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INTRASPECIFIC VARIATION AMONG TREMATODES
OF THE GENUS TELORCHIS

by

Jean Leta Watertor

A Dissertation Submitted to the
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Ames, Iowa
1965
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INTRODUCTION

The data presented in this investigation result from researches conducted at Iowa Lakeside Laboratory in northwest Iowa and at Iowa State University, Ames, from 1962 to 1965.

A collection of larval tiger salamanders, *Ambystoma tigrinum* (Green, 1825), in the vicinity of Lake Okoboji during June, 1962 revealed the presence of a trematode species, *Telorchis bonnerensis* Waitz 1960. The definitive hosts as reported by Waitz (1960) are larval long-toed salamanders, *Ambystoma macrodactylum* (Baird, 1849), and a garter snake, *Thamnophis sirtalis* L., collected near Clarksfork, Bonner County, Idaho. Adult and larval *A. tigrinum* found in the same area did not contain the parasite. Schell (1962) described the life history of *T. bonnerensis*, and reported it to be limited to larval *A. macrodactylum*, since his examination of 70 to 80 metamorphosed salamanders of this species collected in Latah County, Idaho indicated the adult amphibian to be completely free from infection. In the Okoboji region, however, both larval and adult *A. tigrinum* serve as definitive hosts for the Iowa strain of *T. bonnerensis*.

This apparent difference in hosts capable of harboring adults of this species suggested the advisability of establishing the life cycle experimentally and of undertaking a complete study of host-parasite relationships of the two strains. Following the successful completion of the life cycle in laboratory-reared hosts, a study was undertaken to observe the effects of varied hosts on the morphology of this species. Furthermore, a related study was conducted concerning the effects of
varied environmental factors on the adults and larval stages developing within a single host species.

Wharton (1940) in an extensive review of the genus Telorchis doubted the validity of many of the species assigned to it because of the variability of certain morphological characters. He indicated the desirability of establishing experimental infections in the laboratory to ascertain their validity. The need for such studies on intraspecific variations in helminths was also emphasized by Haley (1962). The present experimental study on *T. bonnerensis* should contribute considerably to our knowledge of factors important in the delineation of species within the genus, as well as within other genera of trematodes.
HISTORICAL REVIEW

The Genus *Telorchis*

The history and status of the genus *Telorchis* has been reviewed by Stunkard (1915), Dollfus (1920), Wharton (1940), Skrjabin (1963), and other investigators.

This genus was created in 1899 by Lühe to contain several distome trematodes from reptiles and *D. clava* Diesing, 1850, was designated as type species. A description of the genus included the following characteristics: testes lying tandem at the posterior end of the body; elongate cirrus sac opening to the left of the acetabulum; ovary immediately posterior to cirrus sac and separated from the testes by uterine coils; vitelline follicles situated laterally and extending almost to anterior and posterior ends; diverticula of intestine reaching almost to posterior end of the body; anterior end spinose, with exception of one species; excretory vessel long and branched anteriorly; and oral sucker usually slightly larger than acetabulum, though the same size in one species.

According to Wharton (1940), a day after Lühe proposed the genus, Looss also referred to this group of trematodes under the same name, *Telorchis*, choosing *D. linstowi* as type species. Lühe's genus was considered to have priority over that of Looss and both genera were considered synonymous. However, because of differences in the lateral extension of the uterus which coiled over the intestinal diverticula and the lack of an esophagus in *T. clava* as compared to other species in the genus, Lühe, in 1900, created two subgenera: *Telorchis* with *T. clava* as type, and *Cercorchis* with *T. linstowi* as type.
Barker and Covey (1911) added a third subgenus, *Protenes*, in which the cirrus pouch was always anterior to the acetabulum. This subgenus was raised to generic rank by Stunkard (1915) who separated *Protenes* from the genus *Telorchis* on the basis of the "long distance separating the acetabulum and the genital pore, the dorsolateral location of the latter, and the pre-acetabular position of the cirrus sac". *Protenes leptus* was taken as type.

Although the subgenus *Cercorchis* was accepted by Goldberg, in 1911, Stunkard rejected this division because of intergradation with the genus *Telorchis*. In examination of 100 specimens, the uterus was found to be confined between or overlapping the intestinal ceca and the esophagus was long, short or absent. Perkins (1928) again considered *Cercorchis* as a separate genus from *Telorchis* because of differences in location of the genital pore and testes. In *Telorchis*, the genital pore was distant and to the left of the acetabulum and the testes were oblique or nearly tandem, midway between genital pore and posterior end; while *Cercorchis* possessed a genital pore close to and in front of the acetabulum and the testes in more or less strict tandem at the posterior end. Dollfus (1929) accepted Stunkard's decision.

Because of the wide variation in morphological characters within a single species, Wharton (1940) rejected the criteria of Perkins for reinstating the genus *Cercorchis* and also discarded the genus *Paracercorchis*, proposed by Mehra and Bakhari in 1932. He suggested that only *Telorchis* be recognized as a valid genus. Yamaguti (1953) placed all members of the genus *Cercorchis* in *Telorchis*.

Skrjabin (1963, p. 129) described 54 species within the genus.
Telorchis which includes all of those previously identified as members of the genus Cercorchis. Wharton's (1940) key to the genus Telorchis is presented, but Skrjabin considered it unreliable for precise delineation of species within the genus. However, the key is recognized as being of historical interest and may serve as orienting material for differentiation of species known previous to 1940.

Skrjabin (1964), in a key to genera of the subfamily Telorchinae Looss, 1899, separated Telorchis Luhe, 1899, Cercorchis Luhe, 1900, and Paracercorchis Mehra and Bokhari, 1932, as distinct genera.

Adult Telorchis bonnerensis was described by Waitz (1960) and the life cycle delineated by Schell (1962).

Intraspecific Variation

Stunkard (1957) has noted that many taxonomic problems apparent today were of little concern following the publication of Linnaeus' Systema Naturae in 1758, because of the then accepted idea of fixity of species. However, with the general acceptance of the theory of evolution, this concept of fixity of species is no longer tenable.

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 as opposed to interspecific variability present taxonomic problems.

One criterion generally accepted as essential in differentiating between interspecific and intraspecific variation is that the former has a genetic basis. The genetic organization or "gene pool" is responsible
for the most outstanding characteristics of a species. Gene exchange, interbreeding and the presence or absence of reproductive isolation between populations can be studied directly with the methods of genetics and ecology. Such methods are satisfactory for the study of cross-fertilizing organisms, but 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 data. The concept of species, he stated, must be based on a correlation of larval structure, life history, physiology, host relationships, and morphological comparisons of adults.

Wright (1960) disagreed with Stunkard in that he considered acceptance of the "genetic" species to be a more constructive approach toward trematode taxonomy. Evidence against cross-fertilization between hermaphroditic flukes does not exist and digenetic trematodes do occur in actually or potentially interbreeding natural populations limited by the distribution of their molluscan intermediate hosts. The discontinuous distribution of these hosts provides isolated populations where fluke life-cycles may be completed and furnishes the "gene pools" necessary for speciation. The degree of isolation depends upon geographical features, longevity of adult flukes in their definitive hosts, and mobility of these hosts.

In the past, morphology has been the principal standard used by parasitological taxonomists in naming, identifying, and classifying most groups of parasitic organisms. Chandler (1923) noted that 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. According to Simpson (1943), morphology is the expression of genetic constitution. Blackwelder (1962) observed that data from genetics, ecology, parasitism, physiology, and behavior are usually represented in structure at some level. Therefore, such information is used indirectly in the form of correlated structure. The correlation must, however, be established and reported in each case.

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

The study presented below, preliminary abstracts of which were published by Watertor and Ulmer (1964, 1965), contributes additional knowledge to both these aspects of the host-parasite relationship.
Eggs of Telorchis bonnerensis used for experimental feedings were originally obtained from worms in three naturally infected hosts, namely: T. bonnerensis from larval Ambystoma tigrinum collected at Orleans Pond, Spirit Lake, Iowa; T. bonnerensis from larval Ambystoma macrodactylum collected in the vicinity of Moscow, Idaho; and T. corti from adult Chelydra serpentina collected near Farmington, Iowa. All hosts were sacrificed by decapitation; flukes were removed from the small intestine and placed in 0.3 per cent solution of sodium chloride. After a few hours, eggs were released by the worms and fed to laboratory-reared snails.

Following the initial establishment of infection in experimental definitive hosts, eggs were also obtained from feces of such hosts and from dissection of gravid worms found within the intestines.

Fecal material was thoroughly mixed with creek water and filtered through 10 to 15 layers of cheesecloth. With the use of a dissecting microscope and capillary pipette, the eggs could be transferred to small vials for exposure to snails. Eggs for measurement were obtained from fecal material or were shed by flukes placed in creek water.

Laboratory-reared snails, Physa gyrina Say and Physa integra Haldeman, served as first intermediate hosts for T. bonnerensis and T. corti, respectively. Snails reared from eggs were maintained in aquaria containing creek water, and were fed boiled lettuce and dried maple leaves as needed. A mixture of calcium carbonate and sand was added to the aquaria occasionally. Both multiple and single exposures to fluke eggs
were made. In preliminary, uncontrolled experiments, multiple exposures consisted of exposing a single snail to an indefinitely large number of eggs. In controlled temperature experiments, a single snail was exposed to a known number of eggs. In both single and multiple exposures, eggs were transferred to small vials measuring 1/4 mm. in diameter and containing 2 cc. of boiled creek water. A single snail was placed in each vial for a period of 24 hours. Snails were then removed to larger culture dishes for cercarial development, and were isolated again before cercarial emergence (in uncontrolled experiments), or kept isolated in individual vials containing 10 cc. boiled creek water (in controlled experiments).

Studies of cercariae from laboratory-infected snails were made from both living and fixed specimens. For observation of the excretory system, cercariae were relaxed by means of sodium nembutal (50 mg. per. ml. diluted approx. 1:15 with creek water) and refrigerated one or two days prior to study. Measurements were made from cercariae relaxed for 20 to 30 minutes in a dilute solution of nile blue sulfate and then killed and preserved in hot 10 per cent formalin. Cercariae were observed in shallow dishes of creek water for swimming activities and penetration into intermediate hosts.

Laboratory-reared larval A. tigrinum, Rana pipiens tadpoles, collected as eggs from a pond in Ledges State Park, Boone, Iowa, and a variety of laboratory-reared snails were exposed to cercariae in order to obtain metacercariae for study and feeding experiments.

Measurements of encysted metacercariae were made from specimens dissected from experimentally-infected, laboratory-reared snails (P. gyrina) and fixed in 10 per cent formalin. Metacercariae were excysted
by a pepsin-HCl-trypsin digestion technique, fixed in hot AFA, and stained with Mayer's paracarmine. Measurements of such specimens were used in obtaining the initial growth point for temperature studies.

Metacercariae for feeding experiments were obtained by exposing each laboratory-reared snail (P. gyrina) to 40 actively-swimming cercariae. Such cercariae were isolated for 24 hours with a snail in a small vial containing 2 cc. boiled creek water. Twenty-four hours or more later, the entire snail was fed to experimental definitive hosts in order to obtain adult worms.

Experimental definitive hosts in which T. bonnerensis developed included three species of Ambystoma. Larval and adult A. tigrinum were collected in the Lake Okoboji region and laboratory-reared specimens were hatched from eggs collected at Ledges State Park. Adults and eggs of A. macrodactylum were collected near Moscow, Idaho, by Dr. S. C. Schell. Adults of A. maculatum were purchased from a commercial dealer.

Larval forms of A. tigrinum and A. macrodactylum were maintained in aquaria containing creek water under continuous aeration. After hatching, they were fed nauplii of brine shrimp, Artemia salina, and Daphnia sp. Older larvae were fed fresh beef liver and small oligochaetes, Enchytraeus albidus. After metamorphosis, A. tigrinum and A. macrodactylum were fed raw beef with occasional additions of liver or laboratory-reared snails. Adult A. maculatum, apparently unable to tolerate beef or liver, were fed laboratory-reared snails exclusively.

T. bonnerensis and T. corti developed in three experimental chelonian definitive hosts, Pseudemys scripta elegans, Chrysemys picta belli, and Chelydra serpentina. These hosts were purchased from commercial dealers.
and kept in laboratory aquaria on a diet of beef.

All commercially purchased and naturally collected hosts were main­
tained in the laboratory at least one month before use for experimental purposes. Fecal material was examined for eggs to determine the presence of previous infections. In subsequent feeding experiments with such hosts, only those showing no evidence of previous infection were used in compiling data on adult worms recovered.

Experimental worms recovered from intestinal tracts of hosts were placed in creek water for a short period until sluggish, then straightened on a slide and fixed with AFA without pressure. Whole mounts were stained with Mayer's paracarmine or Harris' hematoxylin and fast green and eosin were used as counterstains. Sections prepared from materials fixed in Bouins, were stained with Harris' hematoxylin and eosin.

Drawings were made with the use of a Leitz microprojector.
SUMMARY OF LIFE CYCLE

The life cycle of *Telorchis bonnerensis*, an intestinal parasite of larval *Ambystoma macrodactylum*, and larval and adult *Ambystoma tigrinum*, involves *Physa gyrina*, *P. propinqua*, and *P. ampullacea* as first intermediate hosts. Embryonated eggs of this species hatch upon ingestion by the molluscan host, and miracidia penetrate the intestinal wall to enter the hemocoel. Here they metamorphose to mother sporocysts, long filamentous structures producing numerous daughter sporocysts.

Daughter sporocysts migrate to various internal organs of the snail, grow rapidly, and produce xiphidiocercariae. Cercariae emerge primarily at night, swim weakly, and penetrate and encyst within a variety of snails, fingernail clams, tadpoles, and salamander larvae. Metacercariae in snails are infective within 12 hours and, upon ingestion of the second intermediate host by the definitive host, the parasites develop to sexual maturity in the anterior small intestine.
ADDITIONAL LIFE CYCLE DATA

Eggs

Viability

Eggs of *Telorchis bonnerensis* are embryonated when shed, but will not hatch if stored in 0.3 per cent saline solution, according to Schell (1962). In the present study, eggs of 8-week *T. bonnerensis*, Idaho strain, maturing in laboratory-reared, adult *Ambystoma tigrinum* remained viable for eight months when maintained at approximately 6°C. in 0.3 per cent saline. Such eggs when ingested by laboratory-reared snails (*Physa gyrina*) proved viable as indicated by cercarial emergence.

Eggs of 48-week *T. bonnerensis*, Iowa strain, maturing in adult *A. tigrinum*, were viable when maintained at approximately 22°C. for one week in creek water before ingestion by snails. Exposure of snails to 48-week eggs at two and three weeks under similar conditions did not produce infection. Eggs of 78-week flukes ingested by snails within 24 hours after being shed into creek water did not result in infection of the snail host.

The viability of *T. bonnerensis* eggs is lengthened by storing them in 0.3 per cent saline at a lowered temperature, whereas maintenance of the eggs in creek water at room temperature, and advanced age of the worms decrease egg viability.

Size

Size of eggs is a characteristic frequently used to distinguish species in the genus *Telorchis*. Comparative measurements of 20 eggs
from the two strains of *T. bonnerensis* obtained from experimentally-infected hosts are given in Table 1.

Table 1. Egg size (in mm.) of Iowa and Idaho strains of *Telorchis bonnerensis* when adults mature in *Ambystoma tigrinum* and *Ambystoma macrodactylum*

<table>
<thead>
<tr>
<th>Definitive host</th>
<th>Iowa strain</th>
<th>Idaho strain</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. macrodactylum</em></td>
<td>0.038 (0.032 to 0.045) by 0.019 (0.016 to 0.019)</td>
<td>0.042 (0.039 to 0.044) by 0.019 (0.018 to 0.021)<em>a</em></td>
</tr>
<tr>
<td><em>A. tigrinum</em></td>
<td>0.040 (0.038 to 0.043) by 0.017 (0.016 to 0.019)</td>
<td>0.038 (0.032 to 0.045) by 0.017 (0.016 to 0.019)</td>
</tr>
</tbody>
</table>

*a*Measurements of eggs according to Waitz (1960).

These measurements indicate that egg size is slightly decreased when adult flukes mature in the unaccustomed definitive host.

**Cercariae**

**Emergence from host**

Schell (1962) noted that most cercariae of *Telorchis bonnerensis* emerge at night. A similar activity of cercariae was observed in the present study. This periodicity could be reversed by placing the snail host in light at night and transferring it to the dark during the day. When the snail host was kept in light for 48 hours, and then transferred to the dark, massive emergence of cercariae occurred within the first hour.
Two laboratory-reared, experimentally-infected snails (Physa gyrina) that had ingested a single egg of T. bonnerensis shed, respectively, 6,081 cercariae in 52 days (daily average 117) and 6,276 cercariae in 48 days (daily average 131). The snails were approximately the same size and were maintained under similar conditions of temperature and availability of food. The largest number of cercariae emerging from a naturally-infected snail (P. gyrina) was 4,192 in 15 days (daily average 299).

The longevity of experimentally-infected, laboratory-reared snail hosts varied from one day to more than nine months. One snail, after producing cercariae for nine months, lived two weeks after cercarial emergence ceased.

Size

The measurements of T. bonnerensis cercariae as reported by Schell (1962) in comparison to measurements obtained in the present study of Iowa and Idaho strains are given in Table 2.

Table 2. Average measurements (in microns) of Iowa and Idaho strains of Telorchis bonnerensis cercariae

<table>
<thead>
<tr>
<th></th>
<th>Idaho^a strain</th>
<th>Idaho strain</th>
<th>Iowa strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of specimens</td>
<td>Not stated</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Body length</td>
<td>180-190</td>
<td>182-208</td>
<td>182-228</td>
</tr>
<tr>
<td>Body width</td>
<td>93-97</td>
<td>76-106</td>
<td>76-91</td>
</tr>
<tr>
<td>Oral sucker diam.</td>
<td>42-43</td>
<td>42-43</td>
<td>35-48</td>
</tr>
<tr>
<td>Pharynx diam.</td>
<td>16-18</td>
<td>14-15</td>
<td>16-20</td>
</tr>
<tr>
<td>Stylet length</td>
<td>21-21</td>
<td>21-22</td>
<td>16-18</td>
</tr>
<tr>
<td>Stylet width</td>
<td>3.6</td>
<td>3.8</td>
<td>3.2</td>
</tr>
</tbody>
</table>

^aMeasurements of cercariae according to Schell (1962).
From the foregoing measurements, it is apparent that considerable variation in size occurs among experimentally-reared cercariae of _T. bonnerensis_ emerging from one snail host during a 24-hour period and also between cercariae of the two strains.

**Excretory system**

The excretory system of _T. bonnerensis_ cercariae is of the reniferid type. According to Schell (1962), the flame cell formula is $2(3 + 3 + 3) + (3 + 3 + 3)$. In the present study, a comparison of the excretory systems of the Iowa and Idaho strains of _T. bonnerensis_ revealed no differences.

**Metacercariae**

**Encystment in host**

Schell (1962) found that several species of snails, fingernail clams, tadpoles and salamander larvae serve as second intermediate hosts for _Telorchis bonnerensis_. In the present investigation, infective metacercariae developed when cercariae encysted in laboratory-reared snails (_Physa gyrina_, _P. integra_, _Lymnea stagnalis_, _Stagnicola reflexa_, _Helisoma_ sp., and _Valvata tricarinata_), tadpoles (_Rana pipiens_) and salamander larvae (_Ambystoma tigrinum_).

When large numbers of metacercariae are present in snails (_P. gyrina_), they may be found throughout the soft tissues, while small numbers are usually encysted in the anterior region of the foot.

**Viability**

Metacercariae were infective for the definitive host within 24 hours.
following encystment according to Schell (1962), when maintained at a temperature of 18 to 22°C. In the present study, metacercariae were infective within 12 hours following encystment in laboratory-reared snails (P. gyrina) when maintained at approximately 22°C. Such metacercariae remained infective as long as nine months, thereby providing a probable means of carrying the infection through the winter.

Size

The diameters of 10 metacercariae (Iowa strain) encysted in a laboratory-reared snail (P. gyrina) varied from 122 to 144 microns as compared to 110 to 128 microns reported by Schell (1962) for diameters of the metacercariae (Idaho strain).

Adults

Incidence of infection

According to Schell (1962), infected Ambystoma macrodactylum larvae collected near Potlatch, Idaho, harbored from one to 72 specimens of Telorchis bonnerensis.

Prior to 1962, T. bonnerensis had not been found in the Lake Okoboji region. Of 90 larval and recently metamorphosed adult A. tigrinum collected during July and August of that year, 30 per cent were infected. Such salamanders harbored from one to 56 worms, with an average number of 5.5. The following year, 34 larval A. tigrinum collected during June were 100 per cent infected, and harbored from one to 179 flukes with an average of 32.5. The largest number of worms obtained from a naturally infected, larval A. tigrinum was 321 collected in July, 1965.
Location within definitive host

Schell (1962) reported *T. bonnerensis* to be found in the anterior part of the intestine of larval *A. macrodactylum*. The present investigation indicated a similar location of adult worms in larval and adult *A. tigrinum* and in all experimentally infected chelonian hosts (*Pseudemys scripta elegans, Chrysemys picta belli, Chelydra serpentina*). However, when large numbers of worms occur, they are distributed along the entire length of the intestine.

Individual flukes may lie free in the intestinal lumen, may be attached to the mucosa, or to another fluke as indicated by Figure 1.

Pathology

The presence of *T. bonnerensis* in the intestine of experimentally-infected amphibian and chelonian hosts appears to have no detrimental effects on such hosts. A slight enlargement of the liver was apparent in adult *A. tigrinum* harboring 200 worms or more and some erosion of the intestinal mucosa occurred when the worm burden reached four or five hundred. However, during the relatively short periods of infection before autopsy, such animals ate normally and appeared to be healthy. No mortality due to unusually heavy infections occurred in amphibian or chelonian hosts.

Definitive Hosts

Natural infections

Specimens of *Telorchis bonnerensis* found by Waitz (1960) were recovered from larval *Ambystoma macrodactylum* collected near Clarksfork,
Bonner County, Idaho. However, T. bonnerensis was not found in adult A. macrodactylum or in four other species of salamanders collected in the same area. These included adult rough skinned newts, Taricha granulosa (Skilton, 1849), adult and larval Ambystoma tigrinum (Green, 1825), adult and larval Pacific giant salamanders, Dicamptodon ensatus (Eschscholtz, 1833) and adult Washington salamanders, Plethodon vandykei idahoensis (Slater and Slip, 1940). Specimens were also obtained from one garter snake, Thamnophis sirtalis.

In the present investigation, gravid adults of T. bonnerensis were found in natural infections of larval and recently metamorphosed adult A. tigrinum collected in the Lake Okoboji region of northwest Iowa. This constitutes a new host and locality record for the species.

Experimental infections

All stages of T. bonnerensis are easily reared under laboratory conditions. Worms used as a source for experimental studies were obtained from adult A. tigrinum collected in Iowa, and larval A. macrodactylum collected in Idaho. Laboratory-reared Physa gyrina were experimentally infected by exposing them to eggs of such adults and all subsequent experimental data were derived from their progeny maintained under laboratory conditions.

Cross-feeding experiments were carried out in which metacercariae from the Iowa strain were infective for laboratory-reared larval A. macrodactylum and metacercariae from the Idaho strain were infective for laboratory-reared adult and larval A. tigrinum. Gravid worms were obtained from all of these experimentally infected hosts as shown in
Figures 2 to 5. Metacercariae from neither the Iowa strain nor the Idaho strain were infective for adult *A. macrodactylum*. The presence of *T. bonnerensis* in larval and adult *A. tigrinum* is at variance with findings of Waitz (1960) who reported that the Idaho strain does not occur in these hosts.

Additional experimentally-infected hosts in which gravid worms of both the Iowa and Idaho strains developed include adult spotted salamanders (*A. maculatum*), and two chelonian hosts, young, red-eared turtles (*Pseudemys scripta elegans*), and painted turtles (*Chrysemys picta belli*). Mature specimens of both strains were obtained from young snappers (*Chelydra serpentina*). Metacercariae of both strains were not infective for tadpoles (*Rana pipiens*), adult African toads (*Xenopus sp.*), and garter snakes (*T. sirtalis*).

Table 3 summarizes the feeding experiments conducted during the course of this investigation. A discussion of morphological variations in adult *T. bonnerensis*, as well as variations in rate of development in various definitive hosts is presented in a later section of this thesis.
Table 3. Summary of feeding experiments involving Iowa and Idaho strains of *Telorchis bonnerensis* in various amphibian and reptilian hosts

<table>
<thead>
<tr>
<th>Strain</th>
<th>Host</th>
<th>Number exposed</th>
<th>Number infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa</td>
<td><em>Ambystoma tigrinum</em> (adults)</td>
<td>31</td>
<td>27</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>A. tigrinum</em> (larvae)</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>A. tigrinum</em> (adults)</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>A. tigrinum</em> (larvae)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>Ambystoma macrodactylum</em> (adults)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>A. macrodactylum</em> (larvae)</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>A. macrodactylum</em> (adult)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>A. macrodactylum</em> (larvae)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>Ambystoma maculatum</em> (adults)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>A. maculatum</em> (adults)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>Rana pipiens</em> (tadpoles)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>R. pipiens</em> (tadpoles)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>Xenopus sp.</em> (adult)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>X. sp.</em> (adult)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>Pseudemys scripta elegans</em></td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>P. s. elegans</em></td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>Chrysemys picta belli</em></td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>C. p. belli</em></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>Chelydra serpentina</em></td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>C. serpentina</em></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Iowa</td>
<td><em>Thamnophis sirtalis</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Idaho</td>
<td><em>T. sirtalis</em></td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
INTRASPECIFIC VARIATIONS OF LARVAL STAGES

Effects of Temperature Stress on Cercarial Development

The developmental rate of trematode larvae is known to be related directly to the temperature at which molluscan intermediate hosts are maintained. Confirmatory evidence of this was demonstrated during the course of this investigation as a result of numerous experiments involving laboratory-reared snails experimentally infected with *Telorchis bonnerensis*.

Twenty-two preliminary experiments were conducted in which 112 laboratory-reared *Physa gyrina* (varying in size from 2 to 8 mm.) were exposed to eggs of *T. bonnerensis*. In these experiments, carried out at room temperature, 111 snails received multiple exposures and 31 were exposed to one egg each. Forty-nine of the multiply-exposed snails became infected, while none of the single exposures resulted in larval production. Smaller snails (2 to 3 mm.) seem unable to tolerate large numbers of eggs, as indicated by the death of such snails before emergence of cercariae. Larger snails (6 to 8 mm.) exposed to a similar number of eggs survived to produce cercariae. The first emergence of cercariae from such experimentally infected snails varied from 17 to 45 days under laboratory conditions. Schell (1962) illustrated a 37-day daughter sporocyst containing a well-developed cercaria, but did not indicate when cercarial emergence began. Snails in Schell's investigation were maintained at temperatures ranging from 18 to 22°C. He indicated that larval development is greatly accelerated by small increases in temperature.
In order to determine more precisely the effects of varying temperatures on cercarial development, additional experiments were carried out in which 220 snails were maintained under controlled temperatures of approximately 4, 10, 22, 30, and 37°C. (44 snails at each temperature). One-hundred ten snails were exposed to 10 to 20 eggs per snail and 110 to 1 egg each. Twenty of the former developed infections while none of the singly-exposed snails showed evidence of infection. Results of this experiment are summarized in Table 4, which includes only the 20 infected snails.

At temperatures of 4°C and 10°C., cercarial production was greatly inhibited, for even after 8½ days at 4°C. and as long as 183 days at 10°C. cercariae failed to emerge. The time required for cercarial emergence at 30°C. was approximately one week less than the time required for appearance of cercariae when snails were maintained at 22°C. The number of snails in which infections developed to cercarial production increased from 1 snail at 4°C. to 8 snails at 30°C. No difference in developmental time was found between single and multiply-exposed snails, or between large and small snails. However, 13 small snails became infected as compared to 7 large snails suggesting a greater resistance to infection on the part of the latter.

That suppression of cercarial development resulting from maintenance of snails at lowered temperature may be modified was shown in a subsequent series of experiments as indicated in Table 5. In these experimental snails maintained at temperatures of 4°C and 10°C., no cercariae emerged even after 183 days. A transfer of these snails to a temperature of 30°C., however, resulted in cercarial emergence within as little as
Table 4. Effect of temperature on cercarial development

<table>
<thead>
<tr>
<th>Type of exposure&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Size of snail</th>
<th>Temperature</th>
<th>First appearance of cercariae (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>4°C.</td>
<td>None in 84</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>10°C.</td>
<td>None in 84</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>10°C.</td>
<td>None in 86</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>10°C.</td>
<td>None in 183</td>
</tr>
<tr>
<td>M</td>
<td>6-8 mm.</td>
<td>10°C.</td>
<td>None in 152</td>
</tr>
<tr>
<td>M</td>
<td>6-8 mm.</td>
<td>10°C.</td>
<td>None in 152</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>22°C.</td>
<td>23</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>22°C.</td>
<td>46</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>22°C.</td>
<td>27</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>22°C.</td>
<td>27</td>
</tr>
<tr>
<td>M</td>
<td>6-8 mm.</td>
<td>22°C.</td>
<td>36</td>
</tr>
<tr>
<td>M</td>
<td>6-8 mm.</td>
<td>22°C.</td>
<td>27</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>30°C.</td>
<td>20</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>30°C.</td>
<td>20</td>
</tr>
<tr>
<td>M</td>
<td>2-4 mm.</td>
<td>30°C.</td>
<td>17</td>
</tr>
<tr>
<td>S</td>
<td>2-4 mm.</td>
<td>30°C.</td>
<td>17</td>
</tr>
<tr>
<td>S</td>
<td>2-4 mm.</td>
<td>30°C.</td>
<td>18</td>
</tr>
<tr>
<td>M</td>
<td>6-8 mm.</td>
<td>30°C.</td>
<td>20</td>
</tr>
<tr>
<td>M</td>
<td>6-8 mm.</td>
<td>30°C.</td>
<td>18</td>
</tr>
<tr>
<td>M</td>
<td>6-8 mm.</td>
<td>30°C.</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>a</sup>M = Multiple exposure of snail to eggs; S = Single exposure of snail to one egg.
Table 5. Effect of altered temperature on cercarial development (all snails multiply exposed)

<table>
<thead>
<tr>
<th>Size of snail</th>
<th>Original temperature</th>
<th>Days at original temperature</th>
<th>No. of cercariae emerging</th>
<th>Altered temperature</th>
<th>Days at altered temperature before cercarial emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4 mm.</td>
<td>4°C.</td>
<td>86</td>
<td>0</td>
<td>30°C.</td>
<td>16</td>
</tr>
<tr>
<td>2-4 mm.</td>
<td>10°C.</td>
<td>86</td>
<td>0</td>
<td>30°C.</td>
<td>13</td>
</tr>
<tr>
<td>2-4 mm.</td>
<td>10°C.</td>
<td>86</td>
<td>0</td>
<td>30°C.</td>
<td>13</td>
</tr>
<tr>
<td>2-4 mm.</td>
<td>10°C.</td>
<td>183</td>
<td>0</td>
<td>30°C.</td>
<td>3</td>
</tr>
<tr>
<td>6-8 mm.</td>
<td>10°C.</td>
<td>152</td>
<td>0</td>
<td>30°C.</td>
<td>3</td>
</tr>
<tr>
<td>6-8 mm.</td>
<td>10°C.</td>
<td>152</td>
<td>0</td>
<td>30°C.</td>
<td>3</td>
</tr>
</tbody>
</table>
three days. Of interest too, was the finding that those snails maintained for longer periods at the lowered temperature, required fewer days at higher temperature before cercarial emergence started. These results present evidence for the opinion of Schell (1962) that sporocyst infections may be carried through the winter.

At 37°C, snail mortality was greatly increased. Only three exposed snails and none of the controls survived for a period of 21 days. No cercariae had emerged from one exposed snail which lived 26 days post infection.

Cercarial development and growth of the snail host was optimal at 30°C. At 40°C and 10°C, snail growth rate was greatly decreased, but survival time was lengthened. No differences were noted in total growth between infected and non-infected snails during the period of cercarial development.

Cercariae developed at each temperature produced viable metacercariae encysted in laboratory-reared Physa gyrina, but no attempt was made to determine whether infectivity was increased or decreased at each respective temperature.

The results obtained in these experiments agree, essentially, with those of Stirewalt (1954) involving Australorbis glabratus, the intermediate host of Schistosoma mansoni, and Gumble et al. (1957) on Oncomelania nosophora, the intermediate host of S. japonicum. Stirewalt indicated temperatures between 26 and 33°C, to be optimal for larval development. Furthermore, her experiments and those on T. bonnerensis, reported in this investigation, show that temperatures above 33°C increase snail mortality while lower temperatures lengthen the
developmental period, decrease the proportion of snails in which infec-
tions developed to cercarial emergence, and arrest cercarial production. Gumble found that only a few cercariae of S. japonicum emerged from the snail host at 10°C. Cercariae of T. bonnerensis are similarly affected.

Effects of Temperature Stress on Metacercarial Development

Temperature is known to effect the viability of cercariae, to alter their subsequent development and to influence the infectivity of the metacercariae.

Hoffman (1958) found that cercariae of Posthodiplostomum minimum did not infect fish at 15°C, but were infective at 18 to 28°C. Metacercariae developed but slightly in fish exposed at room temperature and subsequently maintained at 15 to 18°C for 19 days. Colley and Olson (1963) reported the greatest per cent survival when metacercariae of P. minimum in naturally infected fish were maintained for 48 hours at 15 to 30°C. Schell (1962) reported metacercariae of Telorchis bonnerensis to be infective within 24 hours at 18 to 22°C.

The present study was undertaken to determine the effects of varying temperatures on development of T. bonnerensis metacercariae as indicated by their degree of infectivity in the larval and adult definitive host, Ambystoma tigrinum.

Laboratory-reared snails (Physa gyrina) were each exposed to approximately 40 cercariae shed by one experimentally-infected, laboratory-reared P. gyrina which had been maintained at 22°C. Cercariae from this snail and from the P. gyrina to which they were to be exposed were maintained at temperatures of 10°C, 22°C, and 30°C, respectively,
six hours before exposure. After exposure, each snail remained at the
same temperature for a varying time period before being fed to larval or
adult *A. tigrinum*. Following exposure, hosts were maintained at 22°C.
Larval hosts were autopsied after three weeks and adults after four
weeks. Results of 11 snail exposures are summarized in Table 6.

Table 6. Effects of varying temperatures on development of *Telorchis bonnerensis* metacercariae in *Ambystoma tigrinum*

<table>
<thead>
<tr>
<th>Stage of definitive host</th>
<th>Temperature</th>
<th>Approximate no. of metacercariae fed</th>
<th>Developmental period (hours)</th>
<th>No. of worms recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>10°C</td>
<td>40</td>
<td>51</td>
<td>11</td>
</tr>
<tr>
<td>Larva</td>
<td>22°C</td>
<td>40</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Larva</td>
<td>22°C</td>
<td>40</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Larva</td>
<td>22°C</td>
<td>40</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>Larva</td>
<td>22°C</td>
<td>40</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>Adult</td>
<td>22°C</td>
<td>40</td>
<td>24+</td>
<td>18</td>
</tr>
<tr>
<td>Adult</td>
<td>22°C</td>
<td>40</td>
<td>24+</td>
<td>34</td>
</tr>
<tr>
<td>Adult</td>
<td>22°C</td>
<td>40</td>
<td>24+</td>
<td>34</td>
</tr>
<tr>
<td>Adult</td>
<td>22°C</td>
<td>40</td>
<td>24+</td>
<td>32</td>
</tr>
<tr>
<td>Adult</td>
<td>30°C</td>
<td>40</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Adult</td>
<td>30°C</td>
<td>40</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>
Infective metacercariae developed in snails within 51 hours at 10°C. Whether they developed earlier than this was not ascertained. At 22°C, metacercariae developed to the infective stage by 12 hours, but at 6 hours, metacercariae at this temperature did not develop to maturity when fed to definitive hosts. Infective metacercariae did develop within 6 hours at 30°C, in one exposure only. The highest number of worms were recovered from hosts having been exposed to metacercariae developing for 24 hours or more.

Although *T. bonnerensis* metacercariae are able to develop within 6 hours at 30°C., the temperature and time period producing the highest worm recovery appears to be 22°C, for 24 hours or more.
INTRASPECIFIC VARIATIONS OF ADULTS
FROM HOSTS OF THE SAME SPECIES

Morphological Variations of Adults Resulting from Age

It is well known that growth of helminths may continue after they have attained sexual maturity. Waitz (1960) and Schell (1962) reported that Telorchis bonnerensis grows considerably after becoming sexually mature in Ambystoma macrodactylum. In the present investigation, too, such growth was apparent in experimental studies on T. bonnerensis developing in adult A. tigrinum. Stunkard (1957) has suggested this continuing growth as a major difficulty in the delineation of species of parasitic flatworms.

Morphological characteristics considered to be significant in the determination of species within the genus Telorchis include the following: extent of vitellaria, position of ovary, relative lengths of cirrus sac and metraterm, relation of cirrus sac to ovary, size of suckers, position of acetabulum, comparative lengths of pharynx and esophagus, extent of intestinal crura, and size of eggs.

During the course of this investigation, experimentally infected adult A. tigrinum provided 43 specimens of T. bonnerensis of varying ages. With such experimentally developed worms, morphological variations related to age of the flukes could be thoroughly investigated. Comparative measurements and illustrations of such specimens are presented in Table 7, and Figures 6 to 15. Brief discussions of individual morphological features used by Wharton (1940) in his key to species of the genus, and by Waitz (1960) in his description of T. bonnerensis are
Table 7. Average measurements (in mm.) of experimentally-reared Telorchis bonnerensis at various ages

<table>
<thead>
<tr>
<th>Age (in weeks)</th>
<th>14</th>
<th>18</th>
<th>78</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of specimens</td>
<td>15</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Body length</td>
<td>3.89 (2.82-4.62)</td>
<td>5.19 (4.68-6.15)</td>
<td>5.30 (3.95-6.20)</td>
</tr>
<tr>
<td>Body width</td>
<td>0.54 (0.45-0.62)</td>
<td>0.69 (0.62-0.79)</td>
<td>0.59 (0.51-0.68)</td>
</tr>
<tr>
<td>Oral sucker diam.</td>
<td>0.12 (0.11-0.15)</td>
<td>0.17 (0.15-0.18)</td>
<td>0.18 (0.16-0.20)</td>
</tr>
<tr>
<td>Acetabulum diam.</td>
<td>0.13 (0.11-0.15)</td>
<td>0.16 (0.15-0.17)</td>
<td>0.17 (0.15-0.18)</td>
</tr>
<tr>
<td>Pharynx length</td>
<td>0.07 (0.06-0.08)</td>
<td>0.09 (0.08-0.09)</td>
<td>0.09 (0.08-0.09)</td>
</tr>
<tr>
<td>Esophagus length</td>
<td>0.08 (0.05-0.11)</td>
<td>0.11 (0.06-0.15)</td>
<td>0.07 (0.05-0.15)</td>
</tr>
<tr>
<td>Metraterm length</td>
<td>0.32 (0.27-0.38)</td>
<td>0.11 (0.38-0.46)</td>
<td>0.35 (0.26-0.44)</td>
</tr>
<tr>
<td>Cirrus sac length</td>
<td>0.73 (0.61-0.81)</td>
<td>0.91 (0.82-1.10)</td>
<td>0.71 (0.53-0.85)</td>
</tr>
<tr>
<td>Ovary diam.</td>
<td>0.21 (0.18-0.24)</td>
<td>0.22 (0.20-0.24)</td>
<td>0.17 (0.14-0.20)</td>
</tr>
<tr>
<td>Ovary to ant. end&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.32 (1.13-1.52)</td>
<td>1.72 (1.58-1.97)</td>
<td>1.55 (1.18-1.86)</td>
</tr>
<tr>
<td>Ovary to ant. testis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.96 (1.13-2.18)</td>
<td>2.54 (2.31-2.76)</td>
<td>2.24 (1.75-3.10)</td>
</tr>
<tr>
<td>Acetabulum to ovary&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.35 (0.22-0.45)</td>
<td>0.61 (0.49-0.85)</td>
<td>0.46 (0.30-0.67)</td>
</tr>
<tr>
<td>Acetabulum to ant. end&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.98 (0.87-1.10)</td>
<td>1.12 (1.09-1.16)</td>
<td>1.74 (1.22-1.99)</td>
</tr>
<tr>
<td>Distance between testes</td>
<td>0.01 (0.00-0.23)</td>
<td>0.18 (0.11-0.24)</td>
<td>0.14 (0.07-0.18)</td>
</tr>
<tr>
<td>Testis to post. end&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.11 (0.06-0.20)</td>
<td>0.30 (0.18-0.40)</td>
<td>0.66 (0.06-0.85)</td>
</tr>
<tr>
<td>Length ant. testis</td>
<td>0.31 (0.26-0.36)</td>
<td>0.25 (0.23-0.27)</td>
<td>0.21 (0.17-0.26)</td>
</tr>
<tr>
<td>Vitellaria to testis&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.51 (0.17-0.82)</td>
<td>0.57 (0.36-0.91)</td>
<td>0.67 (0.15-1.37)</td>
</tr>
<tr>
<td>Total length vitellaria</td>
<td>2.13 (1.41-2.50)</td>
<td>3.02 (2.13-3.41)</td>
<td>2.60 (2.00-3.23)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Distance between posterior margin of ovary and anterior end of body.
<sup>b</sup>Distance between posterior margin of ovary and anterior margin of anterior testis.
<sup>c</sup>Distance between posterior margin of acetabulum and anterior margin of ovary.
<sup>d</sup>Distance between anterior margin of acetabulum and anterior end of body.
<sup>e</sup>Distance between posterior margin of posterior testis and posterior end of body.
<sup>f</sup>Distance between posterior extent of vitellaria and anterior margin of anterior testis.
presented below.

**Extent of vitellaria**

*Type description*  "Vitellaria in ungrouped follicles, mostly extra-cecal, beginning near anterior margin of acetabulum and stopping short of anterior testis by approximately twice its length," (Waitz, 1960).

*Experimental results*  The anterior extent of vitellaria, although more than one ovarian diameter anterior to the ovary in all specimens, varied considerably with relation to the acetabulum in the three age groups studied (Figures 6 to 9). Variation from 0.22 mm. anterior to 0.24 mm. posterior to the center of the acetabulum occurred. Variation was found between right and left sides of the same specimen with follicles sometimes anterior on one side and posterior on the other, anterior on both sides, or posterior on both sides. In 4-week, experimental specimens, 53% showed vitellaria extending anteriad on both sides as compared to 7% in 18-week and 15% in 78-week specimens. Vitelline follicles extended posterior to the acetabulum on both sides in 13% of 4-week specimens, 40% in 18-week and 15% in 78-week specimens. Thus, within the range given above, there appears to be no particular pattern in anterior distribution of vitelline follicles with relation to the center of the acetabulum.

Posteriorly, vitellaria terminate short of the anterior testis by 1 1/2 times the length of that organ in 4-week specimens, 2 times its length in 18-week specimens and 3 times its length in 78-week specimens. The total length of vitelline follicles, in comparison to total body
length, decreases with advanced age, being 54% in 4-week, 56% in 18-week, and 48% in 78-week worms. With increasing age, then, there appears to be a corresponding increase in distance of vitellaria from the anterior testis, probably a result of decreasing diameter of the testis, decreasing total length of the follicles, and increasing growth of the posterior region of the flukes.

**Position of ovary**

**Type description** "Ovary just within anterior one-third of body length," (Waitz, 1960).

**Experimental results** In all specimens studied, the ovary was anterior to the middle of the body and always nearer to the acetabulum than to the testis.

In 4-week, 18-week, and 78-week specimens, the ovary was within the anterior one-third of the body length in 40%, 80%, and 100% of the specimens, respectively. In younger specimens, then, and in some older worms, the relationship between ovary and this region of the body is extremely variable.

The ovary was located two ovarian diameters or less behind the acetabulum in 100% of the 4-week specimens, but more than two diameters in 100% of the 18-week and 78-week specimens. In 20% of the 18-week and 38% of the 78-week specimens, this organ was more than three diameters from the acetabulum. The increase in the 78-week specimens is partially due to the decrease in diameter of the ovary, but it is apparent that the position of the female gonad in relation to the acetabulum is subject to considerable variation.
The distance from the ovary to the anterior testis varied within 0.2 mm. of one-half the total body length in 70% of the specimens regardless of age. The remaining 30% varied as much as 0.7 mm. from this point. No pattern could be found between the three age groups making this measurement a more reliable taxonomic character.

**Relative lengths of cirrus sac and metraterm**

**Type description**  "Metraterm approximately one-half the length of cirrus sac," (Waitz, 1960).

**Experimental results**  The metraterm was approximately one-half the length of cirrus sac in all specimens regardless of age. Although the metraterm was often difficult to measure accurately because of the presence of large numbers of eggs, its relative length appears to be a stable taxonomic character.

**Relation of cirrus sac to ovary**

**Type description**  "Cirrus sac extending to the anterior edge of the ovary," (Waitz, 1960).

**Experimental results**  In all specimens of the three age groups, the cirrus sac extended to within one ovarian diameter of the anterior margin of the ovary. In 7% of 4-week and 20% of 18-week specimens, the cirrus sac extended to the anterior edge of this organ. The cirrus sac extended (within 0.2 mm.) beyond the anterior margin in 93%, 50%, and 62% of the 4-week, 18-week, and 78-week worms, respectively. This structure terminated (within 0.12 mm.) short of the ovary in 30% of the 18-week and 38% of the 78-week flukes. The relation of cirrus sac to ovary is, therefore, subject to considerable variation (Figures 10 to 12).
Size of suckers  
Type description  "Acetabulum round, always smaller than oral sucker," (Waitz, 1960).  
Experimental results  The acetabulum was smaller than the oral sucker in 81% of the worms studied and the same size in 19%. Because of the muscular structure of these organs, they are less subject to modifications due to pressure, fixation, etc., and hence provide a rather reliable taxonomic criterion.

Position of acetabulum  
Type description  "Acetabulum median or slightly to one side," (Waitz, 1960).  
Experimental results  In all worms studied, the acetabulum was median and always closer to the ovary than the anterior end. Its position appears to be a stable characteristic.

Comparative lengths of pharynx and esophagus  
Type description  "Esophagus longer than pharynx," (Waitz, 1960).  
Experimental results  The esophagus was shorter than the pharynx in 27%, 20%, and 67%, and longer than the pharynx in 33%, 73%, and 7%, of the 4-week, 18-week, and 78-week worms, respectively. In remaining worms, lengths of esophagus and pharynx were equal. With increasing age, then, esophageal length decreases in comparison to pharyngeal length.

Extent of intestinal crura  
Type description  "Intestinal ceca extending to the posterior end of the body," (Waitz, 1960).
Experimental results  The posterior extent of the intestinal crura varied from the intertesticular zone to the posterior end of the body (Figures 13 to 15). Variation was found between right and left sides of the same specimen, crura sometimes ending in the intertesticular zone on one side and extending to the posterior end of the body on the other side. Crura ended anterior to the posterior margin of the posterior testis in 67%, 13% and 0% of the 4-week, 18-week, and 78-week worms, respectively. That portion of the body posterior to the testes lengthens considerably in the 78-week flukes, making the extent of the crura past the posterior margin of the testis much greater in these specimens. This character appears too variable to be of taxonomic value.

Egg size

Type description "Eggs oval, 0.042 (0.039 to 0.044) by 0.019 (0.018 to 0.021)," (Waitz, 1960).

Experimental results  Average measurements, in mm., of 9 eggs from feces of one adult A. tigrinum harboring 17, 4-week, gravid flukes, was 0.037 (0.035 to 0.040) by 0.017 (0.016 to 0.019). Average measurements of 20 eggs shed by one 4-week fluke, in creek water, were 0.040 (0.038 to 0.042) by 0.017 (0.016 to 0.019). Average measurements of 20 eggs shed by one 78-week fluke, in creek water, measured 0.037 (0.032 to 0.045) by 0.019 (0.016 to 0.026). The range in egg size found between the various aged worms (0.032 to 0.045 by 0.017 to 0.026) is approximately the same as that given by Stunkard (1915) for T. corti (0.031 by 0.015), T. medius (0.043 by 0.021) and T. lobosus (0.032 to 0.036 by 0.018 to 0.019). T. medius and T. lobosus are considered to be synonymous with
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*T. corti* by Yamaguti (1958). The combined, egg-size range of these three worms is 0.031 to 0.043 by 0.015 to 0.021.

**Other variations**

Other variations occurring, with advancing age of the worms, include increased cuticular spination and degeneration of the reproductive organs.

Younger flukes usually are heavily spinose anteriorly. Spination extends to the level of the acetabulum or slightly beyond. In some older specimens, scattered spines were observed as far posterior as the anterior testis. Vitelline follicles in a 78-week specimen (Figure 17) are considerably smaller and fewer in number than those in a 5-week specimen (Figure 16). The uterus in young gravid worms is completely filled with eggs; in older worms, eggs decrease in number or are entirely absent. The testes gradually become smaller and testicular cells diminish in 78-week flukes (Figure 18).

**Summary**

From the foregoing experimental results, it is apparent that considerable variation exists in morphological characteristics used in the delineation of species within the genus *Telorchis*. Such variability, although noted in previous studies by Barker and Covey (1911), Stunkard (1915) and Wharton (1940), was based primarily on a variety of naturally-infected hosts harboring specimens of several species. The present investigation demonstrates conclusively that age variations occur commonly among worms of one species experimentally reared in a single host species.

Metrick (1963) has suggested the use of Amadon's (1949) standard
in the evaluation of helminthological species when comparing one morphological characteristic among a limited number of specimens. According to this method, 97% of specimens "A" should differ from 97% of specimens "B". Applying this method to the per cent of variation found between morphological characters of _T. bonnerensis_ studied here, only the distance of the ovary from the acetabulum would be of specific significance between 4-week, and 18-week and 78-week worms. Other variations observed, however, do present problems when attempting to classify individual worms of varying ages.

Morphological characters that appear to be stable regardless of worm age are anterior extent of vitellaria in relation to the ovary and ovarian diameter, position of the ovary with respect to one-half total body length and its comparative distance from the acetabulum and anterior testis, median position of the acetabulum, comparative lengths of the metraterm and cirrus sac, and comparative size of the acetabulum and oral sucker.

Effects of Temperature Stress on Adult Development

The metabolic rates of adult and larval parasites of poikilothermic animals are greatly influenced by daily and seasonal fluctuations of external environmental temperatures surrounding their hosts. Most studies of temperature effects on helminths have been undertaken with free-living larvae or larval stages within the poikilothermic intermediate hosts.

Recent investigations on larval cestodes include those of Freeman (1952) on _Monoecocestus americanus_ and _M. variabilis_ in the oribatid mite _Liacarus itascensis_, Milleman (1955) on _Oochoristica deserti_, Voge

Temperature effects on larval trematodes have been studied by Stirewalt (1954) on *Schistosoma mansoni* in the snail *Australorbis glabratus*, Gumble et al. (1957) on *S. japonicum* in *Oncomelania nosophora*, Hoffman (1958) and Colley and Olson (1963) on cercariae and metacercariae of *Posthodiplostomum minimum*, and Dinnik and Dinnik (1964) on *Fasciola gigantica* in *Lymnaea natalensis*.

Free-living larval stages of nematodes at various temperatures have been studied by Ciordia and Bizzell (1963) on nematodes of cattle and Balasingam (1964) on nematodes of carnivores. Crewe (1961) studied the juveniles of *Loa loa* in *Chrysops silacea*, and Yanai (1960) studied the development of *Ascaris lumbricoides* eggs when transferred to the peritoneal cavities of poikilothermic animals. Effects of host's body temperature on the adult nematode, *Trichinella spiralis*, in bats was reported by Chute and Covalt (1960).

The thermal response of the larval acanthocephalan, *Leptorhynchoides thecatus*, in the amphipod *Hyalella azteca* was investigated by de Giusti (1949).

Temperature studies on adult trematodes are limited. Willey (1941) related the more rapid maturation of the trematode *Zygocotyle lunata* in ducks, as compared to rats, to the higher body temperature in birds. Izumova (1956) reported that variations in water temperature affect development of the monogenetic trematode, *Dactylogyrus vastator*, a parasite on the gills of fish. This trematode, in the sexually mature state, is very sensitive to fluctuations of water temperature according
to Wohlgemuth (1920). Vernberg and Hunter (1961) found that the in vitro responses to temperature of three adult trematodes, parasitic in a fish, turtle, and bird, respectively, corresponded to the body temperatures of their definitive hosts. McCue and Thorson (1964) observed that the frog lung trematode, Haematoloechus, reacted positively to a thermal gradient.

The purpose of the present study was to determine the effect on morphology, growth and development of Telorchis bonnerensis when the definitive host, Ambystoma tigrinum, was maintained at various constant temperatures.

Preliminary experiments involved laboratory-reared, larval A. tigrinum which had been fed approximately 40 metacercariae of T. bonnerensis developed experimentally in laboratory-reared snails (Physa gyrina). Infected salamanders were maintained at 10°C., 22°C., and 30°C., and the results indicated that temperature does affect the rate of development and growth of the worms and their larval hosts. Further study was undertaken with adult A. tigrinum at temperatures of 10°C., 22°C., 30°C., 34°C., and 37°C.

Growth and development of 356 experimentally-reared worms resulting from such feeding experiments with adult A. tigrinum are illustrated in Graph 1, and Figures 19 to 22. Average measurements of taxonomically significant characters, in specimens from both larval and adult A. tigrinum maintained at 22°C., 30°C., and 34°C., are compared in Table 8.

When developing in adult A. tigrinum maintained at 10°C., worms failed to mature and increased only slightly in size, even after six
Graph 1. Effect of varying temperatures on growth and development of *Telorchis bonnerensis* adults
Graph 1. Effect of varying temperatures on growth and development of *Telorchis bonnerensis* adults.
Table 8. Average measurements (in mm.) of 4-week and adult *Ambystoma tigrinum* maintained at 22°C.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>22°C.</th>
<th>22°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage of development</td>
<td>Adult</td>
<td>Larval</td>
</tr>
<tr>
<td>No. of specimens</td>
<td>15</td>
<td>15</td>
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<tr>
<td>Body length</td>
<td>3.89(2.82-4.62)</td>
<td>4.09(3.61-4.63)</td>
</tr>
<tr>
<td>Body width</td>
<td>0.54(0.45-0.62)</td>
<td>0.50(0.40-0.56)</td>
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<tr>
<td>Oral sucker diam.</td>
<td>0.14(0.14-0.15)</td>
<td>0.17(0.16-0.18)</td>
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<tr>
<td>Acetabulum diam.</td>
<td>0.13(0.11-0.15)</td>
<td>0.15(0.12-0.16)</td>
</tr>
<tr>
<td>Pharynx length</td>
<td>0.07(0.06-0.08)</td>
<td>0.07(0.07-0.09)</td>
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<tr>
<td>Esophagus length</td>
<td>0.08(0.05-0.11)</td>
<td>0.14(0.12-0.16)</td>
</tr>
<tr>
<td>Metraterm length</td>
<td>0.32(0.27-0.38)</td>
<td>0.34(0.28-0.40)</td>
</tr>
<tr>
<td>Cirrus sac length</td>
<td>0.73(0.61-0.81)</td>
<td>0.75(0.65-0.86)</td>
</tr>
<tr>
<td>Ovary diam.</td>
<td>0.21(0.18-0.24)</td>
<td>0.20(0.19-0.21)</td>
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<tr>
<td>Ovary to ant. end</td>
<td>1.34(1.13-1.52)</td>
<td>1.39(1.26-1.52)</td>
</tr>
<tr>
<td>Ovary to ant. testis</td>
<td>1.91(1.14-2.43)</td>
<td>1.92(1.58-2.62)</td>
</tr>
<tr>
<td>Acetabulum to ovary</td>
<td>0.35(0.24-0.45)</td>
<td>0.41(0.28-0.54)</td>
</tr>
<tr>
<td>Acetabulum to ant. end</td>
<td>0.98(0.87-1.10)</td>
<td>0.80(0.72-0.89)</td>
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<tr>
<td>Distance between testes</td>
<td>0.01(0.00-0.23)</td>
<td>0.01(0.00-0.05)</td>
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<tr>
<td>Testis to post end</td>
<td>0.11(0.06-0.20)</td>
<td>0.38(0.28-0.44)</td>
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<tr>
<td>Ant. testis length</td>
<td>0.31(0.26-0.36)</td>
<td>0.29(0.26-0.32)</td>
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<tr>
<td>Vitellaria to testis</td>
<td>0.51(0.17-0.82)</td>
<td>0.31(0.11-0.61)</td>
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<tr>
<td>Total length vitellaria</td>
<td>2.13(1.41-2.50)</td>
<td>2.10(1.82-3.60)</td>
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</tbody>
</table>

\(^a\)Distance between posterior margin of ovary and anterior end of body
\(^b\)Distance between posterior margin of ovary and anterior margin of body
\(^c\)Distance between posterior margin of acetabulum and anterior margin of body
\(^d\)Distance between anterior margin of acetabulum and anterior end of body
\(^e\)Distance between posterior margin of posterior testis and posterior margin of acetabulum
\(^f\)Distance between posterior extent of vitellaria and anterior margin of body
(in mm.) of 4-week Telorchis bonnerensis from larval *tigrinum* maintained at varying temperatures

<table>
<thead>
<tr>
<th></th>
<th>22°C.</th>
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<th>30°C.</th>
<th></th>
<th>30°C.</th>
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<th>34°C.</th>
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<td>Larval</td>
<td>15</td>
<td>Adult</td>
<td>14</td>
<td>Larval</td>
<td>14</td>
<td>Adult</td>
<td>17</td>
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<tr>
<td>09(3.61-4.63)</td>
<td>4.61(4.82-5.61)</td>
<td>3.38(2.88-4.06)</td>
<td>4.42(3.40-5.14)</td>
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<td>50(0.40-0.56)</td>
<td>0.54(0.45-0.62)</td>
<td>0.50(0.33-0.39)</td>
<td>0.61(0.51-0.68)</td>
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<td>17(0.16-0.18)</td>
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<td>0.15(0.13-0.17)</td>
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<td>15(0.12-0.16)</td>
<td>0.13(0.11-0.14)</td>
<td>0.13(0.11-0.16)</td>
<td>0.13(0.12-0.14)</td>
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<tr>
<td>07(0.07-0.09)</td>
<td>0.06(0.05-0.08)</td>
<td>0.08(0.05-0.07)</td>
<td>0.07(0.05-0.08)</td>
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<td>0.09(0.04-0.12)</td>
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<td>34(0.28-0.40)</td>
<td>0.27(0.23-0.33)</td>
<td>0.26(0.19-0.33)</td>
<td>0.25(0.18-0.35)</td>
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<td>75(0.65-0.86)</td>
<td>0.64(0.55-0.79)</td>
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<td>20(0.19-0.21)</td>
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<tr>
<td>39(1.26-1.52)</td>
<td>1.19(1.02-1.35)</td>
<td>1.12(0.88-1.31)</td>
<td>1.24(1.19-1.41)</td>
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<td>92(1.58-2.62)</td>
<td>2.69(1.92-3.33)</td>
<td>1.66(1.30-1.94)</td>
<td>2.33(1.52-2.71)</td>
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<td>30(0.72-0.89)</td>
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<td>11(0.00-0.05)</td>
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<td>0.03(0.02-0.06)</td>
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<td>0.24(0.19-0.44)</td>
<td>0.39(0.28-0.51)</td>
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<td>29(0.26-0.32)</td>
<td>0.26(0.21-0.29)</td>
<td>0.25(0.25-0.26)</td>
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<tr>
<td>51(0.11-0.61)</td>
<td>0.87(0.56-1.13)</td>
<td>0.36(0.16-0.53)</td>
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<tr>
<td>10(1.82-3.60)</td>
<td>2.41(1.46-3.30)</td>
<td>1.83(1.42-2.38)</td>
<td>2.03(1.51-2.33)</td>
<td></td>
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</tr>
</tbody>
</table>

- anterior end of body.
- anterior margin of anterior testis.
- anterior margin of ovary.
- and anterior margin of anterior testis.
- testis and posterior end of body.
- and anterior margin of anterior testis.
weeks. Growth was accelerated at 22°C. and all flukes were gravid by four weeks. Worms from hosts maintained at 30°C. were largest in overall size, showed most rapid growth and all specimens contained well-developed eggs within two weeks. At 34°C., growth appears to be adversely affected, since it is less marked than at 30°C. and, although it is more pronounced in the earlier stages than at 22°C., it decreases at six and eight weeks. The rate of maturation, however, was equal to that of worms maintained at 30°C.

Some variation was noted in growth and rate of development between worms from larval and adult *A. tigrinum* (Table 8). Growth at 22°C. was slightly more rapid than at 30°C. in larvae at four weeks and all worms, both at 22°C. and 30°C., were gravid at two weeks in contrast to those obtained from adult amphibians where only flukes at 30°C. contained eggs at this time.

Infection of laboratory-reared snails (*P. gyrina*) occurred when they were exposed to eggs from 4-week worms developed in a larval host, and also by eggs from 2-week worms from an adult host maintained at 30°C. However, infection did not occur when snails were exposed to eggs from 8-week flukes from an adult host maintained at 34°C.

Adult *A. tigrinum* were unable to tolerate temperatures higher than 34°C. for death occurred within 24 hours at 35 to 37°C. Larval hosts grew best at 22°C., averaging an increase in length of 8 1/2 cm. in four weeks as compared to an increase of 5 cm. at 30°C. and 3 cm. at 10°C.

When one compares measurements of taxonomically important characters among worms reared at varying temperatures, it is seen that except for variation in size of body organs, few differences are apparent. Size
differences are most obvious between eggs recovered from worms developed at the various temperatures. The average size of 20 eggs (0.033 by 0.017 mm.) from 8-week worms developing at 34°C. was smaller than the average egg size (0.037 by 0.019 mm.) previously obtained from a 78-week fluke developed at 22°C. The smallest egg (0.029 by 0.016 mm.) was shed by an 8-week worm at 34°C. and the largest egg (0.043 by 0.019 mm.) by a 4-week worm at 22°C. It appears that higher temperature may be associated with the production of smaller eggs. Degeneration of the reproductive organs, as indicated by a decrease in testicular cells and slightly smaller vitelline follicles, occurred in some 6 and 8-week worms developing at 30°C. and 34°C.

The experimental results obtained in this study indicate that the optimal temperature for growth and development of adult T. bonnerensis is approximately 22°C. when developing in larval hosts and 30°C. when developing in adult A. tigrinum. The variation obtained between larval and adult salamanders might be explained by the fact that the aquatic larvae, being much smaller and more active, have a higher metabolic rate than the more sedentary, terrestrial adults. Fry and Hart (1948), for example, found that the metabolic rate of goldfish was approximately doubled when the fish swam at temperatures between 5°C. and 20°C.

The foregoing experiments indicate that overall growth of adult worms appears to coincide with the growth of their natural definitive host, larval A. tigrinum. The tolerance of this host for temperatures higher than 30°C. was not determined. However, experimentally infected adult A. tigrinum were unable to survive at temperatures higher than 34°C.
The temperature range at which larval and adult helminths live and develop varies considerably from species to species. A direct relationship appears to exist between an increase in temperature and a corresponding increase in rate of growth and development up to a certain degree. After this point is reached, various unfavorable effects may occur. Structural anomalies, decreased infectivity in the definitive host and reduced growth rate, maturation, and size were observed by Heyneman (1958) when cysticercoids of *H. nana* developed above 40°C. Incomplete development of free-living juveniles at 35°C., and death of juveniles in eggs at 40°C. occurred in several species of cattle nematodes according to Ciordia and Bizzell (1963). Voge and Turner (1956) believed such abnormalities to be a result of the increased rate of development which prevents structural organization and differentiation.

The deleterious effects of high temperature appear to be indicated, in the present investigation, by the decrease in overall size of adult *T. bonnerensis*, the reduced size and infectivity of the eggs, and the degeneration of the reproductive structures.

Effects of Host Starvation on Adult Development

The availability of food is of major significance in the rate of development, degree of differentiation, and reproductive capacity of many intestinal helminths.

Starvation of the host has been shown to eliminate intestinal parasites. Ackert et al. (1940) reported that fowl nematodes, *Ascaridia lineata*, were fewer and shorter in glucose-injected chickens than in normally fed birds. Reid (1940, 1942) demonstrated that strobilae of
the fowl cestode, Railletina cesticillus, were expelled when the hosts were starved for 24 to 48 hours. However, the scolex and neck region of these worms remained even after 20 days of host starvation. Beaver, in personal correspondence to Reid (1942), observed that malnutrition of the host resulted in loss of trematode parasites. Burlingame and Chandler (1941) reported that starvation of rat hosts resulted in the loss of the acanthocephalan parasite, Moniliformis dubius.

Read and Rothman (1957) noted that host starvation affects body size, rate of glucose utilization, and glycogenesis in Hymenolepis diminuta. Goodchild (1960) compared growth of H. diminuta when the worm developed in normal, starved and bileless rats. Read and Rothman (1958) found that retarded growth of Moniliformis, due to lack of carbohydrate in the diet of its rat host, was resumed when such hosts were returned to carbohydrate food.

Effects of starvation on larvae of helminths in their intermediate hosts are varied. Von Brand (1938) found that juveniles of the nematode, Eustrongylides ignotus, could remain in the starved host, Fundulus, for a period of 65 days without showing any decrease in glycogen content. Odlaug (1955) noted that Clinostomum metacercariae from starved frogs were in good condition and also showed no decrease in glycogen. Incomplete development of Hymenolepis nana cysticercoids in the starved host, Tribolium confusum, was observed by Schiller (1959). Cheng and Snyder (1962) reported that emergence of Glyphthelmins cercariae from starved snails was decreased to a three or four-day rhythm, but when such snails were fed, large numbers of larvae emerged within 48 hours. Sillman
believed that rate of development and production of *Azygia* cercariae are directly influenced by the nutritional condition of the snail host.

The effects of host starvation on carbohydrate utilization by cestode parasites have been reviewed by Read (1959). In the presence of glucose, the *in vitro* metabolic rate of starved tapeworms is higher than that of unstarved worms. As a result of the increased metabolic rate, glycogen content of starved worms is reduced and von Brand (1952) suggested that its absence may reduce muscular activity to the extent that such worms are unable to maintain their position in the intestine. Furthermore, if worms are eliminated because of their high metabolic rates, worms with lower metabolic rates should be able to withstand longer periods of host starvation.

Parasites of poikilothermic animals generally have lower metabolic demands than those of homoiothermic animals, although Vernberg (1952) found that metabolic rates of poikilotherms change directly with temperature and considerable variation exists between species. It would appear, however, that parasites of poikilothermic vertebrates should be able to endure longer periods of host starvation than those of homoiotherms. Little information, however, is available on this topic.

Metabolic requirements of several trematodes of homoiothermic hosts and a few of poikilothermic hosts have been studied. These include *in vitro* analyses by Bueding (1950) on carbohydrate metabolism of *Schistosoma mansoni* and Goil (1957, 1958a, b, c) on carbohydrate, fat and protein metabolism, and oxygen consumption, respectively, in flukes from buffaloes. Carbohydrate metabolism of *Fasciola hepatica* was determined
by Mansour (1959) and oxygen consumption and carbohydrate metabolism of F. gigantica by Goil (1961a, b). The host-parasite glycogen relationships of several frog flukes were studied by Odlaug (1955) and of Haematoloechus medioplexus by Shields (1963).

The present investigation was undertaken to study the effects of host starvation on Telorchis bonnerensis developing in Ambystoma tigrinum.

Three pairs of adult A. tigrinum, of similar age and size, were fed approximately 40 metacercariae of T. bonnerensis which had been experimentally developed in laboratory-reared snails (Physa gyrina). The salamanders were then isolated in individual containers, and maintained at a temperature of 22°C. One member of each pair was fed pieces of raw beef every two days while the other animal was starved for a period of four weeks. At this time, all animals were autopsied.

A total of 151 experimental worms were found. The average number, size, and gravidity of these flukes, in both starved and unstarved hosts, are illustrated by Grantham. Variation in size and development of individual worms are shown in Figures 23 to 26.

Growth, development and survival of worms from the starved salamanders were greatly inhibited. The few worms which became gravid were smaller than average-sized, gravid worms from unstarved hosts. Except for a slight decrease in size and some diffuseness of the vitelline follicles in flukes from starved hosts, however, the inhibition of growth and development did not result in any apparent structural abnormality but appeared to be simply a retardation or decrease in rate. Such retarded development was also observed by Schiller (1959) when cysticercoids of H. nana developed in starved T. confusum. Schiller
Graph 2. Effects of starvation of definitive hosts on development of adult *Telorchis bonnerensis*
Graph 2. Effects of starvation of definitive hosts on development of adult Telorchis bonnerensis.
believed that some specific growth factor normally present in the hemolymph of the beetles was lacking due to starvation. Other factors such as changes in pH, oxygen supply, bacterial flora and toxic secretions were shown by Ackert et al. (1940) to have little effect on the nematode fauna of starved chickens.

The nutritional requirements of intestinal trematodes are believed to be satisfied primarily by food debris, mucus, and probably bacteria. Schell (1962) observed that adult *T. bonnerensis* were often clustered on undigested food in the intestine of larval *Ambystoma macrodactylum*. A similar activity of these trematodes in *A. tigrinum* was observed during the course of this study, although many worms were also attached to the intestinal mucosa. It is not known if *T. bonnerensis* ingests blood, but the possibility exists that contact with host blood does occur and that these flukes may not be entirely dependent on host-ingested food for nutrition. The presence of haemoglobin in the crura of a related species, *T. robustus*, was believed by Wharton (1941) to indicate ingestion of host blood.

The experimental results obtained indicate that, although adults of *T. bonnerensis* are considerably retarded by starvation of the definitive host, some are able to maintain their position in the intestine and are capable of maturing in the normal length of time.

Effects of Crowding on Adult Development

The detrimental effects of crowding on helminths are well known. The number of worms present in a host is considered by Haley (1962) to be a factor influencing the size of a single species within a host.
The "crowding effect" in cestodes was reviewed by Read (1951). It has been observed in several adult cestodes that, with increasing numbers of worms living in a host, the average size of individual worms decreases. Altering the quantity or quality of carbohydrate in the diet of rats infected with *Hymenolepis diminuta* appears to have little effect on this phenomenon in these worms, according to Read and Phifer (1959). Roberts (1961) found that crowding affects carbohydrate and lipid concentration and possibly metabolism in *H. diminuta*. Heyneman (1958) and Schiller (1959) noted a "crowding effect" when 50 or more cysticercoids of *Hymenolepis nana* developed in the hemocoel of the intermediate host, *Tribolium confusum*.

The effect of population density on the adult nematode, *Nippostrongylus brasiliensis*, was studied by Haley and Parker (1961). Percentage of worm loss from rats with high initial infections of this nematode was much greater and more abrupt than from rats with low initial infections.

Rankin (1937) observed that when large numbers of worms were present in natural infections of the salamander trematodes, *Brachycoelium*, *Plagitura*, and *Megalodiscus*, specimens were usually small, though mature. When these flukes occurred in small numbers they were much larger. More recent experimental work by Boddeke (1960) demonstrated a decreased growth rate of *Prosthogonimus ovatus* when larger numbers of this fluke were present in the bursa Fabricius of the hen. These effects were not apparent in specimens from the oviduct and Boddeke suggested that the larger lumen of this organ removes the possibility of crowding for a similar number of worms. Dawes (1962), in extensive experimental studies
on Fasciola hepatica in mice, stated that variation in size of flukes obtained from the liver did not depend on the number of worms present, and that further study was necessary to determine the reasons for this fact.

Since the "crowding effect" is an important size-controlling factor, the present experimental work was conducted to determine the effect of varying numbers of worms on size of adult Telorchis bonnerensis.

Five adult Ambystoma tigrinum, of similar age and size, were fed approximately 40, 80, 160, 320, and 640 metacercariae of T. bonnerensis experimentally developed in laboratory-reared snails (Physa gyrina). These animals were maintained for one month at a temperature of 22°C on the usual diet of raw beef.

The effects of crowding on 646 experimental worms obtained from these hosts are summarized in Table 9, and Figures 27 to 30. Body size is expressed in terms of total body area.

Results indicate that, with increasing numbers of metacercariae fed, the number of worms recovered increases, while the average body area and per cent of gravid worms present decreases. Per cent survival of metacercariae fed appears to increase to a certain point and then decreases. The decreased number of worms recovered from the feeding of 320 metacercariae, may be due to individual host differences and might be less apparent if more test animals had been used. It is also possible that the worms were lost shortly before autopsy, since the decreased, average-body area appears to indicate the presence of a larger number of flukes during most of the developmental period.

An explanation for the "crowding effect" may be complex. Little
Table 9. Effects of crowding on 1-week, adult *Telorchis donnerensis*

<table>
<thead>
<tr>
<th>Approximate number of metacercariae fed</th>
<th>Number of adults recovered</th>
<th>Per cent survival</th>
<th>Per cent gravid worms</th>
<th>Average body area (sq. mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>18</td>
<td>45</td>
<td>100</td>
<td>2.2 (1.5-2.6)</td>
</tr>
<tr>
<td>80</td>
<td>58</td>
<td>73</td>
<td>98</td>
<td>1.6 (0.7-2.5)</td>
</tr>
<tr>
<td>160</td>
<td>114</td>
<td>71</td>
<td>98</td>
<td>1.3 (0.3-2.2)</td>
</tr>
<tr>
<td>320</td>
<td>79</td>
<td>24</td>
<td>78</td>
<td>0.9 (0.1-2.2)</td>
</tr>
<tr>
<td>640</td>
<td>377</td>
<td>59</td>
<td>88</td>
<td>0.7 (0.1-1.7)</td>
</tr>
</tbody>
</table>

Experimental study appears to have been undertaken with trematodes. The observation by Rankin (1937), that flukes (*Brachycoelium*) from naturally infected salamanders were small but gravid when large numbers of worms were present, appears to be contrary to results found in the present study, where the number of gravid worms decreases with increasing worm burden and many of the non-gravid worms are immature as shown in Figure 27.

The work of Boddeke (1960) with *P. ovatus* suggests space as the limiting factor for rate of development. According to his studies, flukes in the bursa Fabricius demonstrated a much slower growth rate than those in the larger oviduct. The "crowding effect" in tapeworms is believed by Read (1959) to be caused by competition between the worms for utilizable carbohydrate. However, Roberts (1961) observed inhibiting effects on the germinative region, in *H. diminuta*, while most of the
strobi lae were unaffected. He therefore suggested the lack of a vitamin-like nutrient, or action of some inhibitor as an explanation.

The per cent survival of *T. bonnerensis* in the present study seems to indicate that neither the largest nor the smallest number of metacercariae fed was optimal. Similar results were obtained by Haley and Parker (1961), with the nematode, *N. brasiliensis*, during the first 10 days after infection of rats.

Specimens of *T. bonnerensis* exhibiting abnormalities were found in an experimental host harboring the highest population of 377 worms. Both living and fixed flukes showed constrictions at various points of the body as illustrated by Figures 28 to 30. The host intestinal mucosa was slightly eroded or inflamed and such abnormal worms appeared to be trapped in strands of mucus or tissue.
It is well known that morphological and physiological changes may occur when adult trematodes mature in different species of definitive hosts. The experimental studies of Beaver (1937) on *Echinostoma revolutum* developing in avian and mammalian hosts, the work of Rankin (1937) on *Brachycoelium* collected from various species of naturally infected salamanders, and studies by Wharton (1940) on several species of *Telorchis* collected from naturally infected chelonian and amphibian hosts, present evidence for this statement. More recently, Boddeke (1960) studied experimental infections of *Prosthogonimus ovatus* and observed great morphological variations among these flukes from different avian hosts.

The following experiments were conducted to study certain morphological and physiological effects on *Telorchis bonnerensis* when reared in three species of *Ambystoma*, namely: *A. tigrinum*, *A. macrodactylum*, and *A. maculatum*, and three chelonian hosts, *Pseudemys scripta elegans*, *Chrysemys picta belli*, and *Chelydra serpentina*.

Each amphibian and chelonian host was fed approximately 40 metacercariae of *T. bonnerensis* which had been experimentally developed in laboratory-reared snails (*Physa gyrina*). The number of metacercariae fed to larval *A. macrodactylum* may, in some instances, have been less because of the difficulty in force-feeding such small aquatic larvae. All experimental hosts were maintained at approximately 22°C. during the developmental period of the parasites. Specimens of *T. bonnerensis* from the various hosts were then compared with specimens of a similar age.
from *A. tigrinum*. However, when it became apparent that sexual maturation does not occur at the same time but varies among worms from different hosts, another criterion was chosen as a basis for comparison. The earliest time at which eggs first appeared in the uterus (3 to 12 weeks) was consequently used as one criterion. Furthermore, additional comparisons were made after sexual maturity had been reached (24 weeks) when certain morphological characteristics were more apparent than at earlier times.

Comparative measurements and illustrations of *T. bonnerensis* from the various hosts are presented in Tables 10 to 14 and in Figures 31 to 50. Brief discussions of variations in developmental rate and individual morphological features are given below.

**Variations in Rate of Adult Development**

The developmental rate of adult *T. bonnerensis* varies considerably among salamander and turtle hosts (Table 10). Most rapid development

<table>
<thead>
<tr>
<th>Host</th>
<th>First appearance of gravid worms (in weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ambystoma tigrinum</em></td>
<td>3</td>
</tr>
<tr>
<td><em>A. macrodactylum</em> (larvae)</td>
<td>3</td>
</tr>
<tr>
<td><em>A. maculatum</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Chelydra serpentina</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Chrysemys picta belli</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Pseudemys scripta elegans</em></td>
<td>12</td>
</tr>
</tbody>
</table>
Table 11. Average measurements (in mm.) of 3-week Iowa and Idaho strains of Telorchis bonnerensis from adult Ambystoma tigrinum and larval Ambystoma macrodactylum

| Host Strain | A. tigrinum | | A. macrodactylum | |
|-------------|-------------|-------------|-------------|
|              | Iowa        | Idaho       | Iowa        | Idaho       |
| No. of specimens | 5          | 5           | 5           | 2           |
| Body length  | 2.98(2.54-3.38) | 1.88(1.69-2.26) | 2.18(2.03-2.37) | 2.96(2.88-3.05) |
| Body width   | 0.38(0.34-0.39) | 0.29(0.23-0.45) | 0.28(0.17-0.34) | 0.28(0.26-0.28) |
| Oral sucker diam. | 0.13(0.12-0.14) | 0.11(0.08-0.17) | 0.11(0.11-0.11) | 0.12(0.11-0.12) |
| Acetabulum diam. | 0.10(0.09-0.12) | 0.07(0.06-0.11) | 0.08(0.08-0.09) | 0.09(0.09-0.09) |
| Pharynx length | 0.05(0.05-0.06) | 0.05(0.05-0.06) | 0.05(0.05-0.06) | 0.05(0.05-0.06) |
| Esophagus length | 0.08(0.02-0.11) | 0.05(0.03-0.06) | 0.10(0.09-0.12) | 0.11(0.11-0.12) |
| Metraterm length | 0.23(0.11-0.33) | 0.18(0.15-0.21) | 0.13(0.11-0.18) | 0.29(0.23-0.29) |
| Cirrus sac length | 0.56(0.50-0.59) | 0.37(0.29-0.50) | 0.36(0.33-0.54) | 0.56(0.52-0.61) |
| Ovary diam. | 0.16(0.15-0.18) | 0.13(0.11-0.15) | 0.09(0.08-0.09) | 0.11(0.11-0.14) |
| Ovary to ant. end<sup>a</sup> | 1.09(0.90-1.18) | 0.75(0.68-0.85) | 0.87(0.73-0.96) | 1.24(1.18-1.30) |
| Ovary to ant. testis<sup>b</sup> | 1.15(0.96-1.11) | 0.65(0.51-0.85) | 0.93(0.85-1.02) | 1.27(1.21-1.30) |
| Acetabulum to ovary<sup>c</sup> | 0.32(0.26-0.35) | 0.11(0.09-0.18) | 0.19(0.15-0.21) | 0.40(0.37-0.43) |
| Acetabulum to ant. end<sup>d</sup> | 0.39(0.19-0.71) | 0.38(0.17-0.50) | 0.54(0.46-0.58) | 0.62(0.62-0.71) |
| Distance between testes | 0.01(0.00-0.02) | 0.00 | 0.01(0.00-0.02) | 0.00 |
| Testis to post. end<sup>e</sup> | 0.21(0.15-0.24) | 0.11(0.12-0.15) | 0.11(0.08-0.12) | 0.12(0.11-0.14) |
| Length ant. testis | 0.25(0.24-0.26) | 0.17(0.11-0.24) | 0.15(0.12-0.17) | 0.20(0.20-0.20) |
| Vitellaria to testis<sup>f</sup> | 0.11(0.06-0.21) | 0.08(0.03-0.11) | 0.30(0.21-0.40) | 0.29(0.21-0.40) |
| Total length vitellaria | 1.14(1.18-1.86) | 0.94(0.62-1.18) | 0.99(0.85-1.13) | 1.60(1.14-1.69) |

<sup>a</sup>Distance between posterior margin of ovary and anterior end of body.
<sup>b</sup>Distance between posterior margin of ovary and anterior margin of anterior testis.
<sup>c</sup>Distance between posterior margin of acetabulum and anterior margin of ovary.
<sup>d</sup>Distance between anterior margin of acetabulum and anterior end of body.
<sup>e</sup>Distance between posterior margin of posterior testis and posterior end of body.
<sup>f</sup>Distance between posterior extent of vitellaria and anterior margin of anterior testis.
Table 12. Average measurements (in mm.) of 8-week Telorchis bonnerensis from Ambystoma tigrinum and Ambystoma maculatum

<table>
<thead>
<tr>
<th>Host</th>
<th>A. tigrinum</th>
<th>A. maculatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of specimens</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Body length</td>
<td>5.16(4.00-5.92)</td>
<td>3.22(2.43-3.95)</td>
</tr>
<tr>
<td>Body width</td>
<td>0.69(0.62-0.79)</td>
<td>0.37(0.28-0.51)</td>
</tr>
<tr>
<td>Oral sucker diam.</td>
<td>0.16(0.15-0.17)</td>
<td>0.13(0.12-0.15)</td>
</tr>
<tr>
<td>Acetabulum diam.</td>
<td>0.13(0.11-0.14)</td>
<td>0.11(0.08-0.12)</td>
</tr>
<tr>
<td>Pharynx length</td>
<td>0.06(0.03-0.08)</td>
<td>0.06(0.06-0.08)</td>
</tr>
<tr>
<td>Esophagus length</td>
<td>0.06(0.02-0.11)</td>
<td>0.06(0.06-0.12)</td>
</tr>
<tr>
<td>Metraterm length</td>
<td>0.33(0.26-0.44)</td>
<td>0.21(0.12-0.29)</td>
</tr>
<tr>
<td>Cirrus sac length</td>
<td>0.80(0.68-0.88)</td>
<td>0.42(0.26-0.59)</td>
</tr>
<tr>
<td>Ovary diam.</td>
<td>0.19(0.17-0.21)</td>
<td>0.12(0.08-0.15)</td>
</tr>
<tr>
<td>Ovary to ant. end&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.58(1.30-1.75)</td>
<td>1.64(0.85-1.24)</td>
</tr>
<tr>
<td>Ovary to ant. testis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.17(1.81-2.76)</td>
<td>1.51(0.85-1.92)</td>
</tr>
<tr>
<td>Acetabulum to ovary&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.59(0.38-0.75)</td>
<td>0.28(0.18-0.37)</td>
</tr>
<tr>
<td>Acetabulum to ant. end&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.74(0.64-0.84)</td>
<td>0.61(0.46-0.71)</td>
</tr>
<tr>
<td>Distance between testes</td>
<td>0.08(0.02-0.15)</td>
<td>0.03(0.00-0.09)</td>
</tr>
<tr>
<td>Testis to post. end&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.38(0.21-0.49)</td>
<td>0.21(0.12-0.27)</td>
</tr>
<tr>
<td>Length ant. testis</td>
<td>0.26(0.12-0.30)</td>
<td>0.21(0.15-0.29)</td>
</tr>
<tr>
<td>Vitellaria to testis&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.61(0.33-0.84)</td>
<td>0.34(0.09-0.05)</td>
</tr>
<tr>
<td>Total length vitellaria</td>
<td>2.71(1.90-3.24)</td>
<td>1.72(1.10-2.31)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Distance between posterior margin of ovary and anterior end of body.
<sup>b</sup>Distance between posterior margin of ovary and anterior margin of anterior testis.
<sup>c</sup>Distance between posterior margin of acetabulum and anterior margin of ovary.
<sup>d</sup>Distance between anterior margin of acetabulum and anterior end of body.
<sup>e</sup>Distance between posterior margin of posterior testis and posterior end of body.
<sup>f</sup>Distance between posterior extent of vitellaria and anterior margin of anterior testis.
Table 13. Average measurements (in mm.) of 24-hour week Telorchis bonnerensis from Ambystoma tigrinum, Chrysemys picta belli, and Pseudemys scripta elegans

<table>
<thead>
<tr>
<th>Host</th>
<th>A. tigrinum</th>
<th>C. p. belli</th>
<th>P. s. elegans</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of specimens</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Body length</td>
<td>4.15(3.27-4.74)</td>
<td>2.96(2.65-3.21)</td>
<td>4.12(3.84-4.51)</td>
</tr>
<tr>
<td>Body width</td>
<td>0.60(0.51-0.73)</td>
<td>0.28(0.23-0.34)</td>
<td>0.50(0.45-0.56)</td>
</tr>
<tr>
<td>Oral sucker diam.</td>
<td>0.18(0.17-0.18)</td>
<td>0.10(0.09-0.11)</td>
<td>0.11(0.11-0.12)</td>
</tr>
<tr>
<td>Acetabulum diam.</td>
<td>0.16(0.15-0.17)</td>
<td>0.09(0.08-0.10)</td>
<td>0.12(0.11-0.12)</td>
</tr>
<tr>
<td>Pharynx length</td>
<td>0.08(0.08-0.09)</td>
<td>0.06(0.06-0.06)</td>
<td>0.05(0.05-0.05)</td>
</tr>
<tr>
<td>Esophagus length</td>
<td>0.10(0.08-0.12)</td>
<td>0.08(0.06-0.10)</td>
<td>0.11(0.09-0.14)</td>
</tr>
<tr>
<td>Metraterm length</td>
<td>0.28(0.26-0.30)</td>
<td>0.23(0.15-0.30)</td>
<td>0.33(0.27-0.39)</td>
</tr>
<tr>
<td>Cirrus sac length</td>
<td>0.78(0.76-0.84)</td>
<td>0.54(0.47-0.61)</td>
<td>0.73(0.65-0.79)</td>
</tr>
<tr>
<td>Ovary diam.</td>
<td>0.20(0.18-0.23)</td>
<td>0.09(0.09-0.11)</td>
<td>0.16(0.15-0.17)</td>
</tr>
<tr>
<td>Ovary to ant. end&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.38(1.13-1.58)</td>
<td>1.17(1.02-1.35)</td>
<td>1.11(1.35-1.47)</td>
</tr>
<tr>
<td>Ovary to ant. testis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.18(1.62-2.71)</td>
<td>1.16(1.07-1.24)</td>
<td>1.55(1.29-1.80)</td>
</tr>
<tr>
<td>Acetabulum to ovary&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.37(0.24-0.42)</td>
<td>0.13(0.32-0.49)</td>
<td>0.16(0.30-0.53)</td>
</tr>
<tr>
<td>Acetabulum to ant. end&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.77(0.61-0.90)</td>
<td>0.61(0.56-0.70)</td>
<td>0.68(0.64-0.75)</td>
</tr>
<tr>
<td>Distance between testes</td>
<td>0.11(0.06-0.17)</td>
<td>0.03(0.02-0.08)</td>
<td>0.05(0.30-0.61)</td>
</tr>
<tr>
<td>Testis to post. end&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.21(0.15-0.32)</td>
<td>0.26(0.24-0.32)</td>
<td>0.51(0.47-0.56)</td>
</tr>
<tr>
<td>Length ant. testis</td>
<td>0.19(0.09-0.17)</td>
<td>0.11(0.12-0.15)</td>
<td>0.20(0.17-0.23)</td>
</tr>
<tr>
<td>Vitellaria to testis&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.44(0.11-0.78)</td>
<td>0.26(0.21-0.43)</td>
<td>0.50(0.37-0.93)</td>
</tr>
<tr>
<td>Total length vitellaria</td>
<td>2.51(1.93-3.11)</td>
<td>1.20(1.13-1.44)</td>
<td>1.73(1.22-2.10)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Distance between posterior margin of ovary and anterior end of body.

<sup>b</sup>Distance between posterior margin of ovary and anterior margin of anterior testis.

<sup>c</sup>Distance between posterior margin of acetabulum and anterior margin of ovary.

<sup>d</sup>Distance between anterior margin of acetabulum and anterior end of body.

<sup>e</sup>Distance between posterior margin of posterior testis and posterior end of body.

<sup>f</sup>Distance between posterior extent of vitellaria and anterior margin of anterior testis.
took place in the two natural hosts, *A. tigrinum* and *A. macrodactylum*. Since infection may occur in both larval and adult *A. tigrinum* and only in larval *A. macrodactylum*, the former appears to be a more satisfactory host. In addition, the number of worms found in naturally infected *A. tigrinum* larvae was far greater than that obtained from larval *A. macrodactylum*. *A. maculatum* appears to be a less desirable host since maturation rate is decreased and *T. bonnerensis* infections have not been reported to occur naturally in this salamander.

Development in turtles progressed most rapidly in *Chelydra serpentina* and most slowly in *Pseudemys scripta elegans*. Although development was almost as rapid in *C. serpentina* as in *A. tigrinum*, the overall growth of worms was greatly reduced in the former host. Figures 31 to 34 illustrate the variation in such growth amongst 8-week worms from *A. tigrinum* and the three chelonian hosts.

The striking effect of the host on development of *T. bonnerensis* was further emphasized by a series of feeding experiments involving several generations of adult worms developed successively from amphibian to reptilian and again to amphibian definitive hosts (Figures 35 to 37). Adult worms (14 weeks old) were experimentally developed in adult *A. tigrinum* (Figure 35). A filial generation of flukes (12 weeks old) was then developed experimentally in young *P. s. elegans*. The marked decrease in size of gravid worms developing in the chelonian host is shown in Figure 36. At 12 weeks, approximately one-half of these were gravid. Progeny of these worms were then reared in larval *A. tigrinum* resulting in much larger adults within three weeks (Figure 37). Similar results were obtained in a subsequent experiment involving successive generations
of *T. bonnerensis* reared in *A. tigrinum* and passed to *Chrysemys picta bellii* and again to *A. tigrinum*. Here too, worms from the turtle hosts were much smaller and sexual maturity, in some cases, was delayed even more than in *Pseudemys*. The pronounced size differences and delayed maturity in reptilian hosts, consequently, must be considered as host-induced.

With continued passage of *T. bonnerensis* from turtle to turtle, development of the fluke may be accelerated as a result of adaptation to the altered host environment. This was shown by one experiment in which generations of worms were successively passed from adult *A. tigrinum* to young *P. s. elegans* and again to young *P. s. elegans*. Gravid worms appeared within 12 weeks in the first generation and within 8 weeks in the second generation reared in these turtles. Gravid specimens were not found prior to 12 weeks in first-generation infections of *P. s. elegans* during the course of this investigation.

Variations in developmental rate of worms also occurred between individual hosts of the same species and age, and between young and older hosts of the same species. For example, gravid specimens developed in some young *Chrysemys picta bellii* in eight weeks while others did not harbor gravid worms at 12 weeks under similar conditions. Flukes in some older *C. p. bellii* were not gravid even after 24 weeks.

**Morphological Variations of Adults**

**Extent of vitellaria**

The anterior extent of vitellaria was more than one ovarian diameter.
anterior to the ovary in all specimens from both salamanders and turtles. However, considerable variation occurred among these hosts in the distance of follicles both anteriorly and posteriorly from the center of the acetabulum (Figures 41 to 46). This distance varied from 0.21 mm. anterior in 8-week specimens from A. maculatum (Figure 44) to 0.27 mm. posterior in 24-week specimens from C. p. belli (Figure 42). In 8-week worms from A. maculatum follicles always extended to the center of, or anterior to the center of the acetabulum in all specimens examined but never extended further posteriad than the middle of the acetabulum. In 24-week adults from C. p. belli (Figure 42) and P. s. elegans (Figure 43), follicles never extended as far anteriad as the middle of the acetabulum. Greatest variation in extent of vitellaria was shown in specimens reared in A. tigrinum. Here vitelline follicles in 3, 8, and 24-week specimens (the latter shown in Figure 41) extended between the pre- and post-acetabular regions in all worms. Distribution of vitellaria in 3-week specimens from A. macrodactylum (Figures 45 and 46) resembled that of worms from A. tigrinum.

No particular pattern could be found in posterior extent of vitellaria in relation to the anterior testis. This distance averaged from less than one to more than two diameters of that organ in both amphibian and chelonian hosts. In some individual specimens, the distance was greater than four diameters of the testis. Posterior distribution of vitelline follicles is thus highly variable and is probably of little taxonomic significance.

The experimental results obtained in this study demonstrate conclusively the extreme variability of the anterior extent of vitellaria.
in relation to the center of the acetabulum. Extent of vitelline follicles found when worms develop in chelonian and amphibian hosts eliminates this characteristic as a distinguishing one between *T. bonnerensis* and several other species within the genus.

Rankin (1937) considered distribution of vitellaria to be the most variable character in *Brachycoelium*. The follicles were limited to a certain area, but distribution within that area constantly changed with contraction and extension of the worm. Wharton (1940) found the anterior vitelline extent to vary from approximately the base of the metraterm to the acetabulum in *T. robustus*, and within the ovarian level in *T. medius*. Boddeke (1960) also suggested that the position of vitelline glands in *P. ovatus* is dependent on the degree of worm contraction.

The variation in distribution of vitelline follicles observed in *T. bonnerensis* when developing in amphibian hosts is largely confined to the acetabular area. The variation within this area may possibly be due to contraction or extension of the worm at the time of fixation. However, the variation observed in flukes from chelonian hosts is believed to be significant and appears to be host-induced, since the average distance of vitellaria posterior to the center of the acetabulum is greater in specimens from turtles than the average posterior distance found in specimens from amphibian hosts.

**Position of ovary**

In all specimens studied, the ovary was anterior to the middle of the body and always nearer to the acetabulum than to the anterior testis. Variation occurred, however, in the relation of the ovary to the anterior
one-third of the body. In 3-week specimens from A. tigrinum and A. macrodactylum, none of the ovaries were within this region. In 8-week specimens from A. tigrinum and A. maculatum, however, and in 24-week specimens from A. tigrinum, P. s. elegans, and C. p. belii, the ovaries were within the anterior one-third of the body in 100%, 67%, 40%, 50%, and none, respectively. It is apparent that considerable variation occurs in the relationship of the ovary to this point of the body not only with respect to the age of the worm, as previously indicated, but also when worms develop in different host species.

The ovary was located one to three ovarian diameters behind the acetabulum in 3-week specimens from both A. tigrinum and A. macrodactylum. In 8-week worms from A. tigrinum and A. maculatum the distance varied from two to three diameters of the ovary. In 24-week worms from A. tigrinum, P. s. elegans, and C. p. belii this distance varied from one to two, two to three, and three to five diameters, respectively. The distance of the ovary from the acetabulum, in worms from different host species, appears to vary to an even greater extent than in worms of different ages from the same host species.

The distance from the ovary to the anterior testis varied within 0.2 mm. of one-half the total body length in 83% of specimens from A. tigrinum, A. macrodactylum and A. maculatum. No pattern could be found to separate the worms from amphibians on the basis of host or worm age. There appears to be greater variation, however, in chelonian hosts since none of the 24-week flukes from C. p. belii or P. s. elegans came within the 0.2 mm. range, whereas 80% of 24-week specimens from A. tigrinum were within this distance. The range in amphibian hosts was from 0 to
0.5 mm., and in chelonian hosts from 0.3 to 0.8 mm.

**Relative lengths of cirrus sac and metraterm**

The metraterm was approximately one-half the length of the cirrus sac in all specimens regardless of host. However, in a few specimens from both amphibian and chelonian hosts the metraterm was only one-third as long as the cirrus sac.

**Relation of cirrus sac to ovary**

The cirrus sac extended to within one ovarian diameter of the anterior margin of the ovary in all specimens from the various hosts with the exception of one fluke from *A. maculatum* (Figure 47). The ovarian diameter in this worm was 0.09 mm. and the distance of the cirrus sac from the ovary was 0.14 mm. No pattern was found in the relationship of the cirrus sac to ovary in different hosts. Typical variations found in distance between these two organs are shown in Figures 48 and 49. This organ terminated (within 0.14 mm.) short of, and extended (within 0.18 mm.) beyond the anterior margin of the ovary. These measurements are approximately the same as previously obtained in worms of varying ages.

**Size of suckers**

The acetabulum was smaller than the oral sucker in 98% and the same size in 2% of flukes from amphibians regardless of host and age of worms. In *C. p. belli* the acetabulum was smaller in 83% and the same size in 17% of flukes. The greatest variation occurred in specimens from *P. s. elegans* where the acetabulum was larger than the oral sucker in 50%,
smaller in 25%, and the same size in 25%.

Investigations of Boddeke (1960) on *Prosthogonimus ovatus* established that diameters of, and proportions between the oral sucker and acetabulum depended upon whether *P. ovatus* developed in the oviduct or bursa Fabricius of avian hosts. He concluded that the proportion was of taxonomic value only if flukes being compared were of approximately the same age and size and from the same host and organ. In *T. bonnerensis*, the oral sucker was larger or of the same size in all worms from amphibian hosts. Therefore, the larger size of the acetabulum in comparison to the oral sucker in 24-week flukes from *P. s. elegans* is apparently host induced.

**Position of acetabulum**

In all specimens studied the acetabulum was median and always nearer to the ovary than the anterior end. Its position appears to be a stable taxonomic character regardless of host or age of worms.

**Comparative lengths of pharynx and esophagus**

The variation in both amphibian and chelonian hosts was similar to that found previously in worms of varying ages from *A. tigrinum*. However, in 24-week flukes from *P. s. elegans* the esophagus was longer than the pharynx in all specimens studied, while in 18-week flukes from *A. tigrinum*, the esophagus was longer in only 73%.

**Extent of intestinal crura**

Development of flukes in different host species appears to have little effect on this character, since variation is similar to that
found in worms of varying ages developing in the same host species.

**Egg size**

Comparative measurements of 20 eggs from worms developing in different hosts are given in Table 14. The variation in size of eggs

Table 14. Egg size (in mm.) of Telorchis bonnerensis when adults mature in amphibian and chelonian hosts

<table>
<thead>
<tr>
<th>Host</th>
<th>Worm age (in weeks)</th>
<th>Egg size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambystoma tigrinum</td>
<td>4</td>
<td>0.040 (0.038 to 0.042) by 0.017 (0.016 to 0.019)</td>
</tr>
<tr>
<td>A. macrodactylum</td>
<td>4</td>
<td>0.038 (0.032 to 0.045) by 0.019 (0.016 to 0.019)</td>
</tr>
<tr>
<td>A. maculatum</td>
<td>8</td>
<td>0.036 (0.031 to 0.040) by 0.018 (0.016 to 0.019)</td>
</tr>
<tr>
<td>Pseudemys scripta elegans</td>
<td>12</td>
<td>0.040 (0.035 to 0.042) by 0.019 (0.016 to 0.019)</td>
</tr>
<tr>
<td>Chrysemys picta belli</td>
<td>24</td>
<td>0.038 (0.035 to 0.038) by 0.018 (0.016 to 0.019)</td>
</tr>
<tr>
<td>A. tigrinum</td>
<td>24</td>
<td>0.040 (0.035 to 0.043) by 0.017 (0.016 to 0.019)</td>
</tr>
</tbody>
</table>
obtained from such flukes is probably not significant since the overall range (0.032 to 0.045 by 0.016 to 0.019) falls within the range previously obtained for eggs from worms of varying ages (0.032 to 0.045 by 0.017 to 0.026), and worms developing in the same host at varying constant temperatures (0.029 to 0.043 by 0.016 to 0.019).

Beaver (1937) reported that egg size varies with the age of Echinostoma revolutum and was possibly altered by the host. In the present study, eggs from 4 and 24-week worms developing in A. tigrinum are approximately the same size. A slight decrease in egg size was obtained previously, however, from 78-week worms maturing in the same host. Differences in egg size from other amphibian and chelonian hosts appear to be host-induced.

Other variations

In addition to morphological variations already discussed, considerable difference in total body size and size of certain body organs occurs between specimens from various hosts. A decrease in size of vitelline follicles similar to that observed in a few flukes developing in starved A. tigrinum was often seen in worms maturing in P. s. elegans (Figure 50).

Summary

Taxonomic characters of T. bonnerensis showing consistent stability regardless of host or worm age are anterior extent of vitellaria in relation to the ovary and ovarian diameter, comparative distance of ovary from the acetabulum and anterior testis, median position of the acetabulum, and comparative lengths of metraterm and cirrus sac. Those characters appearing less stable when worms develop in varying hosts
include the anterior extent of the vitellaria in relation to the center of the acetabulum, position of the ovary with respect to one-half total body length, extent of cirrus sac in relation to the ovary and ovarian diameter, comparative size of acetabulum and oral sucker, and size of eggs.
COMPARISON OF *TELORCHIS BONNERENSIS* 
WITH RELATED SPECIES

According to Waitz (1960), *Telorchis bonnerensis* most closely resembles *T. corti* Stunkard, 1915, a commonly found trematode of turtles. Morphological characteristics used in differentiation of adults of the two species include the anterior extent of vitellaria, posterior extent of the cirrus sac, and size of eggs.

The anterior extent of vitellaria in *T. corti*, as described by Stunkard (1915), is approximately one-third the distance from the ovary to the acetabulum, and follicles extend anteriorly to the posterior end of the cirrus sac. The cirrus sac extends posteriorly from the genital pore three-fourths of the distance to the ovary. Eggs average 0.031 by 0.015 mm. Stunkard also described *T. lobosus* and *T. medius*, both of which have been considered synonymous with *T. corti* by Wharton (1940). The anterior extent of vitelline follicles in *T. lobosus* is similar to that of *T. corti* on the left side but less on the right side, and in *T. medius*, follicles extend anteriorly to a point midway between the posterior end of the cirrus sac and the anterior margin of the ovary. The cirrus sac extends from the genital pore posteriorly to the ovary, and from genital pore four-fifths to five-sixths of distance to ovary in *T. lobosus* and *T. medius*, respectively. Eggs in *T. lobosus* according to Stunkard, average 0.032 to 0.036 by 0.018 to 0.019, and in *T. medius* 0.042 to 0.043 by 0.021 to 0.026 mm.

Wharton (1940) studied morphological characteristics of *T. corti* from several naturally infected turtle hosts including *P. elegans* and
C. picta and found considerable variation. Anterior extent of vitelline follicles varied from 0.73 mm., in P. elegans, to 0.21 mm. posterior to the center of the acetabulum in C. picta. In P. elegans, the average distance from the ovary to the acetabulum was 1.04 mm. Vitellaria ending 0.73 mm. posterior to the acetabulum would thus be situated approximately one-fourth the distance from ovary to acetabulum as compared to Stunkard's description of one-third this distance. A comparison of these distances in C. picta obtained by Wharton indicates that in this case the follicles would extend one-half the distance from ovary to acetabulum. These differences between T. corti, T. lobosus, and T. medius, which Stunkard considered to be of sufficient validity to separate them from one another, are in all probability examples of intraspecific variations. Differences between experimentally-reared individuals of T. bonnerensis and T. corti shown in the present experimental studies are similar in range, and give support to Wharton's belief that the three species described by Stunkard should be considered synonymous.

In the present study, the anterior extent of vitellaria in 24-week specimens of T. bonnerensis developing in P. s. elegans averaged 0.12 mm., and 0.21 mm. posterior in C. p. belli (Figure 52). In comparing vitellaria in 8-week specimens of T. corti from experimentally-infected C. p. belli (Figure 51) averaged 0.14 mm. posterior to the center of the acetabulum. During the course of this investigation, it was observed that in younger specimens of T. bonnerensis developing in turtles, vitelline follicles also extended further anteriad than in older specimens obtained from such hosts.

In view of the great similarity of variations in the anterior extent
of vitellaria in *T. bonnerensis* and *T. corti* when these two species mature in turtle hosts, it is apparent that this characteristic alone is unreliable in differentiating between the two species.

According to Stunkard (1915) the cirrus sac in *T. corti* extends either from three-fourths the distance between the genital pore and the anterior margin of the ovary, or may reach the anterior margin of the latter. Wharton (1940) found the average distance from ovary to cirrus sac to vary from 0.09 mm. in *C. picta* to 0.25 mm. in *P. elegans*. The average distance from ovary to acetabulum was 0.14 mm. and 1.01 mm., respectively. In both hosts, the cirrus sac extends approximately three-fourths of the distance to the ovary.

In the present study of 24-week *T. bonnerensis* the distance between the cirrus sac and the anterior margin of the ovary averaged 0.08 mm. short of the ovarian margin in *C. p. belli* which is approximately four-fifths the distance from this organ to the acetabulum, and 0.12 mm. past the anterior margin of the ovary in *P. s. elegans*. The cirrus sac in 8-week *T. corti* from experimentally-infected *C. p. belli* ranged from 0.3 mm. short to 0.08 mm. past the anterior margin.

Although the work of Wharton appears to indicate a similar relationship in the distance between the cirrus sac and ovary, and distance between ovary and acetabulum in *T. corti* from two naturally infected chelonian hosts, the experimental results obtained in this study with *T. bonnerensis* and *T. corti* maturing in turtles indicate even more variation in this morphological feature which does not appear sufficiently stable to serve as a criterion for separation of the two species.

As already pointed out in those studies concerned with differences
between flukes of varying age within salamanders of one species, between
flukes in various amphibian and reptilian hosts, as well as those shown
in temperature experiments, the size of eggs from T. bonnerensis is
subject to considerable variation. Considering the range of egg size
from the three synonymous species, T. corti, T. lobosus, and T. medius
reported by Stunkard (1915) there is little difference in egg size
between T. bonnerensis and T. corti.

The presence of T. bonnerensis in natural infections of turtles in
the Lake Okoboji region was not determined in this study. Many adult
flukes identified as T. corti have been collected by other investigators,
however, from turtles in this region. The distinguishing morphological
characteristics between adult worms of the two species are extremely
variable. Because the present investigation has shown conclusively that
T. bonnerensis is capable of developing experimentally in turtles, such
specimens were carefully compared with adult T. corti reared in similar
hosts. On the basis of adult morphology alone, it is not possible to
delineate the two species using those criteria considered by Waitz (1960)
to be of significance, namely: anterior distribution of vitellaria,
extent of cirrus sac, and egg size.

There are, however, cogent reasons for retaining the two species as
separate and distinct entities. These relate to certain aspects of the
life cycles. The first intermediate snail host of T. corti is Physa
integra; three snails serve as first intermediate hosts for T. bonner-
ensis, namely: P. gyrina, P. propinqua, and P. ampullacea. In the
present study, cross-feeding experiments involving laboratory-reared
P. integra and P. gyrina and eggs from T. corti and T. bonnerensis
resulted in infection of *P. integra* with *T. corti* and *P. gyrina* with *T. bonnerensis*, but cross infections did not occur. Negative results occurred when eggs from first generation *T. bonnerensis* developing in turtles were used to infect *P. integra*. Such eggs, however, were infective for *P. gyrina*.

Metacercariae of *T. corti* experimentally developed in laboratory-reared snails (*P. gyrina*) were non-infective when fed to larval and adult *A. tigrinum* and larval *A. macrodactylum*. Such metacercariae, however, were infective when fed to chelonian hosts, *C. serpentina*, *P. s. elegans*, and *C. p. belli*. Although *T. bonnerensis* metacercariae are infective for both amphibian and chelonian hosts, *T. corti* is apparently only infective for the latter.

On the bases of such feeding experiments with intermediate and definitive hosts, *T. bonnerensis* and *T. corti* must still be regarded as distinct species. A comprehensive study of other species currently assigned to the genus *Telorchis*, however, may well result in a considerable decrease in the number of valid species.

This study further emphasizes the necessity for extensive life-cycle experiments to determine meaningful criteria between species of helminths rather than relying solely on characteristics associated with adult morphology.
SUMMARY AND CONCLUSIONS

1. Telorchis bonnerensis Waltz, 1960, previously reported from larval Ambystoma macrodactylum and Thamnophis sirtalis in Idaho, also parasitizes larval and adult A. tigrinum in Iowa. This constitutes a new host record for the species. More than 300 adults may be present in a single definitive host.

2. Experimentally developed life-cycle stages of Iowa and Idaho strains of T. bonnerensis were compared and found to be similar in all respects.

3. Additional experimental hosts for the adult stage include adult and larval A. tigrinum, adult A. maculatum, young Pseudemys scripta elegans, Chrysemys picta belli, and Chelydra serpentina.

4. Eggs of T. bonnerensis retain their viability for as long as eight months if kept in 0.3 per cent saline at lowered temperatures. Viability decreases markedly in creek water at room temperatures, and with increasing age of adult worms.

5. Experimentally-infected snails (Physa gyrina) may shed cercariae for more than nine months: snails infected by ingestion of a single egg produce more than a hundred cercariae daily.

6. Temperature has pronounced effects on the developmental rate of T. bonnerensis larvae in intermediate molluscan hosts. Cercarial production, most rapid at 30°C., is inhibited at 4° and 10°C. At 37°C., snail mortality increases greatly. Suppression of cercarial development when snails are maintained at lowered temperatures may be modified by their transfer to higher temperatures.
7. Infective metacercariae develop in snails within 51 hours at 10°C., 12 hours at 22°C., and 6 hours at 30°C. Rate of infectivity is highest when metacercariae are reared at 22°C. for 24 hours or more. Metacercariae may retain their infectivity for as long as nine months following their encystment in laboratory-reared snails.

8. A thorough morphological study of adult *T. bonnerensis* of varying ages demonstrates conclusively that age variations occur commonly among worms of one species experimentally reared in a single host species. Characteristics used to delineate species in the genus such as anterior extent of vitellaria, posterior extent of cirrus sac and size of eggs vary considerably.

9. Growth and development of adult worms is greatly altered when experimentally-infected definitive hosts (adult *A. tigrinum*) are maintained at various constant temperatures of 10°, 22°, 30°, and 34°C. At 10°C. worms failed to mature and increased in size only slightly. Growth was accelerated at 22°C. and all flukes were gravid by four weeks. Worms from hosts maintained at 30°C. were largest in overall size, showed most rapid growth, and all specimens were gravid within two weeks. Growth is adversely affected at 34°C., but development is similar to that at 30°C. and greater in the earlier stages than at 22°C. Taxonomically important characters are not affected by growth at various temperatures. Deleterious effects of high temperature are indicated by a decrease in overall size of adults, reduced size and infectivity of eggs, and degeneration of reproductive structures.
10. Worms recovered from starved adult *A. tigrinum* were smaller, underwent less development, and were less capable of survival than were worms developed in unstarved hosts. Gravid worms were smaller and fewer than those obtained from unstarved hosts. Retardation of growth and development in flukes from starved hosts did not, however, result in any structural abnormalities, except for a slight decrease in size of vitelline follicles and the tendency for follicles to appear more diffuse in some worms.

11. Experimentally-induced effects of crowding on adult *T. bonnerensis* include an increase in numbers of recoverable worms, a decrease in average body area of individual worms, and a decline in the percentage of gravid worms. At high population levels too, the incidence of abnormal specimens increases.

12. Experimental studies demonstrate marked differences in developmental rates of adult worms when reared in a variety of salamander and turtle hosts. Most rapid development occurs in *A. tigrinum* and in *A. macrodactylum*. In *A. maculatum*, rate of maturation is decreased. Among chelonians, development is most rapid in *Chelydra serpentina*, and least in *Pseudemys scripta elegans*.

13. The rearing of successive generations of adults in amphibians, then in reptiles, and once again in amphibians, results in a pronounced decrease in size and delayed development in the generation reared in turtles. Resumption of normal size and developmental rate occurs when progeny are reared once again in amphibians. Delayed maturity in reptilian hosts thus appears to be host-induced.
14. With continued experimental passage of *T. bonnerensis* from turtle to turtle, development is accelerated as a result of adaptation to altered host environment and the time required for sexual maturity is lessened.

15. A careful study of morphological variations of adult worms experimentally reared in amphibians and reptiles shows conclusively that criteria such as anterior extent of vitellaria, position of ovary, extent of cirrus sac, comparative sizes of suckers, and egg size are subject to considerable variation and cannot be relied upon as entirely valid characteristics in delimiting species.

16. Despite morphological similarities between adult *T. bonnerensis* and the closely related species, *T. corti*, experimental evidence indicates that the two must be retained as separate and distinct species, due to differences in their first intermediate and definitive hosts.
LITERATURE CITED


Freeman, R. S. 1952. Temperature as a factor affecting development of Monoecocestus (Cestoda: Anoplocephalidae) in oribatid mites. Experimental Parasitology 1: 256-262.


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This investigation was supported in part by research grants (G 23597 and GB 2384) from the National Science Foundation.
Plate I

Figure 1. Adult *Telorchis bonnerensis* attached by acetabulum to another adult of the same species

Figures 2 through 5. Three-week adult *T. bonnerensis* (Iowa and Idaho strains) experimentally developed in adult *Ambystoma tigrinum* and larval *A. macrodactylum* (all photographed to same scale)

Figure 2. Iowa strain from *A. tigrinum*

Figure 3. Iowa strain from *A. macrodactylum*

Figure 4. Idaho strain from *A. tigrinum*

Figure 5. Idaho strain from *A. macrodactylum*
Plate II

(All figures drawn to same scale as shown in Figure 15)

Figures 6 through 9. Variations in anterior extent of vitellaria with reference to the center of acetabulum in adult Telorchis bonnerensis of different ages experimentally developed in adult Ambystoma tigrinum

Figure 6. Eighteen-week adult (vitellaria posterior on both sides)
Figure 7. Eighteen-week adult (vitellaria even on both sides)
Figure 8. Four-week adult (vitellaria anterior on both sides)
Figure 9. Four-week adult (vitellaria anterior on one side, posterior on other side)

Figures 10 through 12. Variations in position of the cirrus sac with reference to ovary in adult T. bonnerensis of different ages experimentally developed in adult A. tigrinum

Figure 10. Four-week adult
Figure 11. Seventy-eight-week adult
Figure 12. Seventy-eight-week adult

Figures 13 through 15. Variations in posterior extent of intestinal crura in adult T. bonnerensis of different ages experimentally developed in adult A. tigrinum

Figure 13. Eighteen-week adult
Figure 14. Seventy-eight-week adult
Figure 15. Four-week adult
Plate III

Figure 16. Vitelline follicles in 5-week adult Telorchis bonnerensis experimentally developed in adult Ambystoma tigrinum

Figure 17. Vitelline follicles in 78-week adult T. bonnerensis experimentally developed in adult A. tigrinum (note reduced size and number of follicles)

Figure 18. Testis of 78-week adult T. bonnerensis experimentally developed in adult A. tigrinum (note decrease in testicular cells)
Plate IV

Figures 19 through 22. Effects of varying temperature on adult *Telorchis bonnerensis* experimentally developed in adult *Ambystoma tigrinum*

Figure 19. Four weeks at 10°C
Figure 20. Four weeks at 22°C
Figure 21. Four weeks at 30°C
Figure 22. Four weeks at 34°C

Figures 23 through 26. Effects of starvation on 4-week adult *T. bonnerensis* experimentally developed in adult *A. tigrinum*

Figure 23. Largest, gravid adult from non-starved host
Figure 24. Largest, gravid adult from starved host
Figure 25. Mature adult from starved host
Figure 26. Immature adult from starved host
Plate V

Figures 27 through 30. Effects of crowding on 4-week adult *Telorchis bonnerensis* experimentally developed in an adult *Ambystoma tigrinum* harboring 377 flukes

Figure 27. Immature adult

Figures 28 to 30. Abnormalities in body shape

Figures 31 through 34. Effects of different definitive hosts on experimentally-developed, 8-week adult *T. bonnerensis*

Figure 31. Gravid adult from *A. tigrinum*

Figure 32. Gravid adult from *Chelydra serpentina*

Figure 33. Gravid adult from *Chrysemys picta belli*

Figure 34. Mature adult from *Pseudemys scripta elegans*
Plate VI

Figures 35 through 37. Effect of definitive hosts on successive generations of experimentally-developed adult Telorchis bonnerensis (all photographed to same scale)

Figure 35. Forty-four-week, gravid adult from adult Ambystoma tigrinum

Figure 36. Twelve-week, gravid adult from Pseudemys scripta elegans

Figure 37. Twenty-three-day, gravid adult from larval A. tigrinum

Figures 38 through 40. Effect of definitive hosts on 24-hour, experimentally-developed adult T. bonnerensis

Figure 38. Adult from A. tigrinum

Figure 39. Adult from Chrysemys picta belli

Figure 40. Adult from P. s. elegans
Plate VII

(All figures drawn to same scale as shown in Figure 49)

Figures 41 through 46. Variations in size and distribution of vitellaria in adult Telorchis bonnerensis experimentally developed in various definitive hosts.

Figure 41. Twenty-four-week adult from adult *Ambystoma tigrinum*

Figure 42. Twenty-four-week adult from *Chrysemys picta belli*

Figure 43. Twenty-four-week adult from *Pseudemys scripta elegans*

Figure 44. Eight-week adult from *A. maculatum*

Figure 45. Three-week adult from *A. macrodactylum* (Idaho strain)

Figure 46. Three-week adult from *A. macrodactylum* (Iowa strain)

Figures 47 through 49. Variations in extent of cirrus sac with reference to ovary in adult *T. bonnerensis* experimentally developed in various definitive hosts.

Figure 47. Eight-week adult from *A. maculatum*

Figure 48. Twenty-four-week adult from *C. p. belli*

Figure 49. Twenty-four-week adult from *C. p. belli*
Figure 50. Vitelline follicles in 12-week adult *Telorchis bonnerensis* experimentally developed in *Pseudemys scripta elegans* (note diffuse nature of follicles)

Figure 51. Eight-week, gravid adult *T. corti* experimentally-developed in *Chrysemys picta belli*

Figure 52. Twenty-four-week, gravid adult *T. bonnerensis* experimentally developed in *C. p. belli*