to 2.8:1.0 (2.2:1.0) for *H. longiplexus*. These ratios did not vary geographically (Map 1). These species are also similar in shape of testes and ovary and in general body size. Two features serve to separate these species: the extent of the extracaecal loops and the orientation of the testes.

The extracaecal loops in *H. longiplexus* are the longest in any North American species, and extend anteriorly to near the pharynx (Stafford, 1902; Cort, 1915a). Some variation does exist, but the loops are never found less than 1/2 the distance between the ovary and the pharynx. Uterine loops in *H. breviplexus* are shorter and extend from the anterior border of the posterior testis to the posterior border of the ovary (Cort, 1915a). My observations indicate a slightly greater variation for the left uterine loop. This loop may extend from 1/2 the length of the posterior testis to the level of the anterior border of the anterior testis. However, in *H. breviplexus* the loops never extend anterior beyond the level of the ovary.

The elongate testes are parallel in *H. longiplexus*, that is

they overlap for more than 70% of their length. The testes are unequal in size, the smaller testis occasionally extending 20% of its length anterior of the larger testis. The testes of H. breviplexus are also elongate, but they overlap for only 1/2 their length (Stafford, 1902). This overlap ranged from 35% to 60% in my collections. The testes were never parallel.

The extent of the extracaecal loops and the shape and orientation of testes serve to separate these two species from other North American representatives of Haematoloechus and from one another.

H. varioplexus and H. similiplexus:

The extent of the extracaecal loops, size and shape of testes and ovaries, and O/P ratio are similar for these two species.

The uterine loops in both species commonly extend a distance 40 to 60% along the posterior testis. The ovary and testes are usually unlobed, but lobing in some specimens in both species was noted. The O/P ratio did not differ between these two species. This ratio ranged from 1.3:1.0 to 2.3:1.0 (1.7:1.0) for both species.

The O/A ratio was not reported by Stafford (1902) for either species. Cort (1915a) reported the O/A ratio for H. similiplexus as averaging 4:3 (1.33:1.0). He did not examine specimens of H. varioplexus. Brooks (1976) considered H. similiplexus and H. varioplexus synonyms and reported the O/A ratio as having a range of 1.0:0.69 to 0.84 (1.5:1 to 1.2:1). However, when the data for O/A ratio are plotted according to

host, and not fluke species, the O/A ratio varies with host. The same is true for egg size.

H. varioplexus has an egg length of 0.029, whereas H. similiplexus is reported as having an egg length of 0.039 (Stafford, 1902). Cort (1915a) recorded an egg length range of 0.034 to 0.040 for H. similiplexus. Later, while examining specimens of H. similiplexus from R. pipiens from Oshkosh, Wisconsin, Cort (1915b) noted that the eggs were smaller than previously reported for this species. These individuals had an average egg length of only 0.034, and the limits of variation were from 0.030 to 0.037. Brooks (1976) cited an egg length range for H. varioplexus of 0.032 to 0.037. Bouchard (1951) noted variations in H. similiplexus from the lungs of R. clamitans and R. septentrionalis collected in Maine. He reported that some specimens had an egg length average of 0.029. He also noted that some of these flukes had longer extra-crural uterine folds than reported by Cort(1915a) and Stafford (1902). Figure 55 demonstrates the great variation occurring in egg measurements. This variation appears to be related to host and not geographical influences. Thus H. varioplexus and H. similiplexus cannot be separated.

H. parviplexus:

Irwin (1929) and later Brooks (1976) described the ovary of H. parviplexus as being deeply lobed. My experimental results using H. buttensis show that great variation can occur in the degree of lobing of the ovary and testes, even in worms of the same age. The ovary may be unlobed (Fig. 50) or deeply lobed

(Figs. 51 and 52). Collections of H. parviplexus from five localities show that lobing in ovaries and testes is highly variable and may or may not occur. There is therefore too much variability in this character to be used in species separation.

Uterine folds in H. parviplexus are reported by Irwin (1929) as never reaching the anterior border of the posterior testis; scarcely reaching the posterior testis in some, while in others the loops may extend along 3/4 the length of the posterior testis. A similar degree of variation was found in the 154 specimens examined by me.

I have shown, in this thesis, that the O/A ratio for this species is variable, and may be host-related (Fig. 49) as well as varying geographically.

This species cannot therefore be separated from H. buttensis.

H. buttensis:

Ingles (1936) used a combination of characters to separate his newly-described species, H. buttensis (Fig. 63) from other members of the genus with which it may be confused.

H. buttensis differed from H. parviplexus (Irwin, 1929) notably in the O/A ratio, the shape of the ovary, and the extent of the extracaecal uterine folds. Variations in the shape of the ovary and the extent of the extracaecal uterine folds have been discussed in the previous section. The main feature distinguishing H. buttensis from H. parviplexus was stated to be the O/A ratio, H. buttensis having an average ratio of 1.0:0.7 (1.4:1.0) (Ingles, 1936). Flukes collected from R. pretiosa from

British Columbia had an average O/A ratio of 2.6:1.0 (2.2:1.0 to 2.9:1.0).

H. parviplexus has a ratio of 4:1 (Irwin, 1929). However, Brooks (1976) found the Nebraska collection to range from 2.3:1.0 to 2.6:1.0, and my collections from British Columbia averaged 3.0:1.0 (2.6 to 3.4:1.0). Therefore, the O/A ratio in these two species is too similar to be used as a distinguishing character.

H. buttensis is separated from H. similiplexus Stafford, 1902 (Fig. 64) by having smaller eggs, average 0.027 by 0.014 (0.025 to 0.030 and 0.022 to 0.017 for length and width respectively), and larger testes. Testes measured 0.82 long by 0.64 wide (0.45 to 1.03 by 0.48 to 0.87). Both testes were described as being nearly the same size and shape. Vitellaria were also considered as differently distributed. However, specimens examined by me did not differ in vitelline distribution. Stafford (1902) gave the egg measurements of H. similiplexus as averaging 0.039 by 0.019, but stated that "variations a little above and below occurred." H. similiplexus was described from the lungs of R. virescens (= R. pipiens) and B. lentiginosus (= B. americanus). He described H. varioplexus from the lungs of R. clamitans. The type description was very incomplete, and Stafford trusted his drawings to illustrate its chief characteristics. No scale was given for his drawings.

As shown above, H. buttensis cannot be separated from H. parviplexus or from H. similiplexus and H. varioplexus.

H. uniplexus:

The type description of H. uniplexus Harwood, 1932 (Fig. 67) is based on a single specimen. This fluke is distinguished from all other worms of the genus (considered here) by possessing a single short extracaecal uterine loop. The variations in extracaecal uterine loops have already been discussed for several species. The frequency of occurrence of a single loop in samples collected from numerous localities indicates that this alone is not a good character for separating species in this genus. This species has not been reportedsince its original description.

H. floedae:

Harwood (1932) separated H. floedae from H. parviplexus, which it most closely resembles, by the smaller pharynx, larger acetabulum, the smooth tegument, smaller eggs and the longer longitudinal uterine folds (Figs. 66 and 40 respectively).

The O/P ratio in H. floedae is nearly 1:2; (0.5:1) the ratio does not fall below 2:5 (0.4:1) (Harwood, 1932). In H. parviplexus this ratio is 3:2 (Irwin, 1929).

The O/A ratio in H. floedae is 3:1 (Harwood, 1932). In H. parviplexus this ratic is 4:1 (Irwin, 1929). Eggs of H. floedae vary from 0.021 by 0.017 to 0.017 by 0.013. Eggs of H. parviplexus range in size from 0.023 to 0.029 in length and from 0.016 to 0.019 in width, averaging 0.025 by 0.017.

The lengths of the extracaecal folds of the uterus in H. floedae have been shown, by examining the type specimen, not to extend beyond 1/2 the distance along the anterior testis.

Uterine folds on the ovarian side of the body are often shorter than its mate (Harwood, 1932).

Variations in O/A ratio, egg measurements, and extent of extracaecal loops have previously been discussed for H. parviplexus.

Because of the variation in egg measurements, O/A ratios, and extent of extracaecal loops, it is impossible to separate H. varioplexus, H. similiplexus, H. parviplexus, H. buttensis, H. floedae and H. uniplexus from one another. However, the extracaecal loops never extend beyond the anterior testis. Therefore, this group can be separated from H. longiplexus and H. breviplexus.

H. complexus, H. confusus, and H. oxyorchis:

Ingles (1932) states that " H. confusus more closely resembles H. complexus than any other described lung fluke. It agrees favourably with this latter species in size, type of uterus, the lobed testes and ovary, and in ratio of the oral sucker to the acetabulum. It differs from H. complexus, however, in having spines, in having smaller eggs, in the arrangement of the vitellaria, and in the testes and ovary always being lobed" [sic].

H. oxyorchis differs from H. complexus in having smaller eggs. Egg size was the main feature distinguishing H. oxyorchis from H. complexus.

Type specimens of H. complexus were not available for inspection. However, Seely (1906) states that this species is "without spines" but notes that "this may have been due to

maceration". Eighty-two percent of the specimens examined by me had spines.

The egg sizes of these two species overlap the low egg size range of H. complexus. I feel that there is not enough difference in these measurements to warrant separation of these two species from H. complexus.

I have shown that in collections of H. complexus six percent of the anterior and eleven percent of the posterior testis were lobed.

H. coloradensis:

Cort (1915a) reviewed the North American lung flukes and described H. coloradensis n. sp. in the same paper. He stated that H. coloradensis (Fig. 74) is most closely related to H. complexus (Seely, 1906) (Fig. 68) in having the same body shape and general arrangement of the uterus as that species. Differences between these two species were noted in the O/A and O/P ratios and in size of eggs.

The O/A ratio for H. coloradensis is 5:4 (=1.3:1.0) (Cort, 1915a) and 1.1:1.0 for H. complexus (Seely, 1906). My collections show that the ratios overlap completely for the two species. The ratios ranged from 1.0 to 1.4:1.0 (1.2:1.0) for H. coloradensis and 1.0 to 2.4:1.0 (1.4:1.0) for H. complexus.

The O/P ratio for H. coloradensis is 10:7 (=1.4:1.0) (Cort, 1915a) and was not given for H. complexus by Seely (1906). My data show a great deal of overlap in this ratio for the two species, H. coloradensis has an O/P ratio range of 1.2 to 2.4:1.0 (1.4:1.0) and H. complexus has a range of 1.6 to 2.0:1.0

(1.9:1.0).

The average egg measurement for H. complexus is 0.029 by 0.014 (Seely, 1906) and 0.034 by 0.020 for H. coloradensis as given by Cort, 1915a. Specimens examined by me indicated almost a complete overlap in these measurements for the two species. The average measurement for H. coloradensis was 0.035 by 0.019 and for H. complexus 0.036 by 0.020.

Because of the overlap in measurements used in attempting to separate these two species the separation is not valid.

B. Examining Type Specimens.

A re-examination of available type specimens of North American Haematoloechus spp. indicated some errors in earlier observations.

The absence of spines reported for six species of Haematoloechus has been used to help separate these species from those flukes which bear spines. Ingles (1932) reported that H. oxyorchis differed from H. confusus in being spineless. Similarly, the absence of spines in H. floedae was one character used by Harwood (1932) to distinguish this species from H. parviplexus. Examination of the type specimens of all the above four species demonstrated that all had spines.

H. kernensis was described by Ingles (1932) as having no spines. This feature was used to help separate that species from H. tumidus (described in the same paper), which had spines. H. uniplexus was also described as being spineless (Harwood, 1932), but Harwood did not use this character to separate it from other Haematoloechus spp. Re-examining the type specimens of H. kernensis, H. tumidus and H. floedae demonstrated that all were spined.

The type specimen of H. complexus was not available for examination. This species was described by Seely (1906) as being without spines. Seely stated that "this may have been due, however, to maceration," p. 249. I have shown experimentally that flukes lose their spines when treated in distilled water. Furthermore, I have shown that a certain proportion of flukes of each species collected in the field, including H. complexus, do not have spines.

Because of the errors made in reporting spines, the experimental results demonstrating the loss of spines during pre-fixing treatment, and the variation found in field observations, this character cannot be considered reliable for species separation in this genus.

The shape of testes and ovaries has also been used to separate species in this genus.

Ingles (1932) used these features to help separate H. kernensis from H. tumidus. The ovary is lobed and the testes are unlobed in H. tumidus Ingles, 1932. The shape of the testes was also used by Ingles (1932) to separate H. confusus from H. oxyorchis. Examination of the type specimens of H. confusus and comparison with H. oxyorchis indicated that the shape of the testes of the two species was not different (Figs. 73 and 72). The presence or absence of lobing of ovaries and testes has been shown, in this thesis, to be highly variable for several other members of this genus.

Ingles' (1936) drawing of H. buttensis showed that the left extracaecal uterine loop extended 1/3 the way along the length of the posterior testis, and the right loop did not reach the posterior testis. Examination of the type specimen indicated that both extracaecal loops just reach the level of the posterior testis. Examination of the type specimen of H. floedae demonstrated that the left extracaecal loop extended only a distance 1/2 way along the anterior testis. The right loop extended half-way along the posterior testis. Harwood's drawing of the type specimen shows both loops extending a distance 1/2 way along the ovary. It is very important to be correct in

reporting the variation in this character, as it is used to help separate several species of Haematoloechus. The range of variation in this character has not been reported for many Haematoloechus sp.

PART III. TAXONOMIC DISCUSSION

Haematoloechus Looss, 1899, one of the most commonly encountered genera of frog trematodes, has been reported from practically every continent. Although Looss named the genus in 1899, he renamed it Pneumonoeces in 1902. This was done because the genus Haematoloecha had been given to an hemipteran by Stäl in 1874. Harwood (1932), and Ingles (1932) independently pointed out that the name first chosen by Looss for this genus was not invalidated, and should stand in accordance with the International Code of Zoological Nomenclature. However, some authors, notably Skrjabin and Antipin (1962), retained Pneumonoeces.

Additional generic names have been applied to trematodes of this group. Ward (1917) accepted the genus Pneumonoeces but stated that one group of flukes, in this genus, should be separated as a new genus, to which he applied the name Pneumobites and gave Pneumobites longiplexus (Haematoloechus longiplexus of Stafford, 1902) as the type of the genus. Ward characterized this genus as having elongate lateral and nearly symmetrical testes, and lobed ovary -in contrast to the round, median testes and entire ovary of Pneumonoeces. Extracaecal longitudinal folds of the uterus are more pronounced in Pneumobites, reaching nearly the length of the body. Ward included Pneumobites breviplexus (H. breviplexus of Stafford, 1902) in this new genus.

In my thesis I have demonstrated that the geographical variation in extent of extracaecal loops, and orientation of the testes is constant for the above two species, regardless of

collecting locality. However, the lobed nature of the ovary and testes is highly variable.

Mayr (1969) states that "a genus is a taxonomic category containing a single species, or a monophyletic group of species, which is separated from other taxa of the same rank (other genera) by a decided gap". Mayr (1969) recommended that the size of the gap be in inverse ratio to the size of the taxon. The soundest genera are based on an overall appreciation of the members of the taxon under consideration (Mitchener, 1957) and ideally should be based on the occurrence of correlated character complexes (Mayr, 1969)

To erect new genera based on incomplete knowledge of a few representatives of the world fauna currently recognized under the genus Haematoloechus is clearly unwise.

Variations in extent of extracaecal loops, and orientation of testes are not known for many European species. The characters proposed by Ward(1917) to characterize the genus Pneumobites are present in different combinations in some European species. For example, H. sibericus has round testes but also elongate extracaecal loops, which extend at least as far as is found in H. breviplexus, which Ward included in Pneumobites. Similarly, there are species in which the testes are parallel, but round and smooth (H. nanchangensis reported from Japan). Therefore, I feel that the genus Pneumobites should be rejected, and Haematoloechus retained.

The extent of the extracaecal loops and the shape and orientation of testes serve to separate H. longiplexus and H. breviplexus from one-another as well as from all other North

American representatives of Haemtaoloechus. Because of the consistent character differences, I propose that these two species remain as valid species.

Natural infections of H. longiplexus have most frequently been described from R. catesbeiana, but have been recorded from six species of Ranidae and one of Bufonidae. H. breviplexus has also been reported from R. catesbeiana, but occurs in two other ranids and one bufonid. The above flukes have been reported only from North America and have not so far been reported north of the 50°N parallel or south of the 28°N parallel. The geographic distribution of these species follows closely that described for R. catesbeiana. The occurrence of these flukes in R. catesbeiana and occasionally in R. clamitans may be related to differences in seasonal reproductive timing of the host, or food preferences at the time when infected odonates are available to frogs. Therefore, host specificity of H. longiplexus for R. catesbeiana, as suggested by reported field observations, may be a result of ecological factors. Habitat preferences of R. catesbeiana and R. clamitans were demonstrated by Stewart and Sanderson (1972). The different preferences resulted in R. catesbeiana taking Coenagrionidae (Odonata) as a food item, whereas R. clamitans did not consume any odonates.

Ostiolum Pratt, 1903 was differentiated from Haematoloechus Looss, 1899 only on the absence of extracaecal loops in the former and the presence of one or two loops in the later. Odening (1960a) and Skrjabin (1964) have also accepted this genus as being valid. If this genus is accepted, on the basis of this character designation, two genera would be recognized:

Ostiolum Pratt, 1903, and Haematoloechus Looss, 1899. The genus Osticlum would include five North American species presently recognized in the genus Haematoloechus : H. medioplexus, H. complexus, H. coloradensis, H. oxyorchis, and H. confusus. A single character may justify the creation of a new species but it cannot be the sole criterion for the erection of a new genus (Verster, 1969). If the above criterion was followed it would be necessary to erect at least four new genera: one to accommodate H. uniplexus, in which there is a single extracaecal uterine loop; one to accommodate species with egg lengths greater than 0.058 mm; one to accommodate species with ventral suckers equal to or larger than the oral sucker; and one to accommodate species with round or oval testes.

I have shown, experimentally, and by analysis of field collections, that variations within the genus may result from differences in age and degree of maturity, extent of crowding, species of host, and other factors. Because of these variations, I feel that the genus Ostiolum should not be accepted, and that the genus Haematoloechus should contain the above five species.

I recognize only two of the above five species as being valid. I consider H. medioplexus valid. This species can be distinguished from the other four species of the group by its O/A ratio, which remained constant regardless of collecting locality. This ratio can serve to separate H. medioplexus from all other Haematoloechus sp. which do not contain extracaecal uterine loops.

H. medioplexus occurs primarily in R. pipiens, but has been recorded infrequently from seven other species of Ranidae and

onespecies of Bufonidae. My records show that H. medioplexus has not been recorded above the 50°N parallel or below the 40°N parallel, or west of the Cascade mountains. The life-cycle of H. medioplexus may have developed by adaptation to the early breeding season of R. pipiens. Therefore, metacercariae of H. medioplexus may be available to R. pipiens at a time when it is using the pond most heavily and may not be available to other frogs using the pond later in the season. R. pipiens may be displaced from the ponds when other frogs are using the area. This displacement may result in a shift in food items taken by R. pipiens.

H. complexus is the other species in this group which I consider valid. H. oxyorchis, H. confusus and H. coloradensis are here considered synonyms of H. complexus.

Egg size, and lobation of testes and ovary, as well as absence of spines in H. complexus and H. oxyorchis were the main features previously used to separate these species from one another. The range of egg sizes of H. coloradensis and H. complexus did not vary geographically. However, egg length and width of these two species overlap completely, and are therefore unreliable for separating them. The egg sizes of H. oxyorchis and H. confusus overlap completely with each other and with the lower limit of egg length of H. complexus. I have shown that egg size varies in H. buttensis when this fluke is developed in different frog hosts. I have also shown that egg length varies geographically for several other members of this genus. Separating species on the basis of egg size alone is therefore very unreliable.

Lobation of ovary and testes is also an unreliable character to use for separating species in this genus. I have demonstrated experimentally that lobing in these structures is dependent, in part, on the age of the worm. Furthermore, field collections of H. complexus and H. coloradensis have shown that lobation may or may not be present. This character is therefore unusable for separating species.

For the above reasons, H. complexus, H. oxyorchis, H. confusus and H. coloradensis are considered synonyms. H. complexus (Seely, 1906) is the valid name.

H. complexus uses R. pipiens most frequently as its definitive host, as does H. medioplexus. H. complexus may even have arisen from H. medioplexus. All flukes designated by me as H. complexus occur between the 30° N and 40° N parallels. H. coloradensis has been reported from various localities in Utah, Colorado, Idaho and Nebraska. Two species (H. oxyorchis and H. complexus) recorded from the west coast by Ingles (1932a) may have been derived from what Cort (1915) has called H. coloradensis. "A Little Ice Age" followed some time after the close of the Wisconsin. Dumas (1966)believed the cooler, moister climatic conditions of this age permitted an invasion across the low passes of the Rockies of the eastern pond frog R. pipiens. It is during this time that R. pipiens may have passed on to R. aurora the lung fluke, H. coloradensis, and what Ingles (1932) has called H. oxyorchis and H. confusus. These latter lung flukes are morphologically very similar to H. coloradensis, and the distribution of H. coloradensis in R. pipiens suggests this may be the stock which supplied Ingles with his flukes.

Ingles (1936) states that H. tumidus is always found in frogs which inhabit streams and never in frogs from ponds. The pond-inhabiting frogs are always infected with H. oxyorchis. If my transfer theory is correct, then H. oxyorchis may have been transferred from R. pipiens to Rana aurora draytoni and adapted to a pond-type life cycle. H. tumidus may be the original lung fluke Rana aurora draytoni of the stream type.

What was originally described as H. complexus (Seely, 1906) may also have arisen from H. coloradensis but is now using R. blairi and R. utricularia (both previously considered R. pipiens) as its amphibian host.

Odening (1960a) included in the genus Haematoloechus all those frog lung flukes which contained extracaecal loops. Flukes without extracaecal loops were included in the genus Ostiolum. Odening did not accept Pneumobites Ward, 1917 as being valid. In a previous paper Odening (1958) included all frog lung-flukes in the genus Haematoloechus. The validity of the species which have extracaecal loops is discussed below. H. longiplexus and H. breviplexus have been discussed earlier and will not be included here.

H. kernensis and H. tumidus are separated on the basis of absence of spines in H. kernensis, differences in O/P ratio, and shape of ovary and testes. I have pointed out earlier, in the discussion on examining type specimens, that the O/P ratios are the same for both species, and that spines are present on H. kernensis. The presence or absence of lobing of ovaries and testes has been shown, in this thesis, to be highly variable for several other members of this genus. Therefore, I recommend that

H. tumidus be considered a synonym of H. kernensis. The type description of H. kernensis should be amended to read "tegument with spines".

Some of the R. aurora collected from Kern County, California by Ingles (1932, 1936) contained H. kernensis. This fluke has never been collected outside that vicinity nor hasit ever been reported from any other amphibian host. H. tumidus, also from R. aurora was reported by Ingles (1932, 1936) from Kern County north to the San Francisco Bay region. It is always found in frogs which inhabit streams and never in frogs which inhabit ponds (Ingles, 1936).

The following five species, which also contain extracaecal loops, are considered synonyms of H. varioplexus. They are: H. similiplexus, H. parviplexus, H. buttensis, H. floedae and H. uniplexus. These species are characterized as having extracaecal loops that do not extend to the anterior margin of the anterior testis. This group differs from H. kernensis in having O/A ratios usually greater than 1.1:1.0.

H. uniplexus was previously separated from the other five species by having only one extracaecal loop. I have demonstrated that as much as 8.5% of the specimens in three of the species in the H. varioplexus group also had only one extracaecal loop.

The extent of the extracaecal uterine loops has been important in separating H. parviplexus, H. buttensis, and H. floedae from one another as well as from H. varioplexus. I have demonstrated, experimentally, that the extracaecal uterine folds were in part host-dependent for H. buttensis. Examination of H. buttensis from several localities in B.C., has shown

further that the uterine folds may extend as far as the anterior border of the posterior testis or as little as 1/3 the distance from the end of the worm to the posterior testis. I have also shown a considerable variation in this character for H. varioplexus and H. parviplexus. The descriptions of the extent of extracaecal loops in H. similiplexus and H. floedae lie within this range of variation. It thus becomes impossible to separate these species using this character.

Egg size has been used to separate H. similiplexus from H. varioplexus ; H. buttensis from H. similiplexus and H. floedae from H. parviplexus.

Egg size in some species can vary considerably. The egg size of H. parviplexus varied with host as well as varying geographically. The egg lengths of H. varioplexus and H. similiplexus varied with host.

Species of Haematoloechus occur, under natural conditions, in a variety of amphibian hosts (see Appendix 23 for a list of known definitive hosts). A wider range of potential hosts has been demonstrated experimentally for H. buttensis in this thesis than has been found under natural conditions in British Columbia.

Individual species may differ from each other through slight differences in size, arrangement and location of various structures. Stunkard (1965) states that: "the problem of the taxonomist then is to distinguish between variations within a particular design and between different designs." He went on to state that "consideration must be given to the possibility that representatives of a single species may complete their

development in different host-species and furthermore that, as a result of development in different hosts, individuals of the same species may manifest differences in size and shape, in rate of growth and sexual maturity, and in extent of development of various tissues and organs."

Dronen (1975, 1977) noted that all odonates tested, Anax sp., Libella sp., Tramea sp., and Enallagma sp., became infected with metacercariae of H. coloradensis, and could serve as second intermediate hosts. Schell (1965) used the dragonfly, Aeschna multicolor Hagen, 1861 as an experimental host for H. breviplexus; Dronen (1977) used Libellula sp. In different areas different odonate species may be preferred as second intermediate hosts.

Dronen (Personal Communications) noted that the cercariae of H. breviplexus were larger in the snail, Ferrissia, which he used as first intermediate host than in Gyraulus similaris (Baker, 1919) used by Schell (1965).

Species of Haematoloechus can and do utilize a wide range of hosts. Therefore, it is not advisable to use host-specificity as the sole criterion for diagnosing species.

Stunkard (1957) states "it is abundantly clear that flatworm parasites are able to acquire new hosts, and that they change hosts with differing ecological situations. It is equally clear that development in different host-species and under different physiological conditions of the individual host may profoundly alter the parasite. In many instances, varieties or races peculiar to certain hosts, hostal varieties, may be recognized, but there is no sound reason to believe that they

represent different species". Forms showing marked host specificity could be considered subspecies rather than species.

In view of the demonstrated variability in characters used to separate the above six species I recommend that they become synonyms, and that H. varioplexus be the valid name.

In this thesis I have recommended recognizing only six species of Haematoloechus from Canada and the United States as being valid. They are as follows:

H. longiplexus Stafford, 1902

H. breviplexus Stafford, 1902

H. varioplexus Stafford, 1902 (= H. parviplexus, = H. buttensis, = H. similiplexus, = H. floedae, = H. uniplexus)

H. kernensis Ingles, 1932 (= H. tumidus)

H. medioplexus Stafford, 1902

H. complexus (Seely, 1906) (= H. coloradensis, = H. confusus, = H. oxyorchis).

The above findings suggest several possibilities in regard to relationships between speciation and dispersal of the host, and the lung flukes they contain. These ideas are still highly speculative and incomplete. However, they do add support to current theories of speciation in some North American Ranidae, and help to clarify some aspects of haematoloechid systematics in North America. For these reasons I have outlined my ideas below.

Current species distribution in North America is attributed to Pleistocene and Recent climatic changes (Porter, 1972). The southeastern United States has been the major center of distribution for North American Ranidae (Porter, 1972).

Leopard frogs from North America are comprised of numerous separate species (Pace, 1974). Four of these (Rana pipiens, R. utricularia, R. berlandieri, and R .blairi) were at one time considered a single species, belonging to the R. pipiens Complex. Pace (1974) distinguished the above four species by certain morphological, biochemical, and vocal characteristics. The geographical ranges of these species are in general mutually exclusive with contiguous boundaries, and are depicted in Pace (1974).

Pace (1974) suggested that Florida populations of R. utricularia and Texas populations of R. berlandieri are derived from the same ancestral stock. The Texas population (R. berlandieri), speciated from R. utricularia but the Florida population did not. R. utricularia may have invaded from the south, meeting R. pipiens north of their present zone of interaction (Pace, 1974) (Map 3).

My concept of the H. complexus group (=H. coloradensis, =H. confusus, and =H. oxyorchis) fits, and supports this interpretation. Lung flukes from R. berlandieri are morphologically, more similar to those found in R. utricularia than those recovered from R. blairi or R. pipiens (Fig. 81). This suggests a closer relationship to flukes from R. utricularia.

It is not known where R. blairi survived glaciation, if it was a distinct species at that time (Pace, 1974). Its spread into the midwest followed the post-Wisconsin expansion of the Prairie Peninsula (Smith, 1957). The morphology of lung flukes from R. blairi is intermediate between those from the

southwestern R. pipiens and those from R. utricularia. Similarities between their lung flukes suggest a southwestern origin for R. blairi, perhaps around Arizona and New Mexico, at which time R. blairi may not have been distinct. This hypothesis, while extremely tenuous, nevertheless may account for the greater similarity of H. complexus from R. blairi to H. complexus from R. pipiens than to lung flukes from R. utricularia.

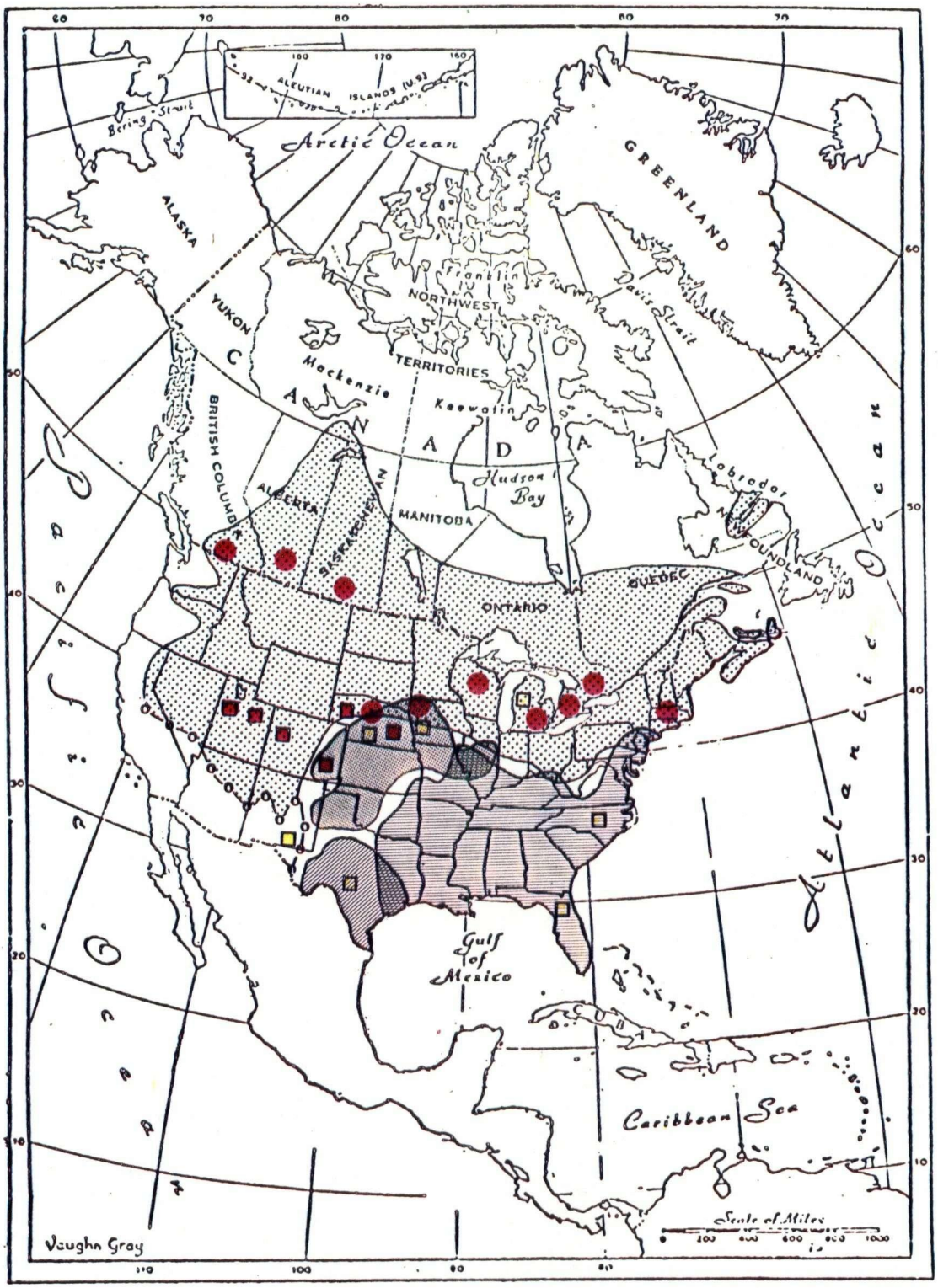
Where did the H. complexus (=H. oxyorchis, =H. confusus), found in R. aurora originate? I suggested earlier that they arose from what was previously considered H. coloradensis. In many respects the flukes from R. aurora, in northern California, are similar to flukes (=H. coloradensis) found in southwestern R. pipiens. This suggests to me a later transfer of flukes to R. aurora from R. pipiens. The ancestral lung fluke was probably what was previously considered H. coloradensis.

R. clamitans and R. catesbeiana are native to eastern United States. The natural western limits are uncertain because of their introduction into many localities (Conant, 1958) (Map 4). The above two species form a natural group distinct from other North American Ranidae (Wallace, et. al., 1973). These frogs contain H. longiplexus and H. breviplexus which I have shown form a group quite distinct from other North American haematoloechids. H. longiplexus and H. breviplexus exhibit very little morphological variation regardless of collecting locality.

I have insufficient data to warrant a good hypothesis concerning the spread of the H. varioplexus group recognized in

this thesis. More specimens are needed from many localities before any predictions can be made.

Map 3. The distribution of the Rana pipiens Complex (After Pace, 1974) and their lung flukes in North America.



H. coloradensis

H. complexus

H. medioplexus

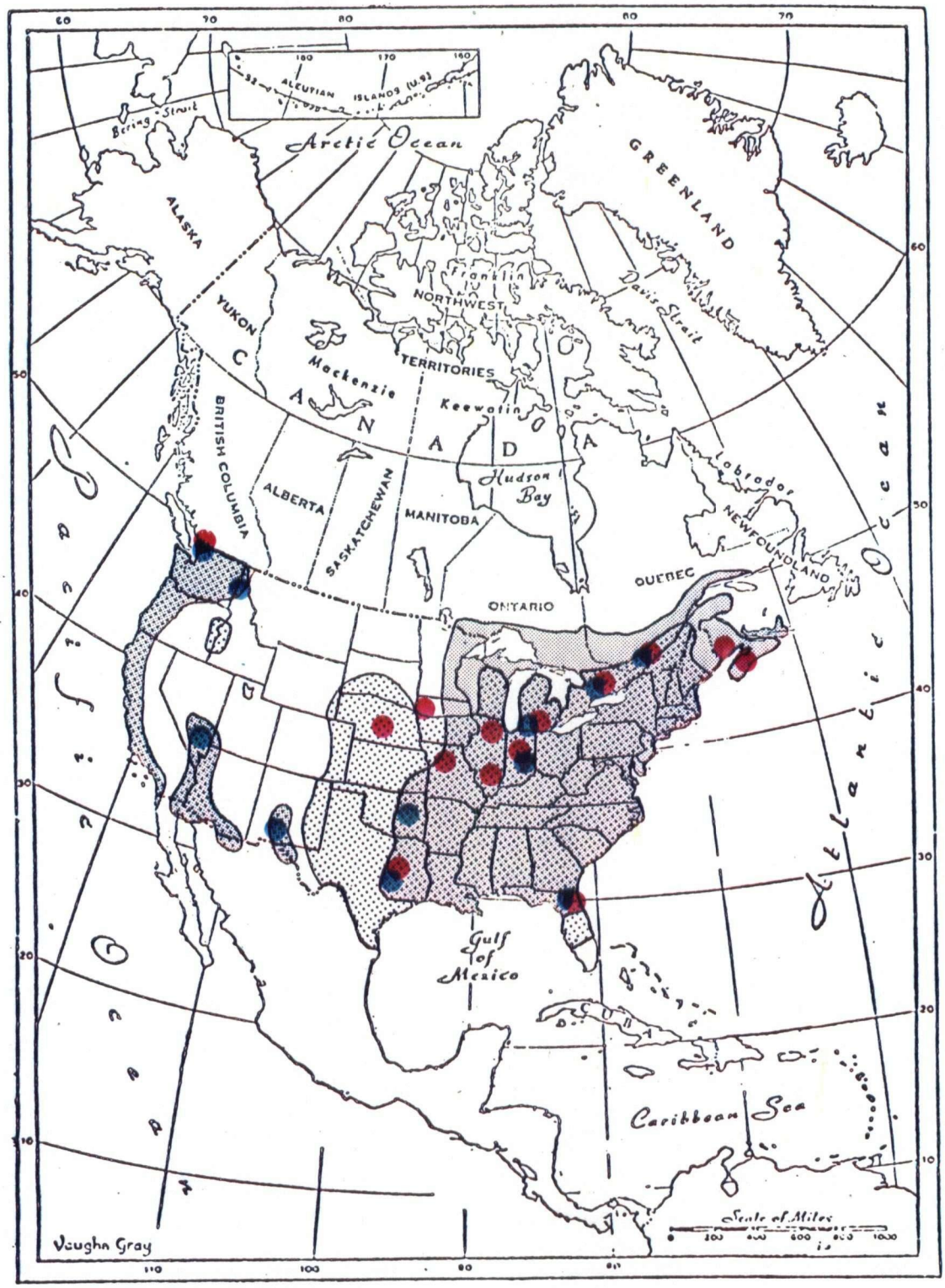
R. berlandieri

R. utricularia

R. pipiens

R. blairi

Map 4. The distribution of R. catesbeiana and R. clamitans (After Conant, 1958) and their lung flukes in North America.



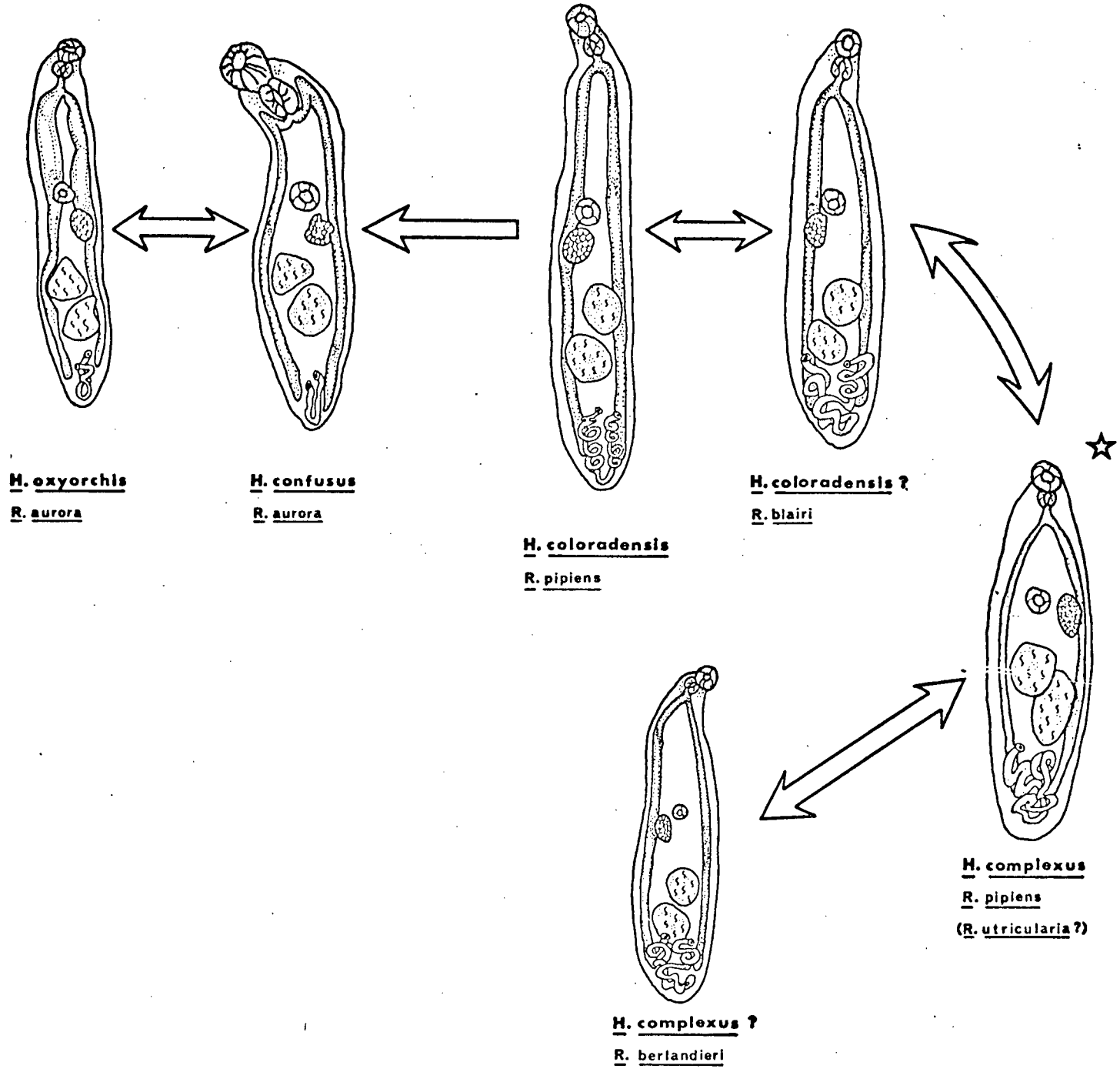
H. longiplexus

H. breviplexus

R. catesbeiana

R. clamitans

Fig. 81 A proposed derivation of the body types which constitute H. complexus (=H. oxyorchis, =H. confusus, =H. coloradensis).


H. oxyorchis
R. aurora
H. confusus
R. aurora
H. coloradensis
R. pipiens
H. coloradensis ?
R. blairi
H. complexus
R. pipiens
(R. utricularia ?)
H. complexus ?
R. berlandieri

A KEY TO THE *HAEMATOLOECHUS* SP. IN CANADA AND THE UNITED STATES

1 Extracaecal uterine loops present 2

1' Extracaecal uterine loops absent 5

2(1) Extracaecal uterine loops extending beyond anterior testis 3

2' Extracaecal uterine loops do not reach anterior testis 4

3(2) Testes parallel or nearly parallel; extracaecal uterine loops extending beyond ovary .....*H. longiplexus*

3' Testes overlap 1/3 to 1/2 their length; extracaecal uterine loops extend to ovary *H. breviplexus*

4(2') Testes elliptical; O/A ratio usually greater than 1.4:1.0; if less than 1.4:1.0 testes not round ....*H. varioplexus*

4' Testes round; O/A ratio 0.8:1.0 to 1.1:1.0 .....*H. kernensis*

5(1') O/A ratio greater than 2.0:1.0 (2.6 to 4.3:1.0) .....*H. medioplexus*

5' O/A ratio 2.0:1.0 or less (1.2 to 2.0:1.0) .....*H. complexus*

SUMMARY

1. Flattening of worms during mounting caused great variation in size and shape of the body, gonads, and suckers. Body size increased with increased temperature of fixative, and when distilled water was used to assemble live flukes before fixation. Tegumental spines were lost during this pre-fixative treatment. The O/A ratio was the only character measured that did not change.

2. Variation in H. buttensis resulted from differences in age and degree of maturity; from extent of "crowding," from type of host and temperature at which it was maintained. The size and shape of H. buttensis was not affected by host size or sex.

3. Adult H. buttensis were reared experimentally in four different species of frogs and three species of insects. Size, shape, and position of ovary and testes, body size, and extent of vitellaria were so variable that they were obviously unreliable for separating species. Stable characters included the O/A ratio, spined tegument, and to some degree, extent of the extracaecal uterine loops.

4. H. longiplexus and H. breviplexus collected from various localities had two characters suitable for species identification: extent of extracaecal uterine loops, and degree of overlap of the anterior and posterior testes. There was was no overlap in these characters, which are therefore, considered good taxonomic features.

5. Examination of specimens labelled H. varioplexus or H. similiplexus indicated that characters previously used to distinguish these species overlapped so much that it was

impossible to make a positive identification. Specimens were therefore treated according to host. When this was done it was noted that the O/A ratio and egg size varied with host.

6. Analysis of the O/A ratios for H. parviplexus showed a significant variation between specimens from B.C. and those from Nebraska. Both a geographic and a host effect was indicated. As well, egg length of worms from Atkinson, Nebraska differed from those of worms from B.C. or Humboldt, Nebraska. Other characters measured did not show significant host or geographic variation.

7. Nine species have previously been described as containing paired extracaecal uterine loops. However, as much as 9% of the specimens examined from field collections contained a single extracaecal loop. This suggested that H. uniplexus, described from a single specimens as having one extracaecal loop, is not a valid species.

8. Examination of type specimens revealed that some errors in published descriptions had been made for H. floedae, H. kernensis, H. oxyorchis, H. uniplexus and H. buttensis. These findings, together with conclusions drawn from experiments on H. buttensis and examination of collections of other lung flukes, led to the conclusion that H. floedae, H. oxyorchis, H. uniplexus and H. buttensis were not valid species.

9. Marked morphological variation and lack of host specificity documented during this study indicated that the following six species are valid:

H. longiplexus Stafford, 1902

H. breviplexus Stafford, 1902

H. varioplexus Stafford, 1902 (= H. similiplexus, = H. buttensis, = H. parviplexus, = H. floedae, = H. uniplexus).

H. medioplexus Stafford, 1902

H. complexus (Seely, 1906) (= H. coloradensis, = H. confusus, =H. oxyorchis).

H. kernensis Ingles, 1932 (= H. tumidus).

A redescription of the above six species was given.

10. A key to the six species recognized in this thesis is provided.

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Appendix 1. Summary of measurements used.

AC Diameter of Acetabulum: The greatest acetabular diameter measured orthogonal to the long axis of the worm.

ATE Anterior Testis from Posterior End. The distance between the posterior end of the anterior testis and the posterior end of the worm.

ATL Anterior Testis Length. Greatest length of the anterior testis measured along the long axis of the worm.

ATS Anterior Testis Shape. Designated as either smooth, mildly lobed, or highly lobed.

ATW Anterior Testis Width. Greatest width of the anterior testis measured orthogonal to the long axis of the worm.

BL Body Length. Greatest body length.

BW Body Width. Measured at the widest part of the worm.

EL Egg Length. The greatest length of an egg.

EW Egg Width. The greatest width of an egg.

Egg measurements were made on eggs found in the most anterior portion of the uterus (Fig. 84).

LVL Length of Left Vitellaria. Distance between the anterior and posterior extent of the vitellaria, exclusive of the central band of vitellaria which extends between the left and right vitelline areas.

O/A Oral sucker width divided by acetabulum width.

OAC Ovary from Acetabulum. The distance between the posterior border of the acetabulum and the anterior border of the ovary.

OAT Ovary from Anterior Testis. The distance between the posterior border of the ovary and the anterior margin of the anterior testis (Fig. 82).

OL Ovary Length. Greatest ovary length measured along the long axis of the worm.

OSH Ovary Shape. Designated as either smooth, mildly lobed or highly lobed.

OSL The Length of the Oral Sucker. The area anterior to the oral sucker, when the sucker is subterminal, is not included (Fig. 82).

OSW Width of the Oral Sucker. This measurement includes only the oral sucker proper, and does not include the area lateral to the oral sucker (Fig. 82).

OW Ovary Width. Greatest ovary width measured orthogonal to the long axis of the worm.

PL Pharynx Length. Includes the entire length of the pharynx, even when the pharynx extends some distance into the oral sucker (Fig. 82).

PTE Posterior Testis from Posterior end. The distance between the posterior margin of the posterior testis and the end of the worm.

PTL Posterior Testis Length. Greatest length of the posterior testis (Fig. 82).

PTS Posterior Testis Shape. Designated as either smooth, mildly lobed, or highly lobed.

PTW Posterior testis width. Greatest width of the posterior testis measured orthogonal to the long axis of the worm.

PW Pharynx Width. Greatest pharynx width.

RUF Length of Right Longitudinal Fold of Uterus. The anterior extent of that part of the uterus which extends to the outside of the left intestinal caecum. The fold is measured from its most posterior extent.

RVL Length of Right Vitellaria. Distance between the anterior and posterior extent of the vitellaria, exclusive of the central band of vitellaria which extends between the right and left vitelline areas.

THS Separation of Testes. This is the distance between the posterior end of the most anterior testis and the anterior end of the most posterior testis.

TO Overlap of Testes. The vertical distance between the testes. A positive value is given when testes actually overlap with one another. A negative value denotes testes separated by some distance.

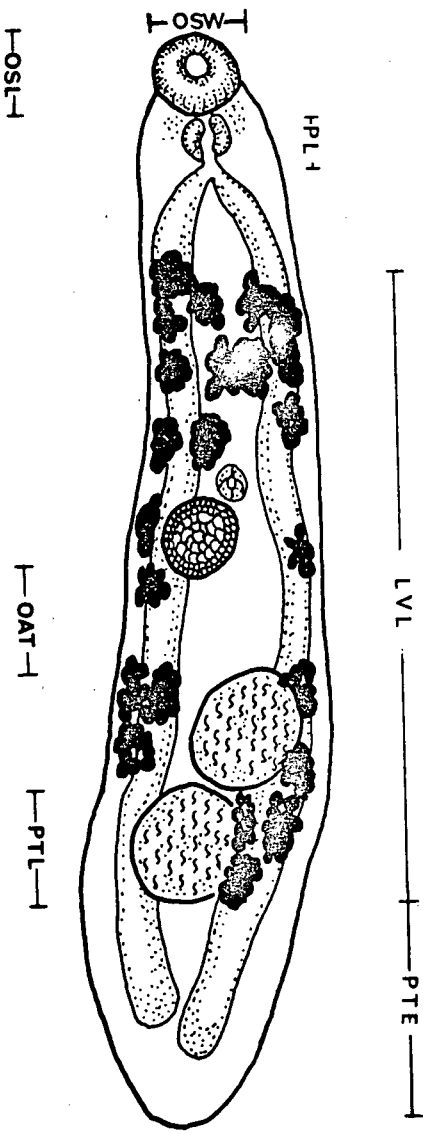
TT Tegument Thickness.

VA Anterior Extent of Vitellaria. Measured between the anterior extent of the vitellaria and the posterior end of the worm.

VE Distance of Vitellaria from end of worm. The distance between the posterior vitelline level and the posterior end of the fluke.

Fig. 82. Delineation of some worm measurements. The uterus has been excluded for clarity.

Fig. 83 Delineation of some worm measurements. The testis, ovary and acetabulum have been omitted for clarity.



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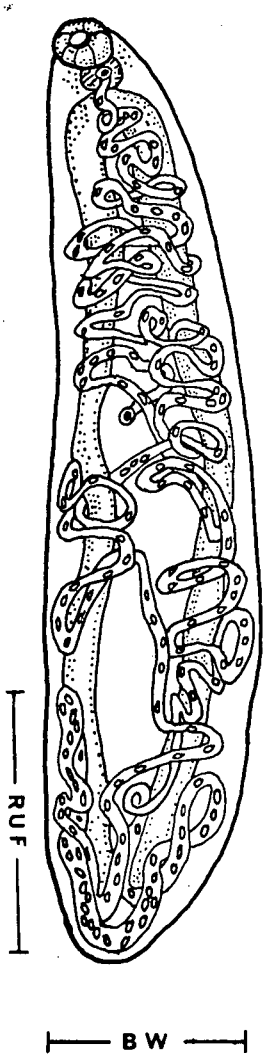

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Appendix 2. Formulae for fixatives and solutions used in experiments.

AFA (alcohol-formol-acetic) fixative

Alcohol, 85%	85 ml
Formalin, commercial	10 ml
Acetic acid, glacial	5 ml

Bouin's (Picro-formol-acetic) fixative

Picric acid, saturated aqueous solution	75 ml
Formalin, commercial	25 ml
Acetic acid, glacial	5 ml

Formalin, 10%

Water, distilled	90 ml
Formalin, commercial	10 ml

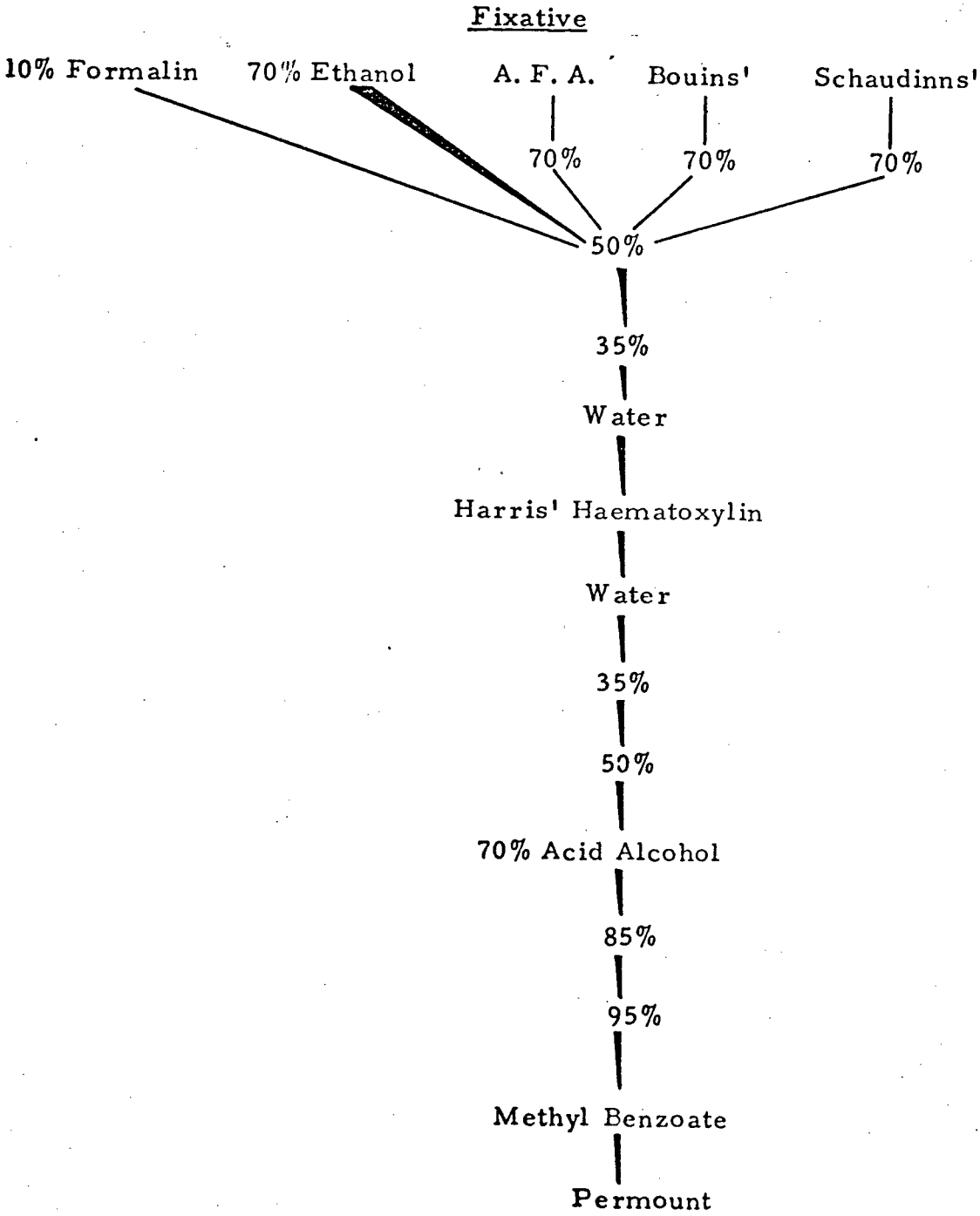
Frog Ringer's Solution

KCl	0.14 gm
NaCl	0.50 gm
CaCl2	0.12 gm
NaHCO3	0.20 gm
Distilled water	100 ml

Schaudinn's fixative

Mercuric chloride, saturated aqueous sol.	66 ml
Alcohol, 95%	33 ml
Acetic acid, glacial	3 ml

Appendix 3. Flow sheet for preparing whole mounts.



Standardized Procedure

Appendix 4. Effect of flattening on character measurements of H. buttensis.

	Unflattened	Weight used	
		5 grams	10 grams
Body length	3.05(2.86-3.60)	4.67(3.85-4.91)	5.43(4.71-5.88)
Body width	0.63(0.55-0.74)	0.95(0.88-1.13)	1.51 (1.27-1.69)
Acetabulum diameter	0.09(0.08-0.09)	0.11 (0.10-0.12)	0.13(0.12-0.14)
Oral sucker length	0.21(0.19-0.23)	0.24(0.21-0.27)	0.27(0.24-0.30)
Oral sucker width	0.23(0.20-0.25)	0.29(0.26-0.31)	0.35(0.32-0.38)
O/A ratio	2.6 (2.6 -2.8)	2.6 (2.5 -2.6)	2.7
Pharynx length	0.13(0.12-0.15)	0.15(0.12-0.16)	0.18(0.15-0.20)
Pharynx width	0.15(0.13-0.16)	0.22(0.19-0.24)	0.31(0.27-0.33)
Ovary length	0.52(0.40-0.61)	0.63(0.59-0.67)	0.66(0.61-0.76)
Ovary width	0.25(0.19-0.31)	0.35(0.28-0.44)	0.41(0.34-0.53)
Ant. Testis length	0.63(0.57-0.74)	0.79(0.68-0.85)	0.83(0.75-0.93)
Ant. testis width	0.32(0.26-0.40)	0.43(0.39-0.53)	0.48(0.42-0.61)
Post. testis length	0.67(0.59-0.78)	0.84(0.79-1.01)	0.89(0.85-1.07)
Post. testis width.	0.41(0.35-0.55)	0.51(0.45-0.66)	0.57(0.50-0.75)

All measurements in millimeters.

Appendix 8. Average measurements of Haematoloechus buttensis of varying ages developed in Rana pretiosa.

	Metacercaria	5 days	14 days	21 days	28 days	60 days
# recovered	10	24	18	20	21	17
Body length	0.68(0.57-0.80)	0.99(0.92-1.11)	1.75(1.61-1.98)	3.10(2.43-3.61)	3.90(3.50-4.59)	6.01(5.58-6.67)
Body width	0.29(0.24-0.35)	0.34(0.33-0.36)	0.51(0.47-0.59)	0.68(0.53-0.85)	0.82(0.78-0.87)	1.26(1.12-1.46)
Acetabulum	0.09(0.08-0.12)	0.05(0.05-0.06)	0.07(0.06-0.07)	0.08(0.07-0.09)	0.10(0.09-0.11)	0.11(0.09-0.12)
Oral sucker length	0.11(0.10-0.13)	0.12(0.11-0.13)	0.16(0.14-0.18)	0.20(0.19-0.22)	0.24(0.23-0.25)	0.25(0.21-0.29)
Oral sucker width	0.12(0.09-0.15)	0.13(0.12-0.13)	0.18(0.17-0.20)	0.22(0.19-0.24)	0.25(0.24-0.26)	0.29(0.25-0.32)
O/A ratio	1.2 (1.0 -1.3)	2.4 (2.2 -2.4)	2.6 (2.5 -2.7)	2.5 (2.1 -2.7)	2.5 (2.4 -2.6)	2.6 (2.4 -2.8)
Pharynx length	0.06(0.04-0.07)	0.08(0.07-0.09)	0.12(0.11-0.13)	0.13(0.12-0.15)	0.15(0.13-0.20)	0.19(0.17-0.23)
Pharynx width	0.06(0.04-0.07)	0.10(0.08-0.11)	0.13(0.12-0.14)	0.15(0.14-0.18)	0.17(0.15-0.18)	0.20(0.18-0.23)
Ovary length	Absent	0.08(0.06-0.10)	0.24(0.20-0.32)	0.48(0.33-0.53)	0.55(0.50-0.62)	0.71(0.71-0.74)
Ovary width	Absent	0.08(0.07-0.09)	0.17(0.04-0.20)	0.23(0.11-0.34)	0.28(0.24-0.35)	0.36(0.31-0.40)
Ant. testis length	0.03(0.02-0.05)	0.13(0.11-0.21)	0.34(0.29-0.37)	0.62(0.51-0.74)	0.60(0.54-0.67)	1.12 (1.05-1.21)
Ant. testis width	0.03(0.02-0.04)	0.09(0.06-0.11)	0.22(0.19-0.29)	0.34(0.25-0.40)	0.42(0.39-0.48)	0.48(0.31-0.59)
Post. testis length	0.04(0.02-0.06)	0.13(0.11-0.17)	0.31(0.26-0.33)	0.65(0.53-0.78)	0.75(0.65-0.90)	1.22(1.15-1.33)
Post. testis width	0.03(0.02-0.04)	0.08(0.05-0.11)	0.22(0.18-0.27)	0.38(0.25-0.54)	0.45(0.35-0.53)	0.52(0.40-0.65)

All measurements in millimeters.

Appendix 6. Measurements and standardized measurement system.

Thirty-five characters were measured for each individual, the number being determined on the basis of those characters most commonly used to identify members of the genus.

Before the measurements were taken, all the slides of the fluke samples were mixed without identification of the fluke species. All the measurements were recorded, the slides then re-identified, and the data re-grouped according to the fluke species. In this way biases due to changes in measurement technique and progressive and personal error were minimized.

Egg sizes vary in different parts of the uterus (Fig. 84). For this reason it is important to choose a site in the uterus from which all egg measurements are done. The most anterior part of the uterus (Site A, Fig. 84) was chosen. The eggs are embryonated when they reach this site and hence are the most fully developed and best represent the true egg size and shape of the species being measured.

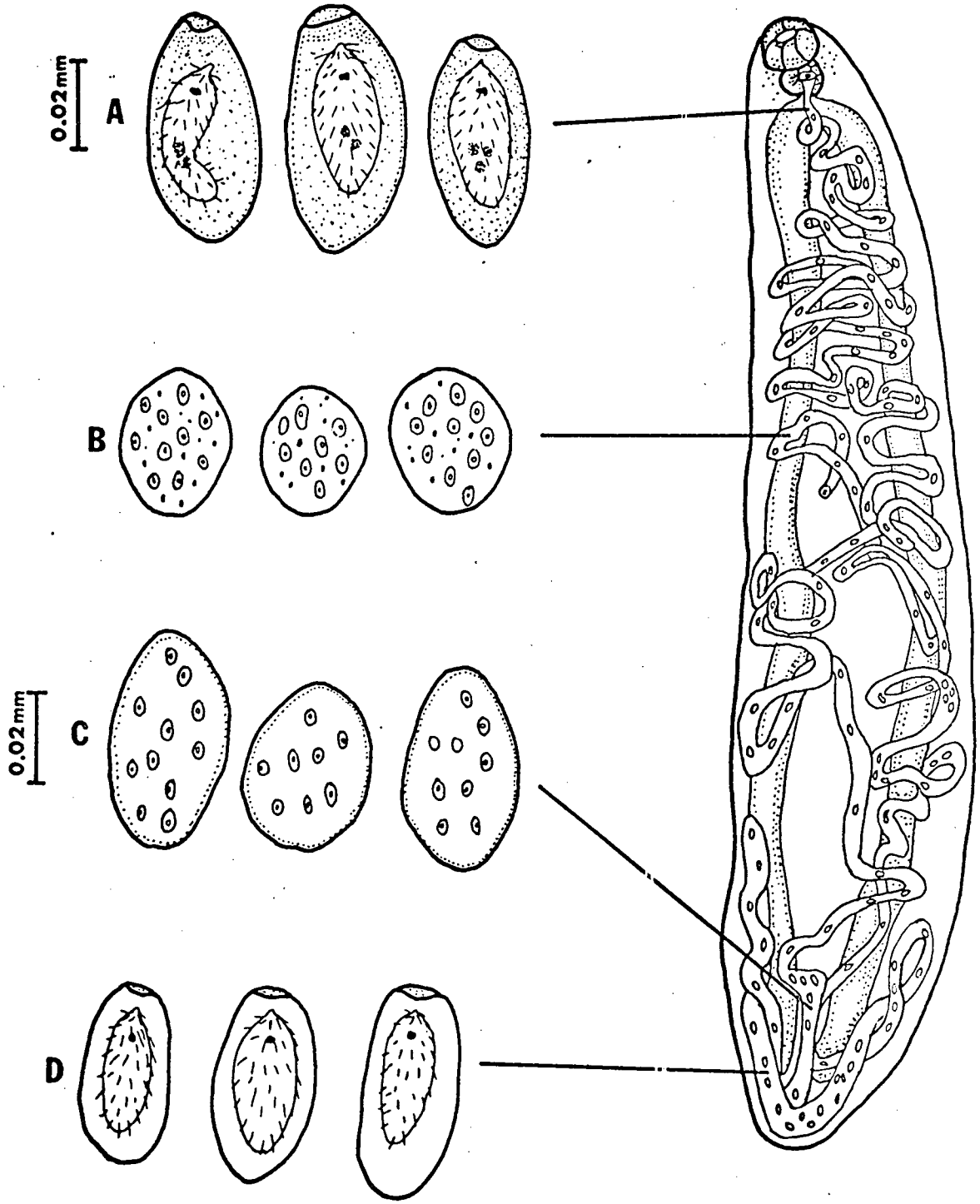
All measurements were made over a one-year period (1976). All 35 characters were measured on one specimen, then a second specimen was measured, and so on until all the specimens had been measured.

All measurements were made by means of a Leitz compound microscope equipped with a linear-graduated ocular micrometer that had been calibrated from a standard stage micrometer to the nearest 0.1 micrometer.

Before measuring a character on each specimen, the correct magnification for that character was set. The specimen was focused on the ventral side and the character was always in

focus. In this study all measurements were first recorded as micrometer units and later converted to millimeter units.

Fig. 84 Variation in size and shape of eggs taken from different parts of the uterus of H. buttensis.


0.02mm
A
B
0.02mm
C
D

Errors and biases in measurement may arise from personal experience, personal visual aberrations, measuring habits, and inadequacies of the measurements system (Kim, Brown, and Cook, 1966).

The reliability of measurements in this study was assessed for each of 28 characters. One individual of each species of Haematoloechus studied was measured on all 28 characters at three widely spaced times (Tables A to O).

Before measuring a character on each specimen, the correct magnification for that character was set. The specimen was focused on the correct side and the character was always in focus. The reliability of measurements for each species was tested and the results are presented in Tables A to O inclusive.

The most unreliable measurement is tegument thickness (TT), with coefficients of variation 33.3% for H. coloradensis and H. complexus; 24.5% for H. longiplexus; 20.0% for H. varioplexus; 19.6% for H. parviplexus; 17.9% for H. oxyorchis; 15.9% for H. confusus; 15.8% for H. kernensis; 13.3% for H. breviplexus and H. buttensis; 17.6% for H. medioplexus; 10.8% for H. similiplexus; 10.6% for H. uniplexux; 10.2% for H. floedae; and 6.0% for H. tumidus.

H. coloradensis had a C.V. of -10.1% for the character, overlap of testes. H. medioplexus had a -10.2% C.V. For the same character.

Table A. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.BREVIPLEXUS

CHARACTER	MEASUREMENTS 1 (JAN 16/76)	2 (APR 10/76)	3 (SEP 10/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	198.0	203.0	201.0	200.7	203.0	198.0	2.5	6.3	1.3
USL	561.0	567.0	566.0	564.7	567.0	561.0	3.2	10.3	0.6
OSW	514.8	510.0	516.0	513.6	516.0	510.0	3.2	10.2	0.6
PL	211.2	209.0	212.0	210.7	212.0	209.0	1.6	2.4	0.7
PW	251.9	257.0	255.5	254.8	257.0	251.9	2.6	6.9	1.0
OL	961.0	950.0	963.0	958.0	963.0	950.0	7.0	49.0	0.7
OW	651.0	652.0	654.0	652.3	654.0	651.0	1.6	2.5	0.2
OAT	-62.0	-59.0	-64.5	-61.8	-59.0	-64.5	2.8	7.6	-4.5
OAC	-372.0	-370.0	-377.0	-373.0	-370.0	-377.0	3.6	13.0	-1.0
ATL	1333.0	1307.0	1340.0	1326.7	1340.0	1307.0	17.4	302.5	1.3
ATW	589.0	576.0	591.0	585.3	591.0	576.0	8.1	66.3	1.4
ATE	2077.0	2055.0	2085.0	2072.3	2085.0	2055.0	15.5	241.5	0.7
PTL	1550.0	1565.0	1542.5	1552.5	1565.0	1542.5	11.5	131.5	0.7
PTW	770.0	749.0	775.0	764.7	775.0	749.0	13.8	190.5	1.8
PTE	1364.0	1355.0	1369.0	1362.7	1369.0	1355.0	7.1	50.5	0.5
TO	961.0	960.0	952.0	957.7	961.0	952.0	4.9	24.5	0.5
THS	-46.0	-46.0	-41.0	-44.3	-41.0	-46.0	2.9	8.3	-6.5
RUF	4681.0	4640.0	4685.0	4668.7	4685.0	4640.0	24.8	616.0	0.5
LUF	4216.0	4200.0	4232.5	4216.2	4232.5	4200.0	16.2	264.0	0.4
RVL	5580.0	5545.0	5590.0	5571.7	5590.0	5545.0	24.5	600.0	0.4
LVL	4402.0	4450.0	4465.0	4439.0	4465.0	4402.0	32.9	1080.0	0.7
VA	5890.0	5850.0	5900.0	5880.0	5900.0	5850.0	26.7	712.0	0.5
VE	651.0	645.0	655.0	650.3	655.0	645.0	5.0	25.5	0.8
BL	8897.0	8950.0	8895.0	8914.0	8950.0	8895.0	31.5	992.0	0.4
BW	1798.0	1780.0	1800.0	1792.7	1800.0	1780.0	11.0	122.0	0.6
TT	12.5	10.0	10.0	10.8	12.5	10.0	1.4	2.1	13.3
EL	20.0	22.0	20.0	20.7	22.0	20.0	1.2	1.3	5.6
EW	16.3	16.0	15.5	15.9	16.3	15.5	0.4	0.2	2.5

Table B. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.BUTTENSIS

CHARACTER	MEASUREMENTS 1 (JAN 17/76)	2 (APR 11/76)	3 (SEP 10/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	101.0	99.5	102.0	100.8	102.0	99.5	1.3	1.6	1.2
OSL	250.8	245.0	253.0	249.6	253.0	245.0	4.1	17.1	1.7
OSW	330.0	325.0	330.0	328.3	330.0	325.0	2.9	8.3	0.9
PL	209.0	204.0	207.0	206.7	209.0	204.0	2.5	6.3	1.2
PW	247.5	245.0	245.0	245.8	247.5	245.0	1.4	2.1	0.6
OL	558.0	547.0	563.0	556.0	563.0	547.0	8.2	67.0	1.5
OW	248.0	250.0	259.0	252.3	259.0	248.0	5.9	34.3	2.3
OAT	-558.0	-540.0	-540.0	-546.0	-540.0	-558.0	10.4	108.0	-1.9
OAC	-53.0	-55.0	-50.0	-52.7	-50.0	-55.0	2.5	6.3	-4.8
ATL	1953.0	1923.0	1955.0	1943.7	1955.0	1923.0	17.9	321.5	0.9
ATW	465.0	455.0	455.0	458.3	465.0	455.0	5.8	33.3	1.3
ATE	2170.0	2140.0	2150.0	2153.3	2170.0	2140.0	15.3	233.5	0.7
PTL	1302.0	1335.0	1335.0	1324.0	1335.0	1302.0	19.1	363.0	1.4
PTW	558.0	545.0	560.0	554.3	560.0	545.0	8.1	66.3	1.5
PTE	1193.5	1175.0	1195.0	1187.8	1195.0	1175.0	11.1	124.0	0.9
TO	403.0	415.0	415.0	411.0	415.0	403.0	6.9	48.0	1.7
THS	-341.0	-345.0	-350.0	-345.3	-341.0	-350.0	4.5	20.3	-1.3
RUF	1891.0	1880.0	1900.0	1890.3	1900.0	1880.0	10.0	100.5	0.5
LUF	1240.0	1240.0	1245.0	1241.7	1245.0	1240.0	2.9	8.5	0.2
RVL	3038.0	3040.0	3045.0	3041.0	3045.0	3038.0	2.8	8.0	0.1
LVL	4278.0	4275.0	4280.0	4277.7	4280.0	4275.0	0.0	0.0	0.0
VA	5161.5	5165.0	5175.0	5167.2	5175.0	5161.5	6.3	40.0	0.1
VE	1023.0	1015.0	1015.0	1017.7	1023.0	1015.0	4.6	21.5	0.5
BL	6153.5	6100.0	6125.0	6126.2	6153.5	6100.0	26.8	720.0	0.4
BW	1302.0	1300.0	1315.0	1305.7	1315.0	1300.0	8.2	66.5	0.6
TT	12.5	10.0	10.0	10.8	12.5	10.0	1.4	2.1	13.3
EL	25.0	25.0	25.5	25.2	25.5	25.0	0.3	0.1	1.1
EW	12.5	12.5	12.0	12.3	12.5	12.0	0.3	0.1	2.3

Table C. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.COLORADENSIS

CHARACTER	MEASUREMENTS 1 (MAY 14/76)	2 (MAY 21/76)	3 (JUN 3/76)	MEAN	MAX.	MIN	STD DEV	VARIANCE	CV
AC	352.0	350.0	353.0	351.7	353.0	350.0	1.5	2.3	0.4
OSL	396.0	396.0	399.0	397.0	399.0	396.0	1.7	3.0	0.4
OSW	335.0	333.0	332.0	333.5	335.5	332.0	1.8	3.3	0.5
PL	305.8	307.0	303.0	305.3	307.0	303.0	2.1	4.2	0.7
PW	257.4	257.0	259.0	257.8	259.0	257.0	1.1	1.1	0.4
OL	506.0	516.0	511.0	511.0	516.0	506.0	5.0	25.0	1.0
OW	423.5	420.0	426.0	423.2	426.0	420.0	3.0	9.1	0.7
OAT	573.5	565.0	569.0	569.2	573.5	565.0	4.3	18.1	0.7
OAC	132.0	125.0	134.0	130.3	134.0	125.0	4.7	22.3	3.6
ATL	527.0	516.0	523.0	522.0	527.0	516.0	5.6	31.0	1.1
ATW	558.0	563.0	565.0	562.0	565.0	558.0	3.6	13.0	0.6
ATE	2294.0	2263.0	2278.0	2278.3	2294.0	2263.0	15.6	242.0	0.7
PTL	496.0	490.0	494.0	493.3	496.0	490.0	3.1	9.3	0.6
PTW	620.0	620.0	618.0	619.3	620.0	618.0	1.2	1.5	0.2
PTE	1927.0	1958.0	1958.0	1947.7	1958.0	1927.0	17.9	322.0	0.9
TO	-99.0	-90.0	-110.0	-99.7	-90.0	-110.0	10.0	100.3	-10.1
THS	312.0	343.0	327.0	327.3	343.0	312.0	15.5	240.3	4.7
RUF	275.0	264.0	280.0	273.0	280.0	264.0	8.2	67.0	3.0
LUF	275.0	275.0	281.0	277.0	281.0	275.0	3.5	12.0	1.3
RVL	3211.0	3200.0	3242.0	3217.7	3242.0	3200.0	21.7	472.0	0.7
LVL	4128.0	4159.0	4128.0	4138.3	4159.0	4128.0	17.9	320.0	0.4
VA	5046.0	5057.0	5035.0	5046.0	5057.0	5035.0	11.0	120.0	0.2
VE	1147.0	1116.0	1147.0	1136.7	1147.0	1116.0	17.9	320.5	1.6
BL	6193.0	6131.0	6213.0	6179.0	6213.0	6131.0	42.7	1824.0	0.7
BW	1395.0	1364.0	1380.0	1379.7	1395.0	1364.0	15.6	242.0	1.1
TT	7.5	5.0	10.0	7.5	10.0	5.0	2.5	6.3	33.3
EL	37.5	37.0	35.0	36.5	37.5	35.0	1.3	1.8	3.6
EW	21.3	20.0	20.0	20.4	21.3	20.0	0.8	0.6	3.7

Table D. RELIABILITY FOR 28 CHAPACTERS MEASURED OF H.COMPLEXUS

CHARACTER	MEASUREMENTS 1 (MAY 14/76)	2 (MAY 21/76)	3 (JUN 3/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	286.0	280.0	289.0	285.0	289.0	280.0	4.6	21.0	1.6
CSL	405.9	400.0	405.0	403.6	405.9	400.0	3.2	10.2	0.8
OSW	401.5	400.0	400.0	400.5	401.5	400.0	0.9	0.8	0.2
PL	198.0	195.0	200.0	197.7	200.0	195.0	2.5	6.3	1.3
PW	231.0	228.0	235.0	231.3	235.0	228.0	3.5	12.3	1.5
CL	456.5	456.0	463.0	458.5	463.0	456.0	3.9	15.3	0.9
CW	319.0	315.0	322.0	318.7	322.0	315.0	3.5	12.3	1.1
OAT	613.8	610.0	616.0	613.3	616.0	610.0	3.0	9.0	0.5
OAC	33.0	33.0	31.0	32.3	33.0	31.0	1.2	1.3	3.6
ATL	744.0	740.0	731.0	738.3	744.0	731.0	6.7	44.5	0.9
ATW	675.8	680.0	683.0	679.6	683.0	675.8	3.6	13.0	0.5
ATE	1555.0	1521.0	1538.0	1538.0	1555.0	1521.0	17.0	290.0	1.1
PTL	899.0	868.0	900.0	889.0	900.0	868.0	18.2	331.0	2.0
PTW	744.0	732.0	737.0	737.7	744.0	732.0	6.0	36.5	0.8
PTE	982.0	970.0	980.0	977.3	982.0	970.0	6.4	41.5	0.7
TO	137.6	135.0	140.0	137.5	140.0	135.0	2.5	6.3	1.8
THS	-367.0	-360.0	-360.0	-362.3	-360.0	-367.0	4.0	16.3	-1.1
RUF	713.0	715.0	719.0	715.7	719.0	713.0	3.1	9.5	0.4
LUF	868.0	860.0	869.0	865.7	869.0	860.0	4.9	24.5	0.6
RVL	3394.0	3372.0	3396.0	3388.0	3396.0	3372.0	13.9	192.0	0.4
LVL	4312.0	4300.0	4290.0	4300.7	4312.0	4290.0	11.0	120.0	0.3
VA	5321.0	5300.0	5300.0	5307.0	5321.0	5300.0	12.3	152.0	0.2
VE	504.6	500.0	495.0	499.9	504.6	495.0	4.8	23.1	1.0
BL	6193.0	6185.0	6183.0	6187.0	6193.0	6183.0	5.7	32.0	0.1
BW	1271.0	1265.0	1275.0	1270.3	1275.0	1265.0	5.0	25.5	0.4
TT	7.5	5.0	10.0	7.5	10.0	5.0	2.5	6.3	33.3
EL	32.5	32.5	35.0	33.3	35.0	32.5	1.4	2.1	4.3
EW	17.5	17.5	17.0	17.3	17.5	17.0	0.3	0.1	1.7

Table E. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.CONFUSUS

CHARACTER	MEASUREMENTS 1 (NOV 9/76)	2 (NOV 10/76)	3 (NOV 11/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	297.9	300.0	296.0	298.0	300.0	296.0	2.0	4.0	0.7
OSL	495.9	493.2	496.0	495.0	496.0	493.2	1.6	2.6	0.3
OSW	436.5	435.6	437.5	436.5	437.5	435.6	1.0	1.0	0.2
PL	378.0	375.6	380.0	377.9	380.0	375.6	2.2	4.9	0.6
PW	328.5	327.5	330.2	328.7	330.2	327.5	1.4	1.9	0.4
OL	333.0	335.0	335.0	334.3	335.0	333.0	1.2	1.3	0.3
OW	306.0	305.0	307.3	306.1	307.3	305.0	1.2	1.3	0.4
OAT	45.0	47.0	45.9	46.0	47.0	45.0	1.0	1.0	2.2
OAC	36.0	34.2	35.0	35.1	36.0	34.2	0.9	0.8	2.6
ATL	513.0	512.0	510.0	511.7	513.0	510.0	1.5	2.3	0.3
ATW	351.0	347.0	349.5	349.2	351.0	347.0	2.0	4.1	0.6
ATE	1278.0	1279.3	1275.8	1277.7	1279.3	1275.8	1.6	2.5	0.1
PTL	486.0	488.0	485.7	486.6	486.0	485.7	1.3	1.7	0.3
PTW	468.0	471.3	470.0	469.8	471.3	468.0	1.7	2.8	0.4
PTE	891.0	887.5	890.5	889.7	891.0	887.5	1.9	3.5	0.2
TO	405.0	400.5	403.4	403.0	405.0	400.5	2.3	5.3	0.6
THS	-99.0	-97.6	-100.0	-98.9	-97.6	-100.0	1.2	1.5	-1.2
RUF	ABSENT	ABSENT	ABSENT						
LUF	ABSENT	ABSENT	ABSENT						
RVL	2187.0	2193.0	2185.3	2188.4	2193.0	2185.3	4.9	24.0	0.2
LVL	1647.0	1651.3	1648.5	1648.9	1651.3	1647.0	3.5	12.0	0.2
VA	2961.0	2956.7	2959.0	2958.9	2961.0	2956.7	2.8	8.0	0.1
VE	765.0	763.0	766.7	764.9	766.7	763.0	1.9	3.5	0.2
BL	3937.5	3935.0	3938.5	3937.0	3938.5	3935.0	0.0	0.0	0.0
BW	882.0	880.1	883.0	881.7	883.0	880.1	1.6	2.5	0.2
TT	13.8	10.0	12.0	11.9	13.8	10.0	1.9	3.6	15.9
EL	30.9	30.0	31.0	30.6	31.0	30.0	0.6	0.3	1.8
EW	22.6	22.5	22.0	22.4	22.6	22.0	0.3	0.1	1.4

Table F. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.FLOEDAE

CHARACTER	MEASUREMENTS 1 (NOV 9/76)	2 (NOV 10/76)	3 (NOV 11/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	153.0	155.0	154.5	154.2	155.0	153.0	1.0	1.1	0.7
OSL	450.0	447.5	449.0	448.8	450.0	447.5	1.3	1.6	0.3
OSW	432.0	430.0	431.3	431.1	432.0	430.0	1.0	1.0	0.2
PL	162.0	160.0	159.3	160.4	162.0	159.3	1.4	2.0	0.9
PW	197.1	198.7	196.0	197.3	198.7	196.0	1.4	1.9	0.7
OL	869.4	871.0	867.7	869.4	871.0	867.7	1.7	3.0	0.2
OW	535.5	537.0	533.0	535.2	537.0	533.0	2.0	4.1	0.4
OAT	189.0	188.0	186.8	187.9	189.0	186.8	1.1	1.3	0.6
OAC	-531.0	-528.0	-530.0	-529.7	-528.0	-531.0	1.5	2.3	-0.3
ATL	1006.0	1005.0	1011.3	1008.1	1011.3	1005.0	3.2	10.0	0.3
ATW	535.5	537.1	538.6	537.1	538.6	535.5	1.6	2.4	0.3
ATE	1417.5	1422.0	1425.0	1421.5	1425.0	1417.5	3.9	15.5	0.3
PTL	1023.8	1025.0	1021.8	1023.5	1025.0	1021.8	1.6	2.5	0.2
PTW	510.3	512.0	508.7	510.3	512.0	508.7	1.6	2.7	0.5
PTE	756.0	758.0	755.0	756.3	758.0	755.0	1.6	2.5	0.2
TO	409.5	407.1	410.0	408.9	410.0	407.1	1.6	2.5	0.4
THS	-126.0	-125.0	-122.0	-124.3	-122.0	-126.0	2.1	4.3	-1.7
RUF	2772.0	2770.0	2765.0	2769.0	2772.0	2765.0	2.8	8.0	0.1
LUF	2929.5	2931.0	2933.4	2931.3	2933.4	2929.5	2.8	8.0	0.1
RVL	2866.5	2868.0	2861.3	2865.3	2868.0	2861.3	4.9	24.0	0.2
LVL	3937.5	3935.0	3944.0	3938.8	3944.0	3935.0	4.9	24.0	0.1
VA	4063.5	4065.0	4060.0	4062.8	4065.0	4060.0	2.8	8.0	0.1
VE	490.5	486.0	492.2	490.2	492.2	486.0	2.1	4.5	0.4
BL	5096.7	5093.0	5100.0	5096.6	5100.0	5093.0	2.8	8.0	0.1
BW	1354.5	1350.0	1356.3	1353.6	1356.3	1350.0	3.2	10.0	0.2
TT	10.1	8.5	8.5	9.0	10.1	8.5	0.9	0.9	10.2
EL	22.1	22.0	22.5	22.2	22.5	22.0	0.3	0.1	1.2
EW	14.3	14.0	13.5	13.9	14.3	13.5	0.4	0.2	2.9

Table G. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.KERNENSIS

CHARACTER	MEASUREMENTS 1 (NOV 9/76)	2 (NOV 10/76)	3 (NOV 11/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	432.0	430.0	433.0	431.7	433.0	430.0	1.5	2.3	0.4
OSL	459.0	460.0	460.0	459.7	460.0	459.0	0.6	0.3	0.1
OSW	451.0	450.0	453.0	451.3	453.0	450.0	1.5	2.3	0.3
PL	286.0	290.0	290.5	289.5	290.5	288.0	1.3	1.8	0.5
PW	294.3	295.0	293.0	294.1	295.0	293.0	1.0	1.0	0.3
OL	630.0	635.0	632.5	632.5	635.0	630.0	2.5	6.5	0.4
OW	427.5	426.5	428.0	427.3	428.0	426.5	0.8	0.6	0.2
OAT	459.0	460.0	447.5	455.5	460.0	447.5	7.0	48.3	1.5
OAC	-52.2	-52.0	-50.0	-51.4	-50.0	-52.2	1.2	1.5	-2.4
ATL	913.5	915.0	912.0	913.5	915.0	912.0	1.6	2.5	0.2
ATW	976.5	973.0	975.0	974.8	976.5	973.0	1.7	3.0	0.2
ATE	2101.1	2100.0	2105.0	2102.0	2105.0	2100.0	3.5	12.5	0.2
PTL	1020.6	1020.0	1018.0	1019.5	1020.6	1018.0	1.4	2.0	0.1
PTW	1039.5	1037.0	1035.3	1037.3	1039.5	1035.3	2.1	4.5	0.2
PTE	1231.7	1234.0	1237.5	1234.4	1237.5	1231.7	2.9	8.5	0.2
TU	157.5	155.0	155.0	155.8	157.5	155.0	1.4	2.1	0.9
THS	-535.5	-537.0	-533.0	-535.2	-533.0	-537.0	2.0	4.1	-0.4
RUF	ABSENT	ABSENT	ABSENT						
LUF	ABSENT	ABSENT	ABSENT						
RVL	3843.0	3841.0	3841.0	3841.7	3843.0	3841.0	0.0	0.0	0.0
LVL	2866.5	2868.0	2865.0	2866.5	2868.0	2865.0	0.0	0.0	0.0
VA	5166.6	5155.0	5158.0	5159.9	5166.6	5155.0	4.9	24.0	0.1
VE	1408.1	1400.0	1410.0	1406.0	1410.0	1400.0	5.8	33.5	0.4
BL	7194.6	7190.1	7197.5	7194.1	7197.5	7190.1	2.8	8.0	0.0
BW	1638.0	1640.5	1637.3	1638.6	1640.5	1637.3	2.9	8.5	0.2
TT	23.1	17.0	19.0	19.7	23.1	17.0	3.1	9.7	15.8
EL	36.0	36.0	35.0	35.7	36.0	35.0	0.6	0.3	1.6
EW	23.1	22.5	22.5	22.7	23.1	22.5	0.3	0.1	1.5

Table H. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.LONGIPLEXUS

CHARACTER	MEASUREMENTS 1 (JAN 15/76)	2 (APR 9/76)	3 (SEP 10/7)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	187.0	189.0	192.0	189.3	192.0	187.0	2.5	6.3	1.3
OSL	573.5	563.0	568.0	568.2	573.5	563.0	5.3	27.6	0.9
OSW	558.0	563.0	555.0	558.7	563.0	555.0	4.0	16.3	0.7
PL	264.0	263.0	262.0	263.0	264.0	262.0	1.0	1.0	0.4
PW	231.0	236.0	231.0	232.7	236.0	231.0	2.9	8.3	1.2
OL	1085.0	1126.0	1069.0	1093.3	1126.0	1069.0	29.4	864.5	2.7
OW	620.0	604.0	626.0	616.7	626.0	604.0	11.4	129.5	1.8
OAT	124.0	113.0	118.0	118.3	124.0	113.0	5.5	30.3	4.7
OAC	-356.0	-323.0	-334.0	-337.7	-323.0	-356.0	16.8	282.3	-5.0
ATL	1240.0	1271.0	1249.0	1253.3	1271.0	1240.0	16.0	254.5	1.3
ATW	558.0	570.0	570.0	566.0	570.0	558.0	6.9	46.0	1.2
ATE	2759.0	2698.0	2727.0	2728.0	2759.0	2698.0	30.5	928.0	1.1
PTL	1550.0	1581.0	1555.0	1562.0	1581.0	1550.0	16.7	278.0	1.1
PTW	620.0	631.0	620.0	623.7	631.0	620.0	6.4	40.5	1.0
PTE	1891.0	1860.0	1907.0	1886.0	1907.0	1860.0	23.9	572.0	1.3
TO	403.0	414.0	414.0	410.3	414.0	403.0	6.4	40.3	1.5
THS	-248.0	-212.0	-237.0	-232.3	-212.0	-248.0	18.4	340.3	-7.9
RUF	4495.0	4526.0	4541.0	4520.7	4541.0	4495.0	23.3	544.0	0.5
LUF	4278.0	4294.0	4290.0	4287.3	4294.0	4278.0	8.0	64.0	0.2
RVL	5733.0	5704.0	5723.0	5720.0	5733.0	5704.0	15.0	224.0	0.3
LVL	4246.0	4259.0	4277.0	4260.7	4277.0	4246.0	15.2	232.0	0.4
VA	6541.0	6572.0	6531.0	6548.0	6572.0	6531.0	21.4	456.0	0.3
VE	651.0	640.0	656.0	649.0	656.0	640.0	8.2	67.0	1.3
BL	7812.0	7800.0	7862.0	7824.7	7862.0	7800.0	33.3	1112.0	0.4
BW	2139.0	2122.0	2150.0	2137.0	2150.0	2122.0	14.2	200.5	0.7
TT	16.3	10.0	12.5	12.9	16.3	10.0	3.2	10.1	24.5
EL	22.5	22.5	25.0	23.3	25.0	22.5	1.4	2.1	6.2
EW	17.5	16.0	17.0	16.8	17.5	16.0	0.8	0.6	4.5

Table I. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.MEDIOPLEXUS

CHARACTER	MEASUREMENTS 1 (JAN 15/76)	2 (APR 9/76)	3 (SEP 10/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	78.8	75.0	82.0	78.6	82.0	75.0	3.5	12.3	4.5
OSL	374.0	363.0	368.0	368.3	374.0	363.0	5.5	30.3	1.5
OSW	283.8	294.0	288.0	288.6	294.0	283.8	5.1	26.3	1.8
PL	242.0	247.0	240.0	243.0	247.0	240.0	3.6	13.0	1.5
PW	198.0	197.0	203.0	199.3	203.0	197.0	3.2	10.3	1.6
OL	744.0	722.0	755.0	740.3	755.0	722.0	16.8	282.5	2.3
OW	341.0	346.0	336.0	341.7	346.0	336.0	4.0	16.3	1.2
OAT	93.0	90.0	96.0	93.0	96.0	90.0	3.0	9.0	3.2
OAC	44.0	39.0	46.0	43.0	46.0	39.0	3.6	13.0	8.4
ATL	868.0	870.0	883.0	873.7	883.0	868.0	8.2	66.5	0.9
ATW	558.0	547.0	535.0	546.7	558.0	535.0	11.5	132.3	2.1
ATE	2573.0	2542.0	2603.0	2572.7	2603.0	2542.0	30.5	928.0	1.2
PTL	620.0	609.0	631.0	620.0	631.0	609.0	11.0	121.0	1.8
PTW	589.0	568.0	589.0	582.0	589.0	568.0	12.1	147.0	2.1
PTE	1643.0	1612.0	1628.0	1627.7	1643.0	1612.0	15.6	242.0	1.0
TO	-124.0	-113.0	-101.0	-112.7	-101.0	-124.0	11.5	132.3	-10.2
THS	-465.0	-467.0	-434.0	-455.3	-434.0	-467.0	18.5	342.3	-4.1
RUF	220.0	208.0	223.0	217.0	223.0	208.0	7.9	63.0	3.7
LUF	176.0	175.0	181.0	177.3	181.0	175.0	3.2	10.3	1.8
RVL	4367.0	4397.0	4339.0	4367.7	4397.0	4339.0	28.8	832.0	0.7
LVL	4345.0	4376.0	4361.0	4360.7	4376.0	4345.0	15.5	240.0	0.4
VA	5611.0	5644.0	5630.0	5628.3	5644.0	5611.0	17.0	288.0	0.3
VE	1302.0	1280.0	1296.0	1292.7	1302.0	1280.0	11.4	129.5	0.9
BL	7626.0	7600.0	7688.0	7638.0	7688.0	7600.0	45.3	2048.0	0.6
BW	826.0	826.0	806.0	812.7	826.0	806.0	11.6	133.5	1.4
TT	4.3	5.0	3.5	4.3	5.0	3.5	0.8	0.6	17.6
EL	25.0	27.5	25.0	25.8	27.5	25.0	1.4	2.1	5.5
EW	18.8	18.0	17.5	18.1	18.8	17.5	0.7	0.4	3.6

Table J. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.OXYORCHIS

CHARACTER	MEASUREMENTS 1 (NOV 9/76)	2 (NOV 10/76)	3 (NOV 11/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	291.6	290.0	289.1	290.2	291.6	289.1	1.3	1.6	0.4
OSL	369.0	373.0	371.5	371.2	373.0	369.0	2.0	4.1	0.5
OSW	351.0	349.0	350.7	350.2	351.0	349.0	1.1	1.2	0.3
PL	207.0	205.0	208.3	206.8	208.3	205.0	1.7	2.8	0.8
PW	243.0	245.0	241.2	243.1	245.0	241.2	1.9	3.6	0.8
OL	585.0	587.0	583.5	585.2	587.0	583.5	1.8	3.1	0.3
OW	333.0	335.0	333.7	333.9	335.0	333.0	1.0	1.0	0.3
OAT	153.0	150.5	155.0	152.8	155.0	150.5	2.3	5.1	1.5
OAC	180.0	178.8	182.0	180.3	182.0	178.8	1.6	2.7	0.9
ATL	729.0	735.0	730.5	731.5	735.0	729.0	3.2	10.0	0.4
ATW	612.0	610.0	613.4	611.8	613.4	610.0	1.7	3.0	0.3
ATE	1602.0	1607.0	1600.0	1603.0	1607.0	1600.0	3.7	13.5	0.2
PTL	675.0	673.5	675.0	674.5	675.0	673.5	1.0	1.0	0.1
PTW	648.0	650.3	647.1	648.5	650.3	647.1	1.6	2.5	0.2
PTE	963.0	961.4	960.5	961.6	963.0	960.5	1.2	1.5	0.1
TO	36.0	34.5	33.6	34.7	36.0	33.6	1.2	1.5	3.5
THS	-117.0	-115.0	-115.0	-115.7	-115.0	-117.0	1.2	1.3	-1.0
RUF	ABSENT	ABSENT	ABSENT						
LUF	ABSENT	ABSENT	ABSENT						
RVL	3906.0	3910.0	3904.0	3906.7	3910.0	3904.0	2.8	8.0	0.1
LVL	2740.5	2742.5	2744.0	2742.3	2744.0	2740.5	0.0	0.0	0.0
VA	4378.5	4380.0	4385.0	4381.2	4385.0	4378.5	2.8	8.0	0.1
VE	540.0	542.0	545.0	542.3	545.0	540.0	2.5	6.3	0.5
BL	5512.5	5516.0	5515.6	5514.7	5516.0	5512.5	2.8	8.0	0.1
BW	976.5	979.0	975.0	976.8	979.0	975.0	2.0	4.0	0.2
TT	6.9	7.0	5.0	6.3	7.0	5.0	1.1	1.3	17.9
EL	23.1	22.5	22.5	22.7	23.1	22.5	0.3	0.1	1.5
EW	15.7	15.0	15.5	15.4	15.7	15.0	0.4	0.1	2.3

Table K. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.PARVIPLEXUS

CHARACTER	MEASUREMENTS 1 (MAY 14/76)	2 (MAY 21/76)	3 (JUN 3/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	87.5	90.0	85.5	87.7	90.0	85.5	2.3	5.1	2.6
OSL	220.0	225.0	230.5	225.2	230.5	220.0	5.3	27.6	2.3
OSW	187.0	187.0	192.5	188.8	192.5	187.0	3.2	10.1	1.7
PL	143.0	148.0	140.0	143.7	148.0	140.0	4.0	16.3	2.8
PW	154.0	159.0	152.0	155.0	159.0	152.0	3.6	13.0	2.3
OL	806.0	795.0	800.0	800.3	806.0	795.0	5.5	30.5	0.7
OW	356.5	351.0	356.0	354.5	356.5	351.0	3.1	9.3	0.9
OAT	186.0	191.0	187.0	188.0	191.0	186.0	2.6	7.0	1.4
OAC	-132.0	-130.0	-128.0	-130.0	-128.0	-132.0	2.0	4.0	-1.5
ATL	682.0	687.0	676.0	682.3	687.0	676.0	4.5	20.5	0.7
ATW	468.0	481.0	476.0	468.3	476.0	461.0	7.5	56.3	1.6
ATE	2339.0	2326.0	2333.0	2333.3	2339.0	2326.0	5.5	30.5	0.2
PTL	744.0	730.0	740.0	738.0	744.0	730.0	7.2	52.0	1.0
PTW	558.0	556.0	570.0	561.3	570.0	556.0	7.6	57.3	1.3
PTE	1225.0	1200.0	1207.0	1210.7	1225.0	1200.0	12.9	166.5	1.1
TD	-138.0	-130.0	-133.0	-133.7	-130.0	-138.0	4.0	16.3	-3.0
THS	-353.0	-347.0	-345.0	-348.3	-345.0	-353.0	4.2	17.3	-1.2
RUF	1561.0	1550.0	1560.0	1563.7	1581.0	1550.0	15.9	252.0	1.0
LUF	1612.0	1612.0	1628.0	1617.3	1628.0	1612.0	9.2	85.5	0.6
RVL	3303.0	3280.0	3332.0	3305.0	3332.0	3280.0	26.1	680.0	0.8
LVL	4173.0	4143.0	4180.0	4165.3	4180.0	4143.0	19.6	384.0	0.5
VA	5275.0	5279.0	5248.0	5267.3	5279.0	5248.0	16.7	280.0	0.3
VE	1101.0	1090.0	1105.0	1098.7	1105.0	1090.0	7.8	60.5	0.7
BL	6422.0	6400.0	6400.0	6407.3	6422.0	6400.0	14.1	200.0	0.2
BW	1166.0	1155.0	1170.0	1164.3	1170.0	1155.0	8.2	66.5	0.7
TT	12.5	8.5	10.0	10.3	12.5	8.5	2.0	4.1	19.6
EL	22.8	22.8	23.5	23.0	23.5	22.8	0.4	0.2	1.6
EW	15.0	15.0	14.0	14.7	15.0	14.0	0.6	0.3	3.9

Table L. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.SIMILIPLEXUS

CHARACTER	MEASUREMENTS 1 (MAY 10/76)	2 (JUL 5/76)	3 (SEP 3/76)	MEAN	MAX.	MIN	STD DEV	VARIANCE	CV
AC	144.1	143.0	145.0	144.0	145.0	143.0	1.0	1.0	0.7
OSL	374.0	370.0	371.2	371.7	374.0	370.0	2.1	4.4	0.6
OSW	341.0	343.0	340.1	341.4	343.0	340.1	1.5	2.2	0.4
PL	198.0	195.5	197.0	196.8	198.0	195.5	1.3	1.6	0.6
PW	173.8	175.0	175.0	174.6	175.0	173.8	0.7	0.5	0.4
OL	627.0	625.0	629.0	627.0	629.0	625.0	2.0	4.0	0.3
OW	506.0	505.0	505.0	505.3	506.0	505.0	0.6	0.2	0.1
OAT	77.0	75.0	80.0	77.3	80.0	75.0	2.5	6.3	3.3
OAC	-186.0	-182.0	-184.0	-184.0	-182.0	-186.0	2.0	4.0	-1.1
ATL	1085.0	1080.5	1087.0	1084.2	1087.0	1080.5	3.3	11.0	0.3
ATW	496.0	500.0	500.0	498.7	500.0	496.0	2.3	5.3	0.5
ATE	2046.0	2045.0	2050.0	2047.0	2050.0	2045.0	2.9	8.5	0.1
PTL	1271.0	1273.0	1268.0	1270.7	1273.0	1268.0	2.5	6.5	0.2
PTW	496.0	491.0	500.0	495.7	500.0	491.0	4.5	20.3	0.9
PTE	1054.0	1055.0	1053.0	1054.0	1055.0	1053.0	1.0	1.0	0.1
TO	310.0	310.0	312.0	310.7	312.0	310.0	1.2	1.3	0.4
THS	20.0	18.0	18.5	18.8	20.0	18.0	1.0	1.1	5.5
RUF	1984.0	1980.0	1990.0	1984.7	1990.0	1980.0	5.1	26.0	0.3
LUF	2573.0	2575.0	2570.0	2572.7	2575.0	2570.0	0.0	0.0	0.0
RVL	4679.0	4684.0	4677.0	4660.0	4684.0	4677.0	2.6	6.0	0.1
LVL	2844.0	2841.0	2843.0	2842.7	2844.0	2841.0	0.0	0.0	0.0
VA	5046.0	5045.0	5049.0	5046.7	5049.0	5045.0	0.0	0.0	0.0
VE	744.0	740.0	745.0	743.0	745.0	740.0	2.5	7.0	0.4
OL	5963.0	5965.0	5960.0	5962.7	5965.0	5960.0	0.0	0.0	0.0
OW	1519.0	1520.0	1517.5	1518.8	1520.0	1517.5	1.9	3.5	0.1
TT	30.0	25.0	25.0	26.7	30.0	25.0	2.9	8.3	10.8
EL	20.0	19.0	20.5	19.8	20.5	19.0	0.8	0.6	3.9
EW	12.5	12.0	12.5	12.3	12.5	12.0	0.3	0.1	2.3

Table M. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.TUMIDUS

CHARACTER	MEASUREMENTS 1 (NOV 9/76)	2 (NOV 10/76)	3 (NOV 11/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	759.2	755.0	761.0	758.4	761.0	755.0	3.1	9.5	0.4
OSL	614.3	610.0	616.0	613.4	616.0	610.0	3.1	9.5	0.5
OSW	693.0	697.0	695.0	695.0	697.0	693.0	2.0	4.0	0.3
PL	497.7	495.3	500.0	497.7	500.0	495.3	2.3	5.5	0.5
PW	472.5	468.1	474.0	471.5	474.0	468.1	3.1	9.5	0.7
OL	1096.2	1095.0	1091.0	1094.1	1096.2	1091.0	2.7	7.5	0.3
OW	645.8	640.5	638.1	641.5	645.8	638.1	3.9	15.5	0.6
OAT	157.5	155.0	159.3	157.3	159.3	155.0	2.2	4.7	1.4
OAC	-434.7	-437.0	-438.5	-436.7	-434.7	-438.5	2.0	3.8	-0.4
ATL	1764.5	1760.0	1766.0	1763.5	1766.0	1760.0	3.5	12.0	0.2
ATW	1102.5	1100.5	1105.2	1102.7	1105.2	1100.5	2.2	5.0	0.2
ATE	2583.0	2590.0	2592.0	2588.3	2592.0	2583.0	4.9	24.0	0.2
PTL	1764.0	1770.0	1768.3	1767.4	1770.0	1764.0	3.9	15.5	0.2
PTW	1354.5	1348.6	1350.0	1351.0	1354.5	1346.6	3.1	9.5	0.2
PTE	1609.7	1600.0	1605.0	1604.9	1609.7	1600.0	4.9	24.5	0.3
TU	693.0	690.0	695.5	692.8	695.5	690.0	2.7	7.5	0.4
THS	283.5	280.0	285.1	282.9	285.1	280.0	2.6	6.8	0.9
RUF	2110.5	2105.2	2103.5	2106.4	2110.5	2103.5	4.6	21.0	0.2
LUF	1856.5	1855.0	1860.1	1857.9	1860.1	1855.0	3.7	13.5	0.2
RVL	5071.5	5065.0	5073.0	5069.8	5073.0	5065.0	4.9	24.0	0.1
LVL	6174.0	6170.0	6175.0	6173.0	6175.0	6170.0	5.7	32.0	0.1
VA	4340.7	4335.0	4333.7	4336.5	4340.7	4333.7	4.0	16.0	0.1
VE	945.0	949.0	947.6	947.2	949.0	945.0	2.0	4.0	0.2
BL	8568.0	8579.0	8567.7	8571.6	8579.0	8567.7	6.3	40.0	0.1
BW	2929.5	2933.0	2930.0	2930.8	2933.0	2929.5	0.0	0.0	0.0
TT	19.4	17.5	17.5	18.1	19.4	17.5	1.1	1.2	6.0
EL	36.4	36.5	37.0	36.6	37.0	36.4	0.3	0.1	0.9
EW	19.8	19.5	20.0	19.8	20.0	19.5	0.3	0.1	1.3

Table N. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.UNIPLEXUS

CHARACTER	MEASUREMENTS 1 (NOV 9/76)	2 (NOV 10/76)	3 (NOV 11/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	83.0	83.0	81.3	82.4	83.0	81.3	1.0	1.0	1.2
OSL	230.5	227.0	231.4	229.6	231.4	227.0	2.3	5.4	1.0
OSW	216.7	215.0	218.0	216.6	218.0	215.0	1.5	2.3	0.7
PL	119.9	118.0	121.4	119.8	121.4	118.0	1.7	2.9	1.4
PW	138.3	137.5	140.0	138.6	140.0	137.5	1.3	1.7	0.9
OL	432.9	431.0	429.6	431.2	432.9	429.6	1.7	2.8	0.4
OW	131.4	130.0	133.0	131.5	133.0	130.0	1.5	2.3	1.1
OAT	-90.0	-92.0	-89.0	-90.3	-89.0	-92.0	1.5	2.3	-1.7
OAC	-184.4	-186.4	-187.0	-185.9	-184.4	-187.0	1.4	1.9	-0.7
ATL	441.0	439.7	443.5	441.4	443.5	439.7	2.0	3.8	0.4
ATW	166.5	166.0	165.0	165.8	166.5	165.0	0.8	0.6	0.5
ATE	855.0	858.7	860.0	857.9	860.0	855.0	2.5	6.5	0.3
PTL	405.0	401.3	407.9	404.7	407.9	401.3	3.3	11.0	0.8
PTW	189.0	187.5	193.0	189.8	193.0	187.5	2.8	8.1	1.5
PTE	496.8	495.0	500.0	497.3	500.0	495.0	2.5	6.5	0.5
TD	81.0	79.0	81.8	80.6	81.8	79.0	1.4	2.1	1.8
THS	27.0	26.0	25.4	26.1	27.0	25.4	0.8	0.7	3.1
RUF	ABSENT	ABSENT	ABSENT						
LUF	549.0	547.0	551.0	549.0	551.0	547.0	2.0	4.0	0.4
RVL	2327.9	2330.0	2324.0	2327.3	2330.0	2324.0	3.7	14.0	0.2
LVL	1679.8	1679.0	1673.0	1673.9	1679.0	1673.0	3.9	15.0	0.2
VA	2595.6	2600.0	2593.4	2596.3	2600.0	2593.4	4.0	16.0	0.2
VE	1004.9	1000.0	1006.6	1004.5	1008.6	1000.0	4.3	18.5	0.4
BL	3266.6	3259.0	3771.1	3432.2	3771.1	3259.0	293.5	86144.0	8.6
BW	711.0	706.4	712.0	709.8	712.0	706.4	3.0	9.0	0.4
TT	9.2	8.0	7.5	8.2	9.2	7.5	0.9	0.8	10.6
EL	20.3	20.5	21.0	20.8	21.0	20.5	0.3	0.1	1.2
EW	16.6	16.0	15.5	16.0	16.6	15.5	0.6	0.3	3.4

Table O. RELIABILITY FOR 28 CHARACTERS MEASURED OF H.VARIOPLEXUS

CHARACTER	MEASUREMENTS 1 (MAY 14/76)	2 (MAY 21/76)	3 (JUN 3/76)	MEAN	MAX	MIN	STD DEV	VARIANCE	CV
AC	165.0	163.0	166.5	164.8	166.5	163.0	1.8	3.1	1.1
USL	517.0	512.0	517.0	515.3	517.0	512.0	2.9	8.3	0.6
OSW	528.0	526.0	529.0	527.7	529.0	526.0	1.5	2.3	0.3
PL	220.0	215.0	221.0	218.7	221.0	215.0	3.2	10.3	1.5
PW	242.0	239.0	241.0	240.7	242.0	239.0	1.5	2.3	0.6
OL	496.0	496.0	499.0	497.0	499.0	496.0	1.7	3.0	0.3
OW	539.0	538.0	539.0	538.7	539.0	538.0	0.6	0.3	0.1
OAT	319.0	311.0	317.0	315.7	319.0	311.0	4.2	17.3	1.3
OAC	-30.0	-25.0	-27.0	-27.3	-25.0	-30.0	2.5	6.3	-9.2
ATL	1209.0	1200.0	1215.0	1208.0	1215.0	1200.0	7.5	57.0	0.6
ATW	465.0	462.5	467.0	464.8	467.0	462.5	2.3	5.1	0.5
ATE	1922.0	1898.0	1911.0	1910.3	1922.0	1898.0	12.1	146.0	0.6
PTL	744.0	740.0	746.0	743.3	746.0	740.0	3.1	9.5	0.4
PTW	310.0	310.0	312.0	310.7	312.0	310.0	1.2	1.3	0.4
PTE	1162.0	1131.0	1151.0	1148.0	1162.0	1131.0	15.7	247.0	1.4
TD	155.0	155.0	175.0	161.7	175.0	155.0	11.5	133.3	7.1
THS	-496.0	-470.0	-481.0	-482.3	-470.0	-496.0	13.1	170.3	-2.7
RUF	3255.0	3100.0	3150.0	3168.3	3255.0	3100.0	79.1	6256.0	2.5
LUF	3007.0	3110.0	3099.0	3072.0	3110.0	3007.0	56.5	3192.0	1.8
RVL	4928.0	4848.0	4939.0	4898.3	4939.0	4848.0	46.2	2136.0	0.9
LVL	4679.0	4649.0	4693.0	4673.3	4693.0	4648.0	23.0	528.0	0.5
VA	5596.0	5550.0	5575.0	5573.7	5596.0	5550.0	23.7	560.0	0.4
VE	551.0	540.0	561.5	550.8	561.5	540.0	10.8	115.6	2.0
SI	6835.0	6804.0	6866.0	6835.0	6866.0	6804.0	31.2	976.0	0.5
BW	1973.0	1942.0	1984.0	1966.3	1984.0	1942.0	21.8	476.0	1.1
TT	22.0	15.0	17.0	18.0	22.0	15.0	3.6	13.0	20.0
EL	22.5	22.0	23.5	22.7	23.5	22.0	0.8	0.6	3.4
EW	15.0	15.0	16.0	15.3	16.0	15.0	0.6	0.3	3.8

Appendix 7. Average measurements (mm) of *H. buttensis* maintained at 12°C

Age (days)	Metacercariae	5	14	21	28	60
No. of specimens	5	13	8	9	11	7
Body length	0.69(0.59-0.77)	0.74(0.63-0.86)	0.76(0.66-0.82)	0.77(0.64-0.80)	0.75(0.68-0.82)	0.80(0.79-0.85)
Body width	0.27(0.21-0.33)	0.29(0.23-0.32)	0.28(0.22-0.32)	0.28(0.23-0.31)	0.29(0.25-0.34)	0.30(0.28-0.36)
Acet. diam.[a]	0.09(0.08-0.10)	0.08(0.07-0.09)	0.08(0.07-0.09)	0.08(0.07-0.10)	0.08(0.07-0.09)	0.08(0.07-0.09)
O.S. diam.[b]	0.11(0.09-0.13)	0.10(0.09-0.14)	0.11(0.08-0.15)	0.11(0.09-0.13)	0.12(0.10-0.14)	0.12(0.11-0.15)
O.S. length[c]	0.10(0.09-0.11)	0.10(0.09-0.11)	0.10(0.09-0.10)	0.11(0.10-0.12)	0.11(0.10-0.13)	0.12(0.11-0.13)
O/A ratio	1.2 (1.1 -1.3)	1.5 (1.3 -1.6)	1.5 (1.2 -1.7)	1.3 (1.2 -1.4)	1.5 (1.4 -1.6)	1.6 (1.5 -1.7)
Phyn. diam.[d]	0.05(0.04-0.07)	0.05(0.04-0.08)	0.06(0.05-0.08)	0.08(0.06-0.09)	0.08(0.08-0.11)	0.09(0.08-0.10)
Phyn. length[e]	0.05(0.04-0.07)	0.05(0.04-0.06)	0.06(0.04-0.08)	0.07(0.06-0.08)	0.07(0.07-0.09)	0.08(0.07-0.09)
Ovary diam.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ovary length	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
A.T. Diam.[f]	0.02(0.02-0.03)	0.02(0.01-0.03)	0.02(0.02-0.03)	0.04(0.03-0.06)	0.07(0.05-0.09)	0.09(0.07-0.11)
A.T. length[g]	0.03(0.02-0.04)	0.05(0.04-0.06)	0.06(0.04-0.07)	0.08(0.06-0.09)	0.10(0.08-0.12)	0.14(0.11-0.20)
A.T. from end[h]	0.02(0.01-0.03)	0.04(0.02-0.07)	0.07(0.05-0.09)	0.09(0.08-0.11)	0.13(0.09-0.15)	0.24(0.20-0.26)
P.T. diam.[i]	0.03(0.02-0.04)	0.05(0.04-0.07)	0.06(0.04-0.08)	0.06(0.05-0.08)	0.07(0.05-0.09)	0.09(0.06-0.11)
P.T. length[j]	0.04(0.03-0.05)	0.06(0.05-0.07)	0.07(0.05-0.08)	0.09(0.06-0.10)	0.09(0.07-0.12)	0.13(0.11-0.17)
P.T. from end[k]	0.01(0.01-0.02)	0.03(0.01-0.04)	0.05(0.03-0.07)	0.07(0.05-0.09)	0.08(0.06-0.11)	0.11(0.10-0.13)

a Diameter of acetabulum
b Diameter of oral sucker
c Length of oral sucker
d Diameter of pharynx
e Length of pharynx
f Diameter of anterior testis
g Length of anterior testis
h Distance of anterior testis from posterior end of worm
i Diameter of posterior testis
j Length of posterior testis
k Distance of posterior testis from posterior end of worm
N.D. Not developed

Appendix 8. Average measurements (mm) of H. buttensis maintained at 20°C

Age (days)	5	14	21	28	60
No. of specimens	21	18	19	11	14
Body length	1.06 (0.94-1.20)	1.78 (1.73-1.88)	3.27 (2.66-3.74)	4.10 (3.55-4.43)	6.30 (5.78-7.01)
Body width	0.35 (0.33-0.37)	0.51 (0.48-0.53)	0.75 (0.70-0.84)	0.81 (0.77-0.84)	1.40 (1.22-1.52)
Acet. diam.[a]	0.05 (0.04-0.06)	0.07 (0.06-0.08)	0.08 (0.07-0.09)	0.11 (0.09-0.12)	0.11 (0.09-0.13)
O.S. diam.[b]	0.12 (0.11-0.13)	0.18 (0.17-0.20)	0.22 (0.19-0.25)	0.26 (0.24-0.28)	0.29 (0.25-0.33)
O.S. length[c]	0.11 (0.10-0.13)	0.16 (0.14-0.19)	0.21 (0.19-0.23)	0.24 (0.23-0.26)	0.25 (0.22-0.29)
O/A ratio	2.3 (2.2-2.6)	2.6 (2.5-2.8)	2.5 (2.2-2.7)	2.5 (2.3-2.7)	2.6 (2.5-2.7)
Phyn. diam.[d]	0.09 (0.07-0.11)	0.14 (0.12-0.16)	0.16 (0.14--.18)	0.17 (0.16-0.19)	0.21 (0.18-0.24)
Phyn. length[e]	0.08 (0.06-0.11)	0.12 (0.10-0.14)	0.13 (0.12-0.16)	0.15 (0.13-0.21)	0.20 (0.17-0.24)
Ovary diam.	0.07 (0.06-0.09)	0.18 (0.14-0.20)	0.23 (0.14-0.36)	0.29 (0.26-0.34)	0.38 (0.33-0.42)
Ovary length	0.08 (0.06-0.11)	0.25 (0.20-0.31)	0.49 (0.33-0.51)	0.56 (0.50-0.63)	0.72 (0.70-0.75)
A.T. diam.[f]	0.09 (0.06-0.11)	0.24 (0.20-0.29)	0.35 (0.26-0.39)	0.44 (0.41-0.49)	0.49 (0.33-0.61)
A.T. length[g]	0.14 (0.11-0.21)	0.35 (0.29-0.39)	0.63 (0.52-0.75)	0.62 (0.57-0.68)	1.15 (1.07-1.31)
A.T. from end[h]	0.24 (0.21-0.27)	0.39 (0.33-0.45)	0.80 (0.45-1.06)	1.12 (0.83-1.39)	1.79 (1.48-2.25)
P.T. diam.[i]	0.08 (0.06-0.11)	0.24 (0.18-0.28)	0.39 (0.27-0.52)	0.46 (0.36-0.54)	0.52 (0.41-0.66)
P.T. length[j]	0.13 (0.11-0.17)	0.32 (0.25-0.32)	0.66 (0.53-0.78)	0.77 (0.66-0.91)	1.23 (1.17-1.35)
P.T. from end[k]	0.12 (0.09-0.13)	0.17 (0.15-0.19)	0.64 (0.39-1.26)	0.51 (0.26-0.63)	1.01 (0.92-1.33)

a Diameter of acetabulum
b Diameter of oral sucker
c Length of oral sucker
d Diameter of pharynx
e Length of pharynx
f Diameter of anterior testis
g Length of anterior testis
h Distance of anterior testis from posterior end of worm
i Diameter of posterior testis
j Length of posterior testis
k Distance of posterior testis from posterior end of worm

Appendix 9. Average measurements (mm) of H. buttensis maintained at 27°C.

Age (days)	5	14	21	28	60
No. of specimens	17	19	13	14	10
Body length	1.98(1.85-2.12)	3.71(3.69-3.83)	3.96(3.88-4.05)	4.63(4.27-4.88)	7.40(6.83-7.94)
Body width	0.37(0.32-0.41)	0.49(0.44-0.52)	0.75(0.69-0.87)	0.79-0.76-0.83)	1.42(1.25-1.59)
Acet. diam.[a]	0.06(0.04-0.07)	0.07(0.05-0.08)	0.08(0.07-0.09)	0.10(0.08-0.13)	0.12 (0.08-0.14)
O.S. diam.[b]	0.13(0.11-0.14)	0.17(0.15-0.19)	0.23(0.18-0.26)	0.27(0.25-0.29)	0.31(0.26-0.33)
O.S. length[c]	0.12(0.09-0.14)	0.17(0.14-0.18)	0.22(0.20-0.24)	0.25(0.23-0.27)	0.26(0.23-0.28)
O/A ratio	2.6 (2.1 -2.8)	2.7 (2.4 -3.0)	2.7 (2.6 -2.9)	2.8 (2.2 -3.0)	2.8 (2.4 -3.1)
Phyn. diam.[d]	0.08(0.07-0.10)	0.12(0.10-0.15)	0.14(0.13-0.17)	0.16(0.14-0.21)	0.19(0.17-0.23)
Phyn. length[e]	0.07(0.06-0.10)	0.10(0.09-0.13)	0.12 (0.11-0.16)	0.13(0.11-0.18)	0.17(0.15-0.21)
Ovary diam.	0.06(0.05-0.08)	0.16(0.13-0.18)	0.19(0.14-0.24)	0.23(0.20-0.31)	0.32(0.29-0.37)
Ovary length	0.07(0.06-0.10)	0.22(0.19-0.27)	0.44(0.31-0.50)	0.50(0.43-0.59)	0.65(0.61-0.70)
A.T. diam.[f]	0.07(0.05-0.08)	0.20(0.18-0.27)	0.29(0.25-0.33)	0.37(0.34-0.42)	0.40(0.31-0.53)
A.T. length[g]	0.12(0.09-0.18)	0.29(0.26-0.35)	0.54(0.49-0.61)	0.51(0.47-0.59)	0.99(0.93-1.17)
A.T. from end[h]	0.68(0.63-0.74)	1.14 (1.11 -1.20)	2.35(2.29-2.41)	3.29(3.01-3.42)	5.31(5.17-5.42)
P.T. diam.[i]	0.07(0.05-0.09)	0.19(0.16-0.23)	0.32(0.24-0.47)	0.38(0.27-0.43)	0.44(0.39-0.54)
P.T. length[j]	0.11 (0.08-0.15)	0.27(0.21-0.29)	0.54(0.48-0.63)	0.65(0.56-0.79)	1.04(0.96-1.17)
P.T. from end[k]	0.35(0.29-0.41)	0.48(0.39-0.53)	1.90(1.78-2.11)	1.52(0.79-1.88)	3.10 (2.95-3.22)

a Diameter of acetabulum
b Diameter of oral sucker
c Length of oral sucker
d Diameter of pharynx
e Length of pharynx
f Diameter of anterior testis
g Length of anterior testis
h Distance of anterior testis from posterior end of worm
i Diameter of posterior testis
j Length of posterior testis
k Distance of posterior testis from posterior end of worm

Appendix 10. Average measurements of H. buttensis of varying ages developed in Rana pretiosa having a snout-vent length of 30-35 mm.

Age (days)	5	14	21	28	60
No. of specimens	26	21	23	19	18
Body length	1.25(1.21-1.33)	1.81(1.78-1.98)	3.33(3.17-3.63)	3.88(3.77-3.95)	6.46(6.28-6.77)
Body width	0.38(0.35-0.52)	0.50(0.57-0.57)	0.73(0.63-0.86)	0.83(0.80-0.94)	1.43(1.34-1.57)
Acetabulum diam.	0.06(0.06-0.07)	0.07(0.07-0.08)	0.09(0.08-0.09)	0.11(0.10-0.12)	0.11(0.10-1.13)
Oral Sucker diam.	0.13(0.12-0.14)	0.18(0.17-0.19)	0.23(0.23-0.27)	0.26(0.23-0.27)	0.28(0.26-0.30)
Oral Sucker length	0.12(0.09-0.13)	0.17(0.16-0.18)	0.22(0.19-0.23)	0.24(0.23-0.26)	0.25(0.23-0.26)
O/A Ratio	2.4 (2.3 -2.5)	2.5 (2.4 -2.7)	2.5 (2.4 -2.7)	2.6 (2.3 -2.7)	2.6 (2.4 -2.8)
Ovary width	0.08(0.07-0.09)	0.17(0.15-0.18)	0.26(0.24-0.28)	0.30(0.28-0.33)	0.39(0.37-0.41)
Ovary length	0.08(0.06-0.09)	0.24(0.22-0.25)	0.48(0.46-0.49)	0.56(0.55-0.59)	0.73(0.68-0.74)
Ant. Testis width	0.10(0.09-0.11)	0.25(0.22-0.28)	0.33(0.28-0.35)	0.39(0.38-0.42)	0.46(0.43-0.49)
Ant. Testis length	0.14(0.13-0.18)	0.35(0.32-0.37)	0.58(0.54-0.60)	0.62(0.58-0.63)	1.17(1.11-1.19)
Post. Testis width	0.09(0.08-0.10)	0.24(0.21-0.26)	0.42(0.39-0.43)	0.47(0.46-0.49)	0.53(0.49-0.54)
Post. Testis length	0.14(0.13-0.16)	0.33(0.30-0.34)	0.67(0.63-0.69)	0.74(0.72-0.77)	1.29(1.24-1.33)

All measurements in millimeters.

Appendix 11. Average measurements of H. buttensis of varying ages developed in Rana pretiosa having a snout-vent length of 45-50 mm.

Age (days)	5	14	21	28	60
No. of specimens	22	23	21	26	25
Body Length	1.11(1.07-1.17)	1.77(1.62-1.79)	3.12(2.88-3.37)	3.91(3.84-4.05)	6.11(5.83-6.72)
Body Width	0.35(0.33-0.38)	0.54(0.46-0.57)	0.70(0.58-0.87)	0.85(0.77-0.92)	1.35(1.06-1.48)
Acetabulum diam.	0.05(0.05-0.06)	0.07(0.07-0.08)	0.09(0.08-0.10)	0.11(0.09-0.12)	0.12(0.10-0.13)
Oral Sucker diam.	0.14(0.13-0.15)	0.19(0.17-0.20)	0.22(0.20-0.24)	0.26(0.25-0.27)	0.28(0.26-0.30)
Oral Sucker length	0.12(0.11-0.14)	0.16(0.15-0.17)	0.21(0.18-0.21)	0.24(0.24-0.26)	0.26(0.23-0.27)
O/A Ratio	2.4 (2.3 -2.5)	2.5 (2.4 -2.7)	2.5 (2.3 -2.6)	2.5 (2.4 -2.6)	2.6 (2.4 -2.7)
Ovary width	0.09(0.08-0.10)	0.18(0.17-0.20)	0.24(0.23-0.27)	0.29(0.28-0.31)	0.36(0.34-0.38)
Ovary length	0.83(0.07-0.10)	0.24(0.23-0.26)	0.48(0.45-0.50)	0.56(0.54-0.57)	0.71(0.68-0.74)
Ant. Testis width	0.09(0.08-0.10)	0.22(0.21-0.25)	0.35(0.32-0.38)	0.42(0.41-0.44)	0.51(0.48-0.53)
Ant. Testis length	0.13(0.13-0.17)	0.36(0.35-0.40)	0.63(0.61-0.66)	0.64(0.62-0.67)	1.14(1.06-1.19)
Post. Testis width	0.09(0.07-0.10)	0.22(0.21-0.25)	0.38(0.35-0.39)	0.44(0.42-0.45)	0.53(0.49-0.54)
Post. Testis length	0.13(0.12-0.14)	0.32(0.32-0.35)	0.66(0.63-0.67)	0.74(0.72-0.75)	1.24(1.20-1.25)

All measurements in millimeters.

Appendix 12. Average measurements of H. buttensis of varying ages developed in Rana pretiosa having a snout-vent length of 55-60 mm.

Age (days)	5	14	21	28	60
No. of specimens	18	16	24	17	17
Body length	1.26(1.24-1.32)	1.76(1.75-1.84)	3.31(3.29-3.47)	3.79(3.63-3.99)	6.44(5.87-6.75)
Body width	0.37(0.34-0.38)	0.47(0.45-0.50)	0.69(0.63-0.71)	0.80(0.73-0.91)	1.37(1.22-1.43)
Acetabulum diam.	0.06(0.06-0.07)	0.08(0.07-0.08)	0.09(0.08-0.10)	0.10(0.09-0.11)	0.11(0.10-0.12)
Oral Sucker diam.	0.12(0.12-0.13)	0.17(0.15-0.18)	0.24(0.23-0.25)	0.25(0.25-0.27)	0.28(0.26-0.29)
Oral Sucker length	0.12(0.11-0.12)	0.18(0.16-0.18)	0.21(0.20-0.22)	0.24(0.23-0.26)	0.25(0.24-0.28)
O/A Ratio	2.5 (2.4 -2.6)	2.5 (2.3 -2.7)	2.6 (2.3 -2.6)	2.7 (2.4 -2.8)	2.6 (2.5 -2.7)
Ovary width	0.08(0.08-0.09)	0.18(0.16-0.20)	0.25(0.21-0.27)	0.32(0.29-0.33)	0.38(0.37-0.40)
Ovary length	0.09(0.08-0.10)	0.25(0.23-0.26)	0.47(0.45-0.48)	0.56(0.54-0.57)	0.71(0.66-0.72)
Ant. Testis width	0.12(0.11-0.14)	0.25(0.23-0.26)	0.33(0.31-0.36)	0.39(0.37-0.41)	0.46(0.41-0.49)
Ant. Testis length	0.14(0.12-0.16)	0.34(0.32-0.38)	0.57(0.55-0.60)	0.63(0.61-0.64)	1.16(1.10-1.21)
Post. Testis width	0.10(0.10-0.13)	0.25(0.22-0.26)	0.41(0.40-0.44)	0.46(0.43-0.47)	0.52(0.49-0.53)
Post. Testis length	0.15(0.13-0.16)	0.34(0.33-0.36)	0.67(0.64-0.67)	0.74(0.71-0.77)	1.26(1.21-1.26)

All measurements in millimeters.

Appendix 13. Average measurements of H. buttensis of varying ages developed in Rana pretiosa having a snout-vent length of 65-70 mm.

Age (days)	5	14	21	28	60
No. of specimens	17	26	15	19	21
Body length	1.25(1.15-1.30)	1.78(1.69-1.80)	3.30(2.97-3.51)	3.86(3.72-4.10)	6.44(6.00-6.99)
Body width	0.36(0.35-0.37)	0.49(0.42-0.52)	0.69(0.52-0.83)	0.82(0.71-0.93)	1.38(1.19-1.52)
Acetabulum diam.	0.06(0.06-0.07)	0.07(0.06-0.08)	0.09(0.07-0.09)	0.10(0.09-0.10)	0.10(0.09-0.11)
Oral Sucker diam.	0.13(0.12-0.13)	0.17(0.16-0.18)	0.23(0.20-0.24)	0.25(0.23-0.26)	0.27(0.25-0.28)
Oral Sucker length	0.11(0.10-0.15)	0.17(0.17-0.19)	0.21(0.20-0.22)	0.24(0.22-0.25)	0.25(0.22-0.27)
O/A Ratio	2.3 (2.1 -2.4)	2.4 (2.3 -2.6)	2.5 (2.3 -2.7)	2.6 (2.5 -2.7)	2.5 (2.2 -2.6)
Ovary width	0.08(0.07-0.09)	0.17(0.15-0.19)	0.25(0.21-0.28)	0.32(0.30-0.35)	0.38(0.35-0.42)
Ovary length	0.09(0.07-0.10)	0.24(0.22-0.26)	0.46(0.45-0.48)	0.55(0.54-0.58)	0.70(0.65-0.72)
Ant. Testis width	0.11(0.09-0.12)	0.24(0.22-0.26)	0.32(0.31-0.36)	0.38(0.35-0.40)	0.45(0.43-0.49)
Ant. Testis length	0.13(0.12-0.17)	0.34(0.31-0.37)	0.57(0.52-0.58)	0.62(0.59-0.65)	1.15(1.04-1.18)
Post. Testis width	0.09(0.08-0.10)	0.24(0.20-0.26)	0.40(0.37-0.42)	0.47(0.44-0.49)	0.52(0.50-0.55)
Post. Testis length	0.14(0.12-0.17)	0.33(0.31-0.35)	0.66(0.64-0.68)	0.74(0.72-0.76)	1.27(1.21-1.28)

All measurements in millimeters.

Appendix 14. Average measurements of *H. buttensis* of varying ages developed in male *Rana pretiosa* having a snout-vent length of 50 mm.

Age (days)	5	14	21	28	60
No. of specimens	22	23	26	19	25
Body length	1.06(0.954-1.18)	1.78(1.59-1.88)	3.00(2.66-3.57)	3.96(3.78-4.61)	6.48(5.83-6.78)
Body width	0.33(0.32-0.36)	0.55(0.48-0.62)	0.66(0.51-0.83)	0.82(0.78-0.87)	1.26(1.18-1.49)
Acetabulum diam.	0.06(0.06-0.07)	0.08(0.07-0.08)	0.08(0.07-0.08)	0.12(0.11-0.13)	0.13(0.13-0.15)
Oral Sucker diam.	0.12(0.11-0.12)	0.18(0.15-0.18)	0.23(0.22-0.25)	0.27(0.26-0.28)	0.29(0.27-0.32)
Oral Sucker length	0.10(0.09-0.11)	0.17(0.16-0.18)	0.23(0.21-0.24)	0.25(0.24-0.27)	0.27(0.26-0.29)
O/A Ratio	2.4 (2.3 -2.6)	2.4 (2.3 -2.7)	2.6 (2.4 -2.7)	2.6 (2.5 -2.8)	2.6 (2.5 -2.7)
Ovary width	0.09(0.08-0.10)	0.18(0.13-0.26)	0.24(0.15-0.36)	0.28(0.21-0.33)	0.37(0.30-0.40)
Ovary length	0.10(0.09-0.11)	0.26(0.25-0.36)	0.52(0.48-0.65)	0.54(0.48-0.61)	0.74(0.72-0.76)
Ant. Testis width	0.12(0.09-0.13)	0.24(0.22-0.28)	0.35(0.29-0.51)	0.43(0.35-0.47)	0.50(0.46-0.61)
Ant. Testis length	0.12(0.11-0.18)	0.35(0.28-0.37)	0.65(0.55-0.72)	0.63(0.58-0.70)	1.16(1.10-1.37)
Post. Testis width	0.09(0.07-0.11)	0.23(0.20-0.30)	0.38(0.28-0.47)	0.45(0.36-0.54)	0.54(0.48-0.62)
Post. Testis length	0.14(0.12-0.17)	0.32(0.29-0.56)	0.66(0.52-0.69)	0.79(0.68-0.93)	1.36(1.20-1.46)

All measurements in millimeters.

Appendix 15. Average measurements of H. buttensis of varying ages developed in female Rana pretiosa having a snout-vent length of 50 mm.

Age (days)	5	14	21	28	60
No. of specimens	27	24	19	21	23
Body length	1.09(1.04-1.22)	1.83(1.71-1.94)	3.09(2.75-3.49)	4.08(3.63-4.45)	6.51(5.93-6.81)
Body width	0.35(0.32-0.36)	0.57(0.50-0.60)	0.67(0.53-0.83)	0.84(0.76-0.88)	1.33(1.27-1.51)
Acetabulum diam.	0.07(0.06-0.08)	0.08(0.07-0.09)	0.09(0.08-0.09)	0.12(0.12-0.13)	0.13(0.12-0.14)
Oral Sucker diam.	0.12(0.12-0.13)	0.18(0.16-0.19)	0.23(0.22-0.25)	0.26(0.26-0.28)	0.29(0.26-0.30)
Oral Sucker length	0.10(0.09-0.11)	0.17(0.16-0.18)	0.23(0.20-0.24)	0.26(0.24-0.27)	0.27(0.26-0.29)
O/A Ratio	2.3 (2.2 -2.5)	2.5 (2.3 -2.7)	2.5 (2.4 -2.7)	2.6 (2.4 -2.7)	2.6 (2.5 -2.8)
Ovary width	0.09(0.08-0.10)	0.18(0.14-0.27)	0.24(0.16-0.34)	0.27(0.22-0.32)	0.37(0.28-0.39)
Ovary length	0.10(0.09-0.11)	0.25(0.25-0.34)	0.50(0.47-0.63)	0.54(0.46-0.59)	0.72(0.68-0.73)
Ant. Testis width	0.12(0.10-0.13)	0.24(0.21-0.26)	0.36(0.31-0.50)	0.44(0.37-0.47)	0.52(0.48-0.59)
Ant. Testis length	0.12(0.12-0.17)	0.37(0.29-0.38)	0.64(0.54-0.74)	0.62(0.57-0.68)	1.15(1.13-1.36)
Post. Testis width	0.09(0.07-0.11)	0.24(0.22-0.32)	0.40(0.29-0.48)	0.46(0.38-0.52)	0.55(0.45-0.58)
Post. Testis length	0.15(0.13-0.17)	0.33(0.31-0.39)	0.67(0.55-0.67)	0.80(0.69-0.89)	1.40(1.25-1.53)

All measurements in millimeters.

Appendix 16 Effects of crowding on 60-day-old Haematoloechus buttensis in Rana pretiosa.

Frog no.	Number of metacercariae	# worms recovered	% survival	# worms per lung		# gravid worms per lung		% gravid worms per lung		*Average body size (LxW)/2
				Right	Left	Right	Left	Right	Left	
1	10	7	70.0	3	4	3	4	100	100	3.72(2.38-5.29)
2	10	10	100	7	3	7	3	100	100	3.92(3.26-5.07)
3	10	6	60.0	4	2	4	2	100	100	3.65(3.62-4.37)
4	20	16	80.0	8	8	6	8	75.0	100	3.29(2.74-3.91)
5	20	15	75.0	9	6	7	6	77.8	100	3.31(2.13-4.73)
6	20	15	75.0	8	7	8	7	100	100	3.47(2.86-4.55)
7	40	25	62.5	14	11	10	7	71.4	63.6	2.80(2.25-3.33)
8	40	27	67.5	9	18	8	11	88.9	61.1	3.07(2.60-3.75)
9	40	32	80.0	18	14	10	9	55.6	64.3	2.85(1.63-3.53)
10	80	43	53.8	20	23	12	12	60.0	52.2	1.99(1.73-2.43)
11	80	27	33.8	11	16	9	7	81.8	43.8	1.80(1.31-2.50)
12	80	52	65.0	31	21	11	15	35.5	71.4	2.05(1.66-2.63)
13	160	85	53.1	50	35	23	19	46.6	54.3	1.20(0.89-1.47)
14	160	101	63.1	49	52	17	21	34.7	40.4	1.27(0198-1.67)
15	160	120	75.0	49	71	19	12	38.8	16.9	1.32(1.00-1.80)

*Measurements in square millimeters.

Appendix 17. Average measurements of H. buttensis of varying ages developed in Rana pretiosa.

Age (days)	5	14	21	28	60
No. of specimens	21	19	18	16	18
Acetabulum	0.054(0.053-0.056)	0.069(0.065-0.075)	0.088(0.076-0.096)	0.098(0.090-0.109)	0.104(0.096-0.110)
Oral sucker length	0.119 (0.115 -0.123)	0.169(0.160-0.181)	0.211 (0.199-0.227)	0.237(0.229-0.243)	0.241(0.211 -0.263)
Oral sucker width	0.129(0.120-0.135)	0.180(0.175-0.190)	0.230(0.198-0.253)	0.251(0.236-0.267)	0.273(0.250-0.286)
O/A ratio	2.4 (2.2 -2.6)	2.6 (2.4 -2.7)	2.6 (2.5 -2.8)	2.6 (2.5 -2.7)	2.6 (2.5 -2.7)
Ovary length	0.082(0.067-0.097)	0.243(0.211 -0.301)	0.501(0.438-0.529)	0.553(0.522-0.603)	0.723(0.691-0.730)
Ovary width	0.079(0.071-0.089)	0.171 (0.153-0.190)	0.246(0.163-0.307)	0.285(0.231-0.337)	0.372(0.337-0.396)
Ant. testis length	0.136(0.117 -0.168)	0.338(0.299-0.365)	0.611 (0.526-0.730)	0.608(0.567-0.667)	1.147 (1.069-1.237)
Ant. testis width	0.088(0.065-0.101)	0.235(0.201-0.273)	0.339(0.253-0.401)	0.411 (0.359-0.467)	0.474(0.380-0.540)
Post. testis length	0.138(0.127-0.151)	0.331(0.288-0.350)	0.669(0.597-0.745)	0.745(0.680-0.837)	1.263(1.216 -1.300)
Post. testis width	0.079(0.059-0.101)	0.233(0.200-0.279)	0.402(0.277-0.513)	0.474(0.400-0.521)	0.528(0.440-0.637)
Body length	1.061(0.979-1.200)	1.777(1.726-1.821)	3.265(2.760-3.715)	3.918(3.650-4.300)	6.413(5.780-7.005)
Body width	0.352(0.329-0.757)	0.507(0.480-0.587)	0.746(0.689-0.987)	0.809(0.771-0.850)	1.399(1.220-1.518)
Egg length	---	0.017(0.016-0.018)	0.024(0.020-0.029)	0.023(0.022-0.024)	0.023(0.022-0.025)
Egg width	---	0.016(0.015-0.017)	0.017(0.016-0.018)	0.018(0.017-0.019)	0.018(0.017-0.018)

All measurements in millimeters.

Appendix 18. Average measurements of H. buttensis of varying ages developed in Rana aurora.

Age (days)	5	14	21	28	60
No. of specimens	17	13	14	15	11
Acetabulum	0.061 (0.055-9.063)	0.077 (0.069-0.081)	0.099 (0.087-0.103)	0.110 (0.101-0.121)	0.111 (0.099-0.122)
Oral sucker length	0.133 (0.126-0.142)	0.189 (0.170-0.204)	0.237 (0.212-0.243)	0.265 (0.287-0.280)	0.270 (0.250-0.291)
Oral sucker width	0.145 (0.137-0.158)	0.201 (0.188-0.206)	0.258 (0.225-0.279)	0.281 (0.250-0.289)	0.306 (0.267-0.322)
O/A ratio	2.4	2.6	2.6	2.6	2.8
Ovary length	Absent	Absent	0.203 (0.148-0.213)	0.388 (0.320-0.416)	0.448 (0.392-0.506)
Ovary width	Absent	Absent	0.075 (0.066-0.082)	0.113 (0.087-0.121)	0.126 (0.113-0.136)
Ant. testis length	0.084 (0.052-0.119)	0.212 (0.188-0.253)	0.390 (0.288-0.427)	0.412 (0.339-0.480)	0.706 (0.619-0.785)
Ant. testis width	0.065 (0.044-0.082)	0.165 (0.128-0.203)	0.254 (0.213-0.304)	0.281 (0.265-0.313)	0.361 (0.305-0.389)
Post testis length	0.133 (0.119-0.184)	0.324 (0.266-0.352)	0.675 (0.583-0.797)	0.778 (0.719-0.850)	1.325 (1.247-1.381)
Post. testis width	0.048 (0.033-0.073)	0.133 (0.096-0.175)	0.225 (0.188-0.264)	0.240 (0.203-0.288)	0.312 (0.276-0.370)
Body length	0.802 (0.753-0.948)	1.439 (1.267-1.680)	2.319 (1.926-2.551)	3.278 (2.981-3.464)	4.951 (4.320-5.219)
Body width	0.265 (0.257-0.288)	0.378 (0.364-0.397)	0.550 (0.497-0.622)	0.634 (0.581-0.714)	1.202 (0.995-1.367)
Egg length	Absent	Absent	Absent	0.019 (0.017-0.020)	0.020 (0.018-0.022)
Egg width	Absent	Absent	Absent	0.016 (0.015-0.018)	0.017 (0.016-0.017)

All measurements in millimeters.

Appendix 19. Average measurements of H. buttensis of varying ages developed in Rana clamitans.

Age (days)	5	14	21	28	60
No. of specimens	14	12	15	13	9
Acetabulum	0.062(0.057-0.065)	0.080(0.071-0.087)	0.101 (0.093-0.105)	0.112 (0.105-0.117)	0.119 (0.109-0.123)
Oral sucker length	0.136(0.128-0.142)	0.193(0.157-0.201)	0.241(0.235-0.241)	0.270(0.262-0.291)	0.275(0.270-0.273)
Oral sucker width	0.147(0.139-0.152)	0.205(0.190-0.211)	0.262(0.257-0.266)	0.286(0.270-0.295)	0.312(0.300-0.324)
O/A ratio	2.4	2.6	2.6	2.6	2.6
Ovary length	Absent	Absent	0.521(0.473-0.562)	0.601(0.512-0.682)	0.870(0.689-0.851)
Ovary width	Absent	Absent	0.453(0.401-0.528)	0.467(0.409-0.521)	0.682(0.614-0.737)
Ant. testis length	0.152(0.117 -0.167)	0.378(0.348-0.417)	0.679(0.613-0.705)	0.693(0.649-0.761)	1.284(1.172 -1.322)
Ant. testis width	0.095(0.077-0.117)	0.252(0.175-0.277)	0.363(0.311 -0.427)	0.440(0.416-0.491)	0.507(0.466-0.582)
Post. testis length	0.160(0.136-0.182)	0.397(0.335-0.427)	0.743(0.669-0.879)	0.827(0.774-0.865)	1.402(1.325-1.457)
Post. testis width	0.140(0.072-0.112)	0.277(0.250-0.289)	0.427(0.401-0.518)	0.502(0.460-0.463)	0.560(0.537-0.648)
Body length	0.823(0.764-0.989)	1.367(1.217 -1.592)	2.247(2.032-2.574)	3.186(2.951-3.324)	4.877(4.429-5.243)
Body width	0.259(0.245-0.277)	0.370(0.348-0.391)	0.538(0.450-0.552)	0.636(0.599-0.682)	1.167 (0.928-1.245)
Egg length	Absent	Absent	Absent	0.018(0.017-0.019)	0.023(0.022-0.025)
Egg width	Absent	Absent	Absent	0.016(0.015 -0.017)	0.017(0.016-0.018)

All measurements in millimeters.

Appendix 20. Average measurements of H. buttensis of varying ages developed in Bufo boreas.

Age (days)	5	14	21	28	60
No. of specimens	19	17	17	21	20
Acetabulum	0.049(0.047-0.051)	0.061(0.057-0.065)	0.076(0.063-0.083)	0.091(0.083-0.099)	0.100(0.082-0.120)
Oral sucker length	0.137(0.128-0.145)	0.188(0.167-0.212)	0.235(0.215-0.257)	0.270(0.255-0.282)	0.291(0.242-0.346)
Oral sucker width	0.135(0.124-0.141)	0.185(0.171-0.206)	0.237(0.195-0.258)	0.264(0.253-0.280)	0.305(0.244-0.372)
O/A ratio	2.7 (2.6 -2.8)	3.1 (2.9 -3.2)	3.1 (2.9 -3.3)	2.9 (2.8 -3.1)	3.0 (2.9 -3.1)
Ovary length	Absent	0.231(0.190-0.304)	0.457(0.313-0.514)	0.527(0.475-0.595)	0.684(0.552-0.825)
Ovary width	Absent	0.085(0.066-0.098)	0.133(0.130-0.194)	0.097(0.086-0.107)	0.132(0.105-0.159)
Ant. testis length	0.096(0.080-0.128)	0.244(0.210-0.271)	0.448(0.367-0.539)	0.473(0.431-0.504)	0.811(0.652-0.983)
Ant. testis width	0.073(0.052-0.085)	0.186(0.148-0.266)	0.285(0.208-0.333)	0.316(0.256-0.389)	0.406(0.326-0.490)
Post. testis length	0.153(0.127-0.201)	0.373(0.314-0.397)	0.776(0.628-0.901)	0.895(0.775-1.070)	1.523(1.227-1.835)
Post. testis width	0.053(0.036-0.069)	0.146(0.117-0.179)	0.248(0.166-0.345)	0.216(0.169-0.252)	0.343(0.271-0.416)
Body length	0.879(0.824-0.962)	1.534(1.429-1.771)	2.695(2.112-3.224)	3.431(3.083-4.037)	5.353(4.325-6.450)
Body width	0.309(0.290-0.321)	0.429(0.422-0.542)	0.625(0.575-0.786)	0.743(0.707-0.784)	1.366(1.104-1.638)
Egg length	Absent	Absent	0.023(0.019-0.025)	0.023(0.023-0.026)	0.023(0.023-0.025)
Egg width	Absent	Absent	0.016(0.014-0.018)	0.018(0.017-0.018)	0.017(0.017-0.018)

All measurements in millimeters.

Appendix 21. Average measurements of *H. buttensis* of varying ages developed in *Rana pretiosa* when *Physa nuttalli* was the first intermediate host.

Age (days)	5	14	21	28	60
No. of specimens	19	17	23	20	19
Body length	1.11(0.97-1.19)	1.81(1.77-1.88)	3.17(2.74-3.59)	4.11(3.58-4.47)	6.64(5.89-6.96)
Body width	0.34(0.32-0.36)	0.57(0.51-0.59)	0.68(0.66-0.79)	0.83(0.80-0.83)	1.32(1.27-1.61)
Acetabulum diam.	0.07(0.05-0.07)	0.08(0.07-0.09)	0.08(0.08-0.09)	0.12(0.10-0.14)	0.14(0.12-0.15)
Oral Sucker diam.	0.12(0.10-0.13)	0.18(0.17-0.21)	0.24(0.22-0.27)	0.27(0.24-0.29)	0.29(0.27-0.33)
Oral Sucker length	0.11(0.10-0.12)	0.17(0.15-0.19)	0.23(0.20-0.24)	0.25(0.24-0.28)	0.28(0.26-0.30)
O/A Ratio	2.5 (2.3 -2.6)	2.5 (2.4 -2.7)	2.6 (2.4 -2.8)	2.5 (2.3 -2.6)	2.6 (2.5 -2.7)
Ovary width	0.10(0.09-0.10)	0.19(0.16-0.21)	0.24(0.17-0.33)	0.29(0.28-0.34)	0.38(0.34-0.42)
Ovary length	0.11(0.10-0.12)	0.27(0.22-0.32)	0.52(0.39-0.54)	0.56(0.53-0.63)	0.74(0.73-0.77)
Ant. Testis width	0.12(0.07-0.14)	0.25(0.22-0.32)	0.36(0.28-0.39)	0.44(0.43-0.52)	0.55(0.36-0.61)
Ant. Testis length	0.12(0.11-0.18)	0.36(0.30-0.39)	0.65(0.55-0.74)	0.65(0.60-0.71)	1.18(1.00-1.36)
Post. Testis width	0.09(0.08-0.10)	0.24(0.20-0.26)	0.39(0.29-0.47)	0.46(0.38-0.57)	0.55(0.47-0.69)
Post. Testis length	0.15(0.12-0.17)	0.33(0.27-0.34)	0.67(0.55-0.77)	0.80(0.76-0.92)	1.38(1.27-1.48)

All measurements in millimeters.

Appendix 22 Summary of some measurements given in type descriptions.

	H. buttensis Ingles (1936)	*H. similiplexus* Stafford (1902)	*H. similiplexus	*H. varioplexus* Stafford (1902)	*H. floedae* Harwood (1932)
Acetabulum	0.31 (0.24-0.37)	(0.38) (0.41)			
Oral sucker length	0.33(0.18 -0.14)	(0.44) (0.51)			
Oral sucker width	0.46 (0.29-0.47)	(0.41)(0.57)			(3.6-4.4)
O/A ratio	1:0.7 (1:0.6-1.08)	(1.1:1.0) (1.4:1.0)	4:3		3:1
Pharynx length	0.22				
Pharynx width	0.21				
Ovary length	0.44 (0.26-0.55)		(0.29) (0.46)		(0.65-0.83)
Ovary width	0.32 (0.20-0.37)		(0.21) (0.43)		(0.32-0.45)
Ant. testis length			(0.34) (0.48)		(0.7 - 1.1)
Ant. testis width			(0.34) (0.45)		(0.32-0.65)
Post. testis length	0.82 (0.45-1.03)		(0.46) (0.56)		(0.8-1.2)
Post. testis width	0.64 (0.43-0.87)		(0.34) (0.58)		(0.34-0.7)
Body length	7.4 (3.2 -10.0)	(3 - 8)	1.9 (1.8-5.8)	10.5	(4.4 -10.0)
Body width	1.3 (0.7 -2.2)		(0.67-1.96)	2.0	(1.2-1.6)
Egg length	0.027(0.025-0.030)	0.039	0.038(0.034-0.04)	0.029	(0.017-0.021)
Egg width	0.014(0.011 -0.017)	0.019	0.018 (0.017-0.021)	0.018	(0.013-0.018)

*A redescription from Cort, 1915a.

Appendix 22 continued

	H. uniplexus Harwood 1932	H. parviplexus (Irwin 1929)	H. breviplexus Stafford (1902)	*H. breviplexus	H. longiplexus Stafford (1902)
Acetabulum	0.08		0.16		0.23
Oral sucker length					(0.46) (0.38)
Oral sucker width	0.23		0.32	(0.4) (0.28)	(0.46) (0.49)
O/A ratio	1:3	4:1	1:2	2:1	
Pharynx width	0.14			(0.19) (0.195)	
Ovary length		(1.16) (0.79)		1.57	
Ovary width		0.495 (0.314)		0.92	
Ant. testis length	0.5	1.023 (1.04)		1.71	
Ant. testis width	0.16	0.578 (0.495)		1.03	
Post. testis length	0.48	(1.469) (1.02)		2.31	
Post. testis width	0.16	(0.528) (0.512)		1.1	
Body length	4.25	(3.9-8.49	12.0	(5.8) (9.4)	7.0 or 8.0 (15)
Body width	0.7	(0.85-1.6)	(2.0-2.5)	(1.8) (2.74)	About 2.0 (3.0)
Egg length	(0.021-0.017)	0.025(0.023-0.029)	0.022	0.023(0.021-0.026)	0.022
Egg width	(0.017-0.013)	0.017(0.016-0.019)	0.017	0.014(0.013-0.016)	0.017

Appendix 22 continued

	*H. longiplexus	H. complexus Seely (1906)	H. kernensis Ingles (1932)	H. tumidus Ingles (1932)
Acetabulum	0.7	0.38	0.44	0.68
Oral sucker length	0.36		0.44	
Oral sucker width	0.42	0.4		0.61
O/A ratio	(5:2 - 2:1)		1:1 (7:6-15:16)	5:6
Pharynx length	0.22			0.49
Pharynx width	0.18		0.31	
Ovary length	0.85	0.7		0.87
Ovary width	0.72	0.32	0.37 (0.58)	
Ant. testis length	1.08	1.1		
Ant. testis width	0.27	0.92	0.97	0.16
Post. testis length	1.26	1.4		
Post. testis width	0.32	0.92	9.96	0.15
Body length	4.6	(5-8)	6.3 (5.5-7.0)	3.1 (6.4-9.7)
Body width	2.0	1.7	1.54	2.5
Egg length	0.025(0.022-0.087)	0.029	0.030(0.025-0.039)	0.032(0.030-0.036)
Egg width	0.015(0.014-0.017)	0.014	0.016	0.017

Appendix 22 continued

	H. oxyorchis Ingles (1932)	H. confusus Ingles (1932)	*H. coloradensis	H. medioplexus Stafford (1902)	*H. medioplexus
Acetabulum	0.32	0.32	0.7	(0.12) (0.15)	0.08
Oral sucker length		0.44		(0.22)	(0.39) (0.40)
Oral sucker width	0.41	0.46		(0.31) (0.24)	(0.37) (0.35)
O/A ratio	5:4 (7:6-8:5)	4:3 (5:4-9:5)	5:4 (7:6-4:3)		4:1
Pharynx length		0.34			0.29
Pharynx width	0.31	0.28			0.26
Ovary length	0.58	0.40	(0.30 - 0.49)		0.56
Ovary width	0.51	0.19	(0.22 - 0.42)		0.28
Ant. testis length		0.51	(0.42 - 0.64)		0.56
Ant. testis width	0.76	0.44	(0.46 - 0.60)		0.64
Post testis length		0.50	(0.46 - 0.74)		0.64
Post. testis width	0.78	0.42	(0.44 - 0.86)		0.6
Body length	5.8 (3.8-6.5)	3.9 (3.3-4.9)	8.1	11.0	(3.9-7.8)
Body width	0.87	0.9	1.55	1.25	(0.59-1.2)
Egg length	0.027(0.026-0.030) 0.026(0.024-0.029)		0.034(0.032-0.039)	0.028	0.026(0.022-0.029)
Egg width	0.017	0.015	0.020(0.018-0.021)	0.018	0.015(0.013-0.017)

Appendix 23 Known Hosts of Adult Haematoloechus sp. in Canada and the United States

Host	Haematoloechus							
	brevilexus	buttensis	coloradensis	complexus	confusus	floedae	kernensis	longilexus
Ranidae								
Rana aurora							13	
R.aurora draytoni					9		9	
R.blairi			41	41				41
R.boylii		13						
R.catesbeiana	1,4,12,30,37, 38,42.	38				8,15,28, 33.		1,8,11,12,15, 29,32,35,36, 38,41.
R.clamitans	4,23,42.					8		8,21.
R.grylio								15,17.
R.montezume				18				
R.palmipes								
R.palustris								
R.pipiens	1		4,27,29,31, 40,41.	3,4,10,12, 18,22,36, 41.	25			32,36.
R.pretiosa	32(experimental)							32
R.pretiosa Hybrids								
R.sylvatica								
R.septentrionalis								21
R.sphenocephala				3,4,12.				
R.sylvatica								
Bufonidae								
Bufo americanus	4							
B.microscaphus			27,31.					
B.woodhousii			27,31.	41				41
Hylidae								
Hyla chrysoscelis				41				

1.Stafford(1902)
2.Stafford(1905)
3.Seely(1906)
4.Cort(1915)
5.Fortner(1923)
6.Irwin(1929)
7.Krull(1931)
8.Harwood(1932)
9.Ingles(1932)
10.Innes(1933)
11.Cheatwright and Hefley (1934)
12.Brandt(1936)
13.Ingles(1936)
14.Bennett(1938)
15.Manter(1938)
16.Caballero(1941)
17.Parker(1941)
18.Caballero(1942)
19.Rankin(1945)
20.Uribe-Piedrahita(1948)
21.Bouchard(1951)
22.Ollaug(1954)
23.Najarian(1955)
24.Turner(1958)
25.Cheng and Provenza(1960)
26.Cheng(1960)
27.Francsen and Grundman(1960)
28.Loftin(1960)
29.Waitz(1961)
30.Knight, Barbay and Morrison (1965)
31.Farry and Grundman(1965)
32.Scholl(1965)
33.Jacobs and Morrison(1966)
34.Campbell(1968)
35.Clark and Longest(1970)
36.Ulmer(1970)
37.Hollis(1972)
38.Babero and Golling(1974)
39.Cain and French(1975)
40.Bronen(1975)
41.Brooks(1976)
42.Rosen and Manis(1976)
43.Kennedy(unpublished)

Appendix 23 cont. Known Hosts of Adult Haematoloechus sp. in Canada and the United States.

Host	Haematoloechus medioplexus	oxyorchis	parviplexus	similiplexus	tumidus	uniplexus	varioplexus
Ranidae							
Rana aurora					13		
R.aurora draytoni		9,10.			9		
R.blairi	41						41
R.boyli							
R.catesbeiana	39		14,38,41.	1,34,35.			1
R.clamitans	19,21.		6,14,23,29, 43.	21			
R.grylio							
R.montezume	18		16				
R.palmipes	20						
R.palustris	19,21.						
R.pipiens	1,4,5,18,19, 29,36,37,41.			1,4,5,36.			41.
R.pretiosa				24			
R.pretiosa Hybrids							
R.sylvatica							
R.septentrionalis	21			21			
R.sphenocephala						8	
R.sylvatica							23
Bufonidae							
Bufo americanus	1			1,4,36.			
B.microscaphus							
B.woodhousii			41				41
Hylidae							
Hyla chrysoscelis							

1.Stafford(1902)
2.Stafford(1905)
3.Seely(1906)
4.Cort(1915)
5.Fortner(1923)
6.Irwin(1929)
7.Krull(1931)
8.Harwood(1932)
9.Ingles(1932)
10.Ingles(1933)
11.Frowbridge and Hefley (1934)
12.Brandt(1936)
13.Ingles(1936)
14.Bennett(1938)
15.Manter(1938)
16.Caballero(1941)
17.Parker(1941)
18.Caballero(1942)
19.Rankin(1945)
20.Uribe-Piedrahita(1948)
21.Bouchard(1951)
22.Delaug(1954)
23.Najarian(1955)
24.Turner(1958)
25.Cheng and Provenza(1960)
26.Cheng(1960)
27.Frandsen and Grundman(1960)
28.Loftin(1960)
29.Kaitz(1961)
30.Knight,Barbay and Morrison (1965)
31.Parry and Grundman(1965)
32.Schell(1965)
33.Jacobs and Morrison(1966)
34.Campbell(1968)
35.Clark and Longest(1970)
36.Ulmer(1970)
37.Hollis(1972)
38.Babero and Golling(1974)
39.Cain and French(1975)
40.Bronen(1975)
41.Brooks(1976)
42.Rosen and Manis(1976)
43.Kennedy(unpublished)