Intraintestinal sites of infection for the trematode species *Plagitura salamandra* Holl 1928 and *Plagitura parva* Stunkard 1933 in red-spotted newts *Notopthalmus v. viridescens* (Rafinesque)

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Jeremy T. Clay

Marshall University
Huntington, WV

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Advisor: James E Joy

Committee: Donald Forster

Committee: Paul Green

Department of Biological Sciences

Leonard J Deutsch

Dean of the Graduate College

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Abstract

The red-spotted newt, *Notopthalmus v. viridescens* (Rafinesque) is a common salamander in the eastern United States. Studies on the parasites are available, however these studies examined total parasite fauna. This study examines two species, *Plagitura salamandra* Holl 1928 and *Plagitura parva* Stunkard 1933 found in the small intestine of the red-spotted newt. A total of 225 newts was collected and examined for the two *Plagitura* species. No difference was found in prevalence of infection, mean intensity, SVL, and weight between male and female newts. *Plagitura parva* was found in 24.4 % of the newts while *P. salamandra* was found in 32.9 % of the newts. While examining the small intestine for site of infection, a greater number of *P. parva* was found in the anterior and middle portion of the small intestine ($X^2 = 9.138$), while a greater number of *P. salamandra* was found in the posterior small intestine ($X^2 = 223.397$) suggesting competitive exclusion between the two species.

Chapter I - Introduction

Purpose of Study

The red-spotted newt, *Notopthalmus viridescens viridescens* (Rafinesque), is a common salamander in the eastern portion of the United States, primarily east of the Mississippi River. There has been some work done on parasites of the red-spotted newt, particularly Jackson and Beaudoin (1967) and Price and Buttner (1982). Each of those studies examined total parasite fauna of the newt. The two papers reported *Plagitura* sp., but ecological studies on this trematode species are lacking. Then, too, this is the first report of *Plagitura* from West Virginia.

Two *Plagitura* species, *Plagitura parva* Stunkard 1933 and *P. salamandra* Holl 1928 are found in the small intestine of the newt. This investigation is concerned with the prevalence of *P. parva* and *P. salamandra*, the infection rate (as mean intensity of infection), and specific location of trematode infection in the host's alimentary tract.

Description of Host Species

Notopthalmus viridescens viridescens Life Cycle

The red-spotted newt has a distinctive four-stage life cycle: egg, aquatic larval, terrestrial red eft, and the aquatic adult. Adults mate from March to June.

The eggs are laid singly on aquatic plants, decaying leaves or other detritus. Depending on water temperature eggs hatch in 20 to 35 days (Bishop 1941, Gage 1891).

The larvae are generalist carnivores and remain in the water from hatching till two to five months old, however few larvae overwinter in the water. High larval mortality allows 1 to 2% of larvae to metamorphose to the terrestrial red eft stage (Gill 1978).

Once on land the eft is brightly colored and very active. This coloration is indicative of its toxic nature. The efts, found in leaf litter, are opportunistic feeders and aggregate around rotting mushrooms where invertebrates are very active (MacNamara 1977). The eft stage ranges from one year to seven years duration depending on locality.

The eft stage is abundant in forest floor habitats until maturity, then the eft migrates to breeding habitats where the efts transform to mature adults (Petranka 1998).

The mature adult remains in the water for courtship and mating, but can travel onto land to feed, overwinter, or dispose of ectoparasites. The adult may travel onto land if water level lowers and temperature reaches high levels (Gill 1978, Hurlbert 1969).

Notopthalmus viridescens viridescens Identification

The aquatic adults have lungs and are light yellow dorsally and dark olive green ventrally. The skin is slightly granular and black spots are

scattered over the entire body. Red spots located ventrally are bordered by black accents. The tail makes up about 50 % of the entire length and is keeled above and below.

Sexual dimorphism in this species is exhibited. The male cloaca is round and bulbous while the female cloaca is elliptical and has a slit-like vent. This characteristic can be seen year round. Mating adult males possess black pads, or excrescences, on the toes and inner thighs of the hind legs. This characteristic is only seen in mating season on the males.

Red efts are terrestrial and have coarse granular skin. The skin ranges from bright vermilion to dull red in coloration. The red spots as seen in the adults are also seen in this stage (Petranka 1998).

The hatchlings are round with a balancer on both sides of the head. They are yellowish green in coloration with dark bars extending the length of the back (Bishop 1941). Older larvae are light brown to yellowish brown and have a slender body and a blunt snout. A dark stripe extends from the snout through the eyes (Petranka 1998).

Host Taxonomy and Distribution

Rafinesque originally named the red-spotted newt *Triturus viridescens* in 1820. Other generic names were used in the mid-1800's, including *Notopthalma*, *Triton*, and *Malge.* In 1962, the International Commission established *Notopthalmus viridescens* as the official taxonomic name (Piascik 1997).

The red-spotted newt is the second most widely distributed salamander in North America. Populations follow the extent of the Appalachian Mountains from Southern Canada to Northern Georgia and Alabama and from the Atlantic coast west to Michigan and western Kentucky (Figure 1) (Petranka 1998).

Parasite Taxonomy

Holl (1928) erected the generic name *Plagitura* to contain a species of flukes found in the newt *Notopthalmus viridescens viridescens* in Durham, North Carolina. The description of the species is based on the type specimen represented in Figure 1A. Holl named the parasite *Plagitura salamandra*.

Kelley (1934) reported on the great variation of the species from specimens collected in Massachusetts, Pennsylvania, Ohio, and Virginia. This paper noted significant differences in body size, sucker size, and form and location of the gonads. She also noted that the cuticle was armed with spines, whereas Holl (1928) reported that the cuticle was unarmed. Stunkard (1936) noted these differences. He found a difference in body morphology and in a primary host, the gastropod *Helisoma anceps* (Conrad). He named the other species *Plagitura parva* (Figure 1B).

Owens (1946) studied the life history of *Plagitura salamandra* and determined the primary intermediate host to be the snail *Pseudosuccinea columnella*, whereas Stunkard had determined the primary intermediate host as *Helisoma anceps*. Thus, the difference in primary intermediate host

utilization is an important factor distinguishing these two trematode species, along with other characteristics like sucker size and location of gonads.

Description of Trematode Species

Plagitura parva

The species *Plagitura parva* (Fig. 1B) is found in the small intestine of *Notopthalmus v. viridescens*, the red-spotted newt. Once the eggs (Fig. 2A) of this trematode species are excreted in the feces, they have to incubate in water for about two days before hatching. A ciliated miracidium is released from the egg and this larva infects the primary intermediate host, a gastropod mollusc, *Helisoma anceps*. While in the intermediate host, the miracidia penetrate the snail's intestine and migrate to the adjacent lymph spaces. The miracidia then transform into mother, or primary, sporocysts. As germinal cells multiply in the sporocyst, germ masses form (Fig. 2B). For about ten days, the primary sporocyst is irregularly oval but later the germ masses increase in size and bring about a distention that causes the sporocyst wall to elongate and become a shapeless, thin-walled sac. The birth pore is located at the anterior end of the primary sporocyst where the developed secondary, or daughter sporocyst, emerges.

Ultimately many daughter sporocysts emerge, and they become associated with interlobular connective tissue and the lymph spaces of the digestive gland of the snail intermediate host. They may be also found in the peripheral sinuses near the snail's body wall. Young daughter sporocysts

develop. Inside the daughter sporocyst, germ masses develop. The daughter sporocyst increases in size and fills with larvae. These larvae increase in size and develop into tailed forms called cercariae (Fig. 2C). These cercariae emerge from birth pores on one end of the daughter sporocyst.

Once the tailed cercariae emerge, they migrate through tissues toward the cephalic end of the snail. Sometimes, the cercariae develop and encyst inside the daughter sporocyst, which causes the snail to be used as a secondary intermediate host. Development is usually completed upon emergence from the daughter sporocyst.

At first, infected snails give off cercariae in great numbers. Several hundred may emerge from a snail in twenty-four hours. Some cercariae do not mature before escaping from a snail and die soon after emergence. If immature cercariae survive, they are uninfective and cannot penetrate into a second intermediate host and soon die. Cercariae emerge both at day and night, and if a light source is present, they tend to migrate toward the light. While swimming, the cercarial body is situated concave with the ventral side up while the tail lashes vigorously from side to side. This takes the cercaria upward and forward. It begins to sink as soon as the body relaxes and the tail ceases to beat. After a time swimming, the cercariae creep along the substrate of the pond.

Once the appropriate second intermediate host is found, the cercariae, with help from penetration gland excretions and a stylet imbedded in the oral sucker (Fig. 3), burrows into one of numerous species of aquatic animals (e.g.

hellgrammites, and various species of snails) which serve as a secondary intermediate hosts. The cercariae encyst in this secondary intermediate host using cystogenous glands. A capsule forms around the encysted cercaria and this life cycle becomes, by definition, the metacercarial stage. The metacercariae may undergo additional development while encysted, but do not increase in size.

Newts become infected by ingesting a secondary intermediate host infected with the metacercaria. In the newt intestine, the cyst splits on one side and the worm escapes. The cyst wall, although thin and flexible, is tough and can tear the metacercaria as it escapes. Since very little development occurs in the metacercarial stage, the second intermediate host is utilized primarily as a transfer agent.

Once escaping the cyst in the intestine of the newt, the metacercaria begins development to the adult. After two days, some development has occurred but little size difference can be seen. Twelve days after ingestion, adult organs are clearly outlined, but the female reproductive system has not attained full development (Fig. 4). Eighteen days after ingestion, all the organs of the trematode are actively functional (Fig. 5) (Stunkard 1936).

Plagitura salamandra

The species *Plagitura salamandra* also occurs in the small intestine of the red-spotted newt. The eggs, once excreted through the vent in the feces, need to incubate in pond water for about four days before becoming infective.

The eggs hatch once the primary intermediate host, a gastropod *Pseudosuccinea columnella* (Say), ingests them. Thirty-six hours after ingestion the miracidia that emerge from the eggs are found attached to the outer wall of the intestine. All attempts on infecting specimens of *Helisoma anceps* were negative (Owens 1946).

Once attached to the outer intestinal wall, the miricidia begin transformation to primary, or mother, sporocysts. These primary sporocysts are localized to the lymph spaces adjacent to the outer intestinal wall. Figure 6A shows the sporocyst with germ masses. Once the germ masses begin to mature, they enlarge the mother sporocyst. Each mature germ mass, and there are many such forms developing, becomes a secondary or daughter sporocyst. The secondary sporocyst emerges form the birth pore and migrates to the host's liver. These secondary sporocysts (Fig 6B) are usually larger than the primary sporocyst. The germ masses become differentiated and clustered into cercaria-like larvae toward the anterior end. Once mature enough, the tailed cercariae emerge through the birth pore and begin the process of escaping the snail.

Before leaving the snail, the cercariae (Fig. 7A) undergo rapid development. The behavior is similar to that of *Plagitura parva* stated in the preceding section. The cercariae utilize *Sympetrum, Leucorhinia,* and other odonates, adult backswimmers from the family Notonectidae, and *Pseudosuccinea columnella* as second intermediate hosts. Once inside the second intermediate host, the cystogenous gland excretes a substance to

form the cyst (Fig. 7B). The cyst wall is thin and colorless. The cyst description is similar to the one of *Plagitura parva*, as stated in the preceding section.

As with *P. parva*, newts become infected when ingesting infected secondary intermediate host. Upon ingestion, the fluke emerges from the cyst and continues its development to the adult form.

Chapter II - Materials and Methods

Study Site

The study site was located at a marshy area in Shoals, WV (USGS Topographic Map; Lavalette Quad; MG ^{3}7120 ^{47}4310). This area is an oxbow of Twelvepole Creek in Wayne County, West Virginia. It is a low elevation site about 200 m above sea level. The marsh is about 100 m north from the intersection of Plymale Branch Road and Rt. 75 (Figs. 9 A and B). Figures 10 and 11 show the marsh in the February collection of 1995. The depth and surface area of the pond depends on the climatic conditions. During spring and winter, the oxbow connects with Twelvepole Creek. Summertime conditions cause the marsh water to evaporate severing the connection with the creek.

Surrounding the oxbow are different zones: open water, limnetic, margin and outermost zone. The open water zone is dominated by least duckweed, *Lemna minor* L. The limnetic zone is dominated by buttonbush, *Cephalanthus occidentalis* L.; Black willow, *Salix nigrum* Marshall; buttonbush, *C. occidentalis* L.; and swamprose, *Rosa palustrus* Marshall encircle the margin of the pond. The outermost zone is encircled by Americana elm, *Ulmus americana* L.; green ash, *Fraxinus pennlylvanica* Marshall; river birch, *Betula nigra* L.; and sugar maple, *Acer sacchrum* Marshall (Evans 1972).

The site is an area where humans are moving in and causing some habitat alteration. The study was prematurely stopped prior to the fall collection of 1999 because of habitat alteration by the landowner.

Collection

Newts were collected over a period of four years, 1996 to 1999 (Table 1). After capture, newts were transported to the lab in a twenty-liter bucket containing marsh water at ambient temperature. Once back in the lab, winter specimens were maintained in a refrigerator at approximately 4° C. Summer newts were kept in an aquarium at ambient temperatures. All newts were necropsied within 48 hours upon return from the field.

Newts were killed by pithing. After pithing, the newt was sexed based upon external morphological characteristics using the excrescences on the males. If host sex was ambiguous, the sex was determined by identifying the reproductive organs when the newt's abdomen was opened. The newt was weighed to the nearest 0.01 gram using a Mettler BB300 balance and the snout-vent length (SVL) measured to the nearest 0.1 mm using Vernier calipers. Mean weights and SVL's are shown in Table 2. The small intestine of each newt was removed and divided into three regions of equal lengths. The three regions were designated as anterior, middle, and posterior small intestine. Each region of the intestine was then slit lengthwise with sharpened jewelers forceps to expose the contents.

As the intestines were cut, *Plagitura parva* and *P. salamandra* individuals were extracted. Moving from anterior to posterior, when a fluke

was found, the numbers of flukes and the location of finding were recorded. Next, the flukes were flattened by slight coverslip pressure and fixed with 10 percent buffered formalin for approximately five minutes. Each fluke was preserved in 10 percent buffered formalin in a properly labeled bottle, including host number and location in the gut, until staining. Semichon's acetic carmine stain was used (Meyer and Olsen, 1975). Selected worms were dehydrated in an ethanol series, cleared in xylene, and mounted in Canada Balsam. After staining, all individuals were identified.

Statistical Analyses

A correlation of SVL and weight was performed on the data. Infection differences in prevalences were evaluated with a chi-square 2 X 2 contingency table. Differences in SVL and weight of male versus female newts were compared using a t-test. T-tests were also used to determine if infected and uninfected newts' size (i.e. SVL and weight) differed significantly. Finally, a chi-square goodness of fit test was performed to determine if a difference exists in the location of the trematodes in the small intestine; the null hypothesis being that trematode being that trematode individuals of each species were equally distributed throughout all three designated intestinal regions.

Statistical analyses were performed according to Sokal and Rohlf (1995). P values of < 0.05 were considered significant for all tests. Prevalence of infection and mean intensities follows the definition of Bush et al. (1997).

Chapter III - Results

Overview

Two species of *Plagitura*, *Plagitura parva* and *P. salamandra*, were found in the small intestine of the red-spotted newt, *Notopthalmus v. viridescens.* A total of 225 newts was collected from Shoals Marsh, located in Shoals, West Virginia (Fig. 9 B) during the course of the study. Table 1 shows the dates of collection for the different years. Table 2 shows the mean snout-vent length (SVL) and mean weight of all newts.

One hundred twenty newts were infected in this study. The infections were a single infection, either *Plagitura parva* or *P. salamandra*, dual infection involving both species *P. parva* and *P. salamandra*, and infections with the metacercarial stage (i.e., immature form) that cannot be identified to species. Seven newts (3.1%) were infected with metacercariae. One hundred thirteen of the newts (50.2%) were infected with adult *P. parva*, adult *P. salamandra*, or both adult trematodes. Forty newts (17.8%) were infected with *P. parva* only (single infection) and 59 (26.2%) newts were infected with *P. salamandra* only (single infection). Fourteen newts were infected with both species together (6.2%).

A total of 54 newts, counting single and dual infections, had an infection of *Plagitura parva* while 73 newts, counting single and dual infections, had an infection of *P. salamandra*. One hundred eighty two individuals of *P. parva* were recovered from infected newts. Two hundred

forty nine individuals of *P. salamandra* were recovered from the infected newts. The mean intensity of *P. parva* was 3.43 parasites per infected individual (182/53). The mean intensity of *P. salamandra* was 3.40 parasites per infected individual (249/73). Table 3 shows the mean intensities of infection do not significantly differ between the two species ($P = 0.95$). Table 4 shows the mean intensity of infected males and the mean intensity of infected females did not differ either ($P = 0.48$).

Metacercaria

Seven newts were infected with the metacercarial stage, four of the newts in winter months (December and February) and three in the summer (August).

As a part of the life cycle of *Plagitura parva* and *P. salamandra*, these worms undergo a metacercarial stage of development. At this stage of development, the flukes are not fully mature and cannot be identified to species based on adult characteristics used for identification. Thus only the newts infected with adult *P. parva* and *P. salamandra* were included in the calculations for the statistical analyses of the regression or chi-square analyses.

Host t-tests

The first type of analysis performed was on SVL and weight of males and females using unpaired two-tailed student t-tests. Table 5 shows that

mean SVL of male newts at 47.9 mm was larger than mean SVL of female newts at 47.8 mm. The difference was not significantly different (t = 0.194; df = 223; P = 0.85). Mean weight of males at 3.68 did not differ significantly from mean female weight at 3.92 g (t = 1.609; df = 223; P = 0.11).

The next analysis was an unpaired, two-tailed student t-test on infected and uninfected newts. Table 6 shows that SVL of infected newts, 49.2 mm, was significantly larger than the SVL of uninfected newts, 46.3 mm (t = 4.65; df = 223; P <0.001). This table also shows that the weight of infected newts at 4.09 g was also significantly larger than the 3.40 g of uninfected newts (t = 5.53; df = 223; P < 0.001).

Another unpaired, two-tailed student t-test was performed on infected males and infected females (Table 6). Seventy-five infected males had a mean of 48.8 mm which was not significantly different from the mean of the forty-five infected females of 48.7 mm (t = 0.163; df = 118; P = 0.25). Table 7 also shows that the infected male weight of 4.00 g was not significantly different from the 4.25 g of infected females (t = 1.164; df = 118; P = 0.25).

Chi-square Contingency Tables for Prevalences

This section determines if the prevalences of different variables are significantly different. A chi-square 2 X 2 contingency table was constructed to test if differences exist between prevalences of numerous sets of data. Yates's correction of continuity was included in all of the calculations for the chi-square 2 X 2 contingency tables. The first contingency table was to test

the prevalence of infection of male versus female newts. Altogether 50.0 percent males were infected (70/140) while 52.9 percent of the female newts were infected (45/85). Table 8 shows no significant difference in prevalence of infection (X^2 = 0.003; df = 1; P = 0.96). This analysis allowed infected males and infected females to be lumped together (i.e., increasing total sample size) for future statistical analyses.

A second chi-square 2 X 2 contingency table constructed was for the infections of *Plagitura parva* and *P. salamandra* examined separately. This tested the differences in prevalences of these species in the newt. Fifty-four newts had an infection of *P. parva* (24.4%) while 73 newts were infected with *P. salamandra* (32.9%). As seen in Table 9, there is no difference in the infection of *P. parva* and *P. salamandra* (X^2 = 3.346; df = 1; P = 0.054). This analysis allows the numbers of infected newts with *P. parva* to be lumped together with the newts infected with *P. salamandra*.

A chi-square 2 X 2 contingency table was constructed to compare a type of infection, i.e., if the newt was infected with adult flukes or flukes in the metacercarial (i.e. pre-adult) stage of development. The newts infected with adult trematodes 50.2 percent (113/225) were significantly different from the 3.1 percent (7/225) of newts that were infected with the metacercarial stage of the fluke's life cycle. Table 10 shows there is a difference in the type of infection, whether it was an adult or metacercarial infection (X^2 = 114.832; df = 1; P < 0.001). This shows that there is a highly significant difference of prevalence in adult infections than in metacercarial infections.

A chi-square 2 X 2 contingency table was constructed to test another type of infection. Table 11 is concerned with the infection of adult trematodes, i.e. if the type of infection, single infection, either *P. parva* or *P. salamandra*, and dual infection, both species, differed. As seen in Table 11, the newts that had a single infection, n = 99, was significantly different from those newts that harbored a dual infection, n = 14 (X^2 = 73.91; df = 1; $P < 0.001$). This shows that newts were far more likely to be infected with single infections, that is either *P. parva* or *P. salamandra* in the small intestine, than dual infections, that is both species of *P. parva* and *P. salamandra*.

Correlation and Regression Analyses

The correlation analysis determines if one variable is dependent on the other variable. The correlation performed on the data determines if newt weight is correlated to host SVL. Figure 11 shows a scatter diagram of the weight of the newt and the SVL of the newt. Shown with the regression line, as the host length increases, the weight also increases ($\hat{y}$ = -2.90 + 0.139x; F = 176.40). From the data, the Pearson's correlation coefficient (r) of 0.664 shows a high correlation between weight and SVL of the newts. Since these are highly correlated ($P < 0.01$), there is no need to regress the numbers of *P. parva* and *P. salamandra* individuals against both host weight and host SVL.

The regression analysis determines if a relationship exists between two variables; the null hypothesis being that the slope of the regression is equal to zero (i.e. H_0: b = 0). If the slope of the line produced is equal to zero,

then there is not a relationship between the two variables. The first regression carried out was on host SVL and number of individuals of *P. parva* found in the small intestine of the newt. Figure 12 shows a scatter plot of the data and the regression line. The regression line shows no relationship between the SVL of the newt and the numbers of *Plagitura parva* in the small intestine (b = 0; $F_{0.05, 51}$ = 0.333). Also, there is no correlation between the number of *P. parva* individuals and the host SVL (r = 0.081).

The next regression carried out was done on the relationship of host SVL and numbers of *P. salamandra* found in the small intestines of the newt. Figure 13 shows a scatter plot of SVL of infected newts with *P. salamandra* versus the number of individuals of *P. salamandra*. This shows that the number of *P. salamandra* individuals is a function of host SVL ($\hat{y}$ = -11.61 + 0.305x; $F_{0.05, 71}$ = 14.13).

Goodness-of-fit

A goodness-of-fit test performed was on the type of infection, whether it was a single infection, either *P. parva* or *P. salamandra*, a dual infection, both *P. parva* and *P. salamandra*, or a metacercarial infection. The single infection contributes most to the total chi-square value (X^2 = 131.150; df = 2; $P < 0.001$) (Table 12). There is not a uniform infection for the trematodes in the newt.

A second goodness-of-fit test is looking for uniform distribution of a variable throughout a sample. The distribution of the two parasites, *Plagitura*

parva and *P. salamandra*, along the small intestines of newts is not uniform. As stated in the Materials and Methods section, the small intestine was divided into three equal sections; the anterior, middle, and posterior small intestine. Table 13 shows the goodness-of-fit test for *Plagitura parva*. A total of 182 individuals of *P. parva* was found in fifty-four newts. As can be seen in Table 10, the proportion of the number of observations decreases from the anterior small intestine to the posterior small intestine. The results do not show a uniform distribution for *P. parva* (X^2 = 9.138; df = 2; P = 0.011). The numbers of *P. parva* located in the posterior portion of the small intestine contributes most (63% of the total to the total X^2) to the chi-square value.

A final goodness-of-fit test was performed on the distribution of *Plagitura salamandra*. A total of 249 individuals of *P. salamandra* was found in seventy-three infected newts. The results do not show a uniform distribution for *P. salamandra* in the small intestine of the newt (X^2 = 223.397; df = 2; P < 0.001). The high number of *P. salamandra*, which were found in the posterior portion of the small intestine, contributed most (66.4% of the total X^2) to the total chi-square value (Table 14).

Chapter IV – Discussion

Two *Plagitura* species, *P. parva* and *P. salamandra*, were recovered from the small intestine of a total of 225 red-spotted newts, *Notopthalmus v. viridescens*. Seven newts were infected with a metacercarial (pre-adult) stage of *Plagitura* sp. These juvenile forms could not be identified to species because they had not developed certain adult characteristics needed to identify the flukes.

The data for the metacercarial stage was not included in the statistical calculations for type of infection of adult trematodes or for the regression analyses of adult trematodes.

Host t-tests

The first statistical analysis performed was a t-test on the snout-vent length (SVL) and the weight of male and female newts. The mean SVL and weight of male and female newts were not significantly different ($P = 0.85$, $P = 0.11$, respectively). Caetano and LeClair (1996) and Dyer, et al. (1980) found that the male and female salamanders seem to be equal in length. This allows for the inclusion of male and female SVL and weight data for future analyses. If a difference in the data had existed, the male and female data would have to be treated as independent groups for analyses.

A second t-test was performed on the SVL and weight of infected versus uninfected newts. In both calculations of host SVL and weight, the

infected newts were found to be larger than the uninfected newts ($P < 0.001$, $P < 0.001$, respectively). Joy and Dowell (1994) and Tucker and Joy (1996) show that host weight in uninfected frogs was significantly different than in infected frogs. However, those studies show that infected frogs weighed less than uninfected frogs, which is the opposite of what was discovered in this study.

The infected male SVL and weight and infected female SVL and weight were compared with a student t-test. In both tests, there were no difference on host size ($P = 0.87$, $P = 0.25$ respectively). Thus, infected male data were combined with the data for infected females.

Chi-square Contingency Tables for Prevalences

The next series of statistical analysis performed was the chi-square 2 X 2 contingency tables for prevalences. All contingency tables include the Yates's correction for continuity. The first table was to determine if adult trematode prevalence in male and female newts differed significantly. Table 8 shows no difference of trematode prevalence between male and female newts ($P = 0.96$). Fortner (1923, p. 85) stated that the "-- percentage of infection between the two sexed does not differ to any great extent." The current study agrees with other studies, which reported that male and female amphibians show no differences in infections of helminths (Jackson and Beaudoin 1967, Dyer et al., 1980, Price and Buttner 1982, Joy and

Pennington 1998). Muzzall and Peebles (1991) showed that the prevalences in male and female frogs were not different.

A 2 X 2 contingency table was constructed to test if a difference exists between the infection of adult *P. parva* and *P. salamandra* in infected newts. Table 9 shows no difference in prevalence of adult *P. parva* and *P. salamandra* in the small intestine of infected newts ($P = 0.054$).

The type of infection of the flukes, that is either the metacercarial (pre-adult) stage of development or the adult stage of development, was the next variable tested. Adult newts were far more likely ($P < 0.001$) to be infected with adult *Plagitura parva* or *P. salamandra* than with metacercarial forms. This study corroborates Stunkard (1936) by showing that the flukes of *P. parva* mature rapidly from the metacercarial (pre-adult) stage to the adult stage. In a period of 12 days, the organs were clearly outlined but the female reproductive system was not yet mature (Fig 4). In a period of eighteen days the reproductive organs were found to be fully active and eggs were produced (Fig 5). Owens (1946) did not test to see how long a *P. salamandra* metacercaria to develop to adult. Owens did, however, show a metacercaria at eleven and one half days of development from the newt intestine.

A final 2 X 2 contingency table was constructed to determine if the newts with single infections differed significantly from the newts which harbored dual infections. Single infections included the newts that had either species *P. parva* or *P. salamandra* while dual infections included the newts that had both trematode species. Table 11 shows a significant difference in

the type of infection ($P < 0.001$). When one species of *Plagitura* is present, it is more likely to be the only species of *Plagitura* found in the small intestine. When the dual infection was found, it could have been that competition between the two species had not begun or that their spatial separation in the host's intestine was sufficient enough to preclude competition.

Correlation and Regression Analyses

A correlation of newt SVL and weight was performed. The Pearson's correlation coefficient value of 0.664 shows a definite correlation between the host SVL and host weight ($P < 0.01$). The test show that as host SVL increases, the weight also increases, as one would expect. These data will allow for the assumption of regression analyses of host weight to coincide with the data from the regression analyses of host SVL.

A regression analysis of the number of individuals of *P. parva* as a function of host SVL was performed (Fig 12). There is no relationship between the number of *P. parva* individuals found in the small intestine of an infected newt and the SVL of an infected newt ($F_{0.05, 51} = 0.333$). The numbers of individuals found in the small intestine are not affected by the host SVL, even though there is a negative slope of the regression line ($\hat{y} = 6.83 - 0.069x$). Joy and Dowell (1994) and Tucker and Joy (1996) show that the number of *Glypthelmins pennsylvaniensis* is not a function of host weight. Wetzel and Esch (1996) show that there is not a correlation in the number of

Halipegus occidualis and the host (*Rana clamitans*) size. The numbers of *P. parva* in the host intestine agrees with the findings of those studies.

A regression analysis was performed on the total number of individuals of *P. salamandra* in the small intestine of an infected newt as a function of host SVL (Fig 13). The number of *P. salamandra* individuals in the small intestine of an infected newt increase significantly with the host SVL ($\hat{y}$ = -11.61 + 0.305x; $F_{0.05, 71}$ = 14.13). Wetzel and Esch (1996) show a correlation between numbers of *Halipegus eccentricus* and host (*Rana clamitans*) size. The present study agrees with those findings. Joy and Dowell (1994) and Tucker and Joy (1996) show that the number of *Glypthelmins pennsylvaniensis* is not a function of host weight. The numbers of *P. salamandra* in the small intestine do not agree with those studies. Larger newts incorporate larger prey in their diet (Petranka 1998). *Plagitura salamandra* is the larger species and may be located in the larger prey for the newt.

Goodness-of-fit

The goodness-of-fit test checks for uniform distribution of a variable throughout a sample. Table 12 shows the type of infection found in the newts. The type of infection, defined here, is a single infection, either species of *Plagitura*, dual infection (i.e., both species of *Plagitura*), or metacercarial (pre-adult) infection. A total of 120 newts were infected; of these, 99 newts had a single infection, 14 newts had a dual infection, and seven newts had a

metacercarial infection. The chi-square value of the single infection, 87.025, was the greatest contributor to the total chi-square value of 131.150 (Table 10). This shows that the single infection was the predominant type of infection in the newts. When compared to the numbers of dual infections, 14, there is a significant difference in the prevalence. This test shows a type of exclusion of one species when the other is present. If this were not true, then the numbers of dual infections would be equal to the numbers of single infection.

A goodness-of-fit test was performed on distribution of *P. parva* in the small intestine of the newt. The null hypothesis states that *P. parva* is uniformly distributed in the small intestine. Table 13 shows that the number of *P. parva* found in the posterior portion of the small intestine, 42 (5.761), contributes most to the total chi-square value. A small number of *P. parva* is found in the posterior small intestine than expected. Table 10 shows that the single infection is more prevalent than the dual infection. Therefore, one can conclude that *P. parva* is predetermined to reside in the anterior and middle small intestine.

A goodness-of-fit test was performed on the distribution of *P. salamandra* in the small intestine of the newt (Table 14). A similar null hypothesis was formed for *P. salamandra* being *P. salamandra* is uniformly distributed in the small intestine. The number of *P. salamandra* found in the posterior small intestine, 194 (148.446), contributes most to the total chi-square value. The number of *P. salamandra* found in the posterior small

intestine is much greater than expected. Table 10 shows that the single infection is more prevalent than the dual infection. Therefore, one can conclude that *P. salamandra* is significantly predisposed to reside in the posterior small intestine. Joy and Scott (1997) and Joy and Thomas (1997) noted the location of a nematode and an acanthocephalan, respectively, in the small intestine of the red-spotted newt. Most of the worms collected in those studies indicate that each species was found in the anterior portion of the small intestine.

Chapter V – Summary

Tables 13 and 14 show the distribution of *P. parva* (13) and *P. salamandra* (14) in the small intestine of the newt. A total of 99 newts had a single infection, while 14 newts had a dual infection. The small number of dual infections along with the large number of single infections shows competitive exclusion when one species is present. Also the trematodes were predisposed to reside in a certain area of the small intestine. Even when only a single infection is present, the parasites are found in a certain area of the small intestine. *Plagitura parva* was found to reside in the anterior and middle small intestine while *P. salamandra* was found to reside in the posterior small intestine, thus suggesting competitive exclusion.

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Table 1. Dates of collection of *Notopthalmus v. viridescens* and number collected by host sex.

		Number Collected		
Month	Year	♂♂	♀♀	Total
Feb/Mar	1995	37	8	**45**
May/Jun	1995	8	8	**16**
Aug	1995	0	21	**21**
Oct	1995	11	11	**22**
Dec	1995	13	7	**20**
Feb/Mar	1996	20	3	**23**
Feb/Mar	1997	10	5	**15**
Feb/Mar	1999	41	22	**63**
	Totals	**140**	**85**	**225**

Table 2. Mean snout-vent length (SVL) and weight of individuals of *Notopthalmus v. viridescens* both sexes combined with one standard deviation.

	Mean ± 1 Standard Deviation
Snout-Vent Length (mm)	47.88 ± 4.84
Weight (g)	3.77 ± 1.01

Table 3. Unpaired, two-tailed student t-test determining if the mean intensity of the trematode *Plagitura parva* is different from the mean intensity of the trematode *P. salamandra.*

T-test for mean intensities for *Plagitura parva* and *P. salamandra.*		
	P. parva	*P. salamandra*
Mean Intensity	3.43	3.40
t statistic	0.057	
P value	0.95	

Table 4. Unpaired, two-tailed student t-test determining if the mean intensity of infected males (n=79) is different from the mean intensity of infected females (n=47).

T-test for mean intensities for infected males and infected females.		
	Infected ♂♂	Infected ♀♀
Mean Intensity	3.53	3.11
t statistic	1.97	
P value	0.48	

Table 5. Unpaired, 2 tailed student t-tests for snout-vent length (SVL) and weight of male and female newts (n = 225).

Table 5A. SVL (mm) of male and female newts		
	♂♂	♀♀
Mean	47.9	47.8
t statistic	0.194	
P value	0.85	

Table 5B. Weight (g) of male and female newts		
	♂♂	♀♀
Mean	3.68	3.92
t statistic	1.609	
P value	0.11	

Table 6. Unpaired, 2 tailed student t-tests for snout-vent length (SVL) and weight of infected and uninfected newts (n = 225).

Table 6A. SVL (mm) infected and uninfected newts		
	infected	uninfected
Mean	49.2	46.3
t statistic	4.650	
P value	< 0.001	

Table 6B. Weight (g) of infected and uninfected newts		
	infected	uninfected
Mean	4.09	3.40
t statistic	5.533	
P value	< 0.001	

Table 7. Unpaired, 2 tailed student t-tests for snout-vent length (SVL) and weight of infected male and infected female newts (n = 120).

Table 7A. SVL (mm) infected males and infected females		
	Infected ♂♂	Infected ♀♀
Mean	48.8	48.7
t statistic	0.163	
P value	0.87	

Table 7B. Weight (g) of infected males and infected females		
	Infected ♂♂	Infected ♀♀
Mean	4.00	4.25
t statistic	1.164	
P value	0.25	

Table 8. Chi-square 2 X 2 contingency table of infected males and infected females to test the null hypothesis (H_0) that infected males' prevalence of 50.0%, was equal to the infected females' prevalence of 52.9%. Total chi-square value includes the Yates's correction for continuity.

	Infected newts	Uninfected newts	Total	Percent prevalence
♂♂	70	70	140	50.0
♀♀	45	40	85	52.9
Total	115	110	225	

Accept H_0: $X^2 = 0.003$; df = 1; $P = 0.96$

Table 9. Chi-square 2 X 2 contingency table of *Plagitura parva* and *Plagitura salamandra* to test the null hypothesis (H_0) that the prevalence of infection of *P. parva* (24.4%) was equal to the prevalence of infection of *P. salamandra* (32.9%). Total chi-square value includes the Yates's correction for continuity.

	Infected newts	Uninfected newts	Total	Percent prevalence
Plagitura parva	54	171	225	24.4
Plagitura salamandra	73	152	225	32.9
Total	127	323	450	

Accept H_0; $X^2 = 3.346$; df = 1; $P = 0.054$

Table 10. Chi-square 2 X 2 contingency table of the type of infection to test the null hypothesis (H_0) that adult infection, of 52.2%, was equal to metacercarial infection, of 3.1%. Total chi-square value includes the Yates's correction for continuity.

	Infected newts	Uninfected newts	Total	Percent prevalence
Adult Infection	113	112	225	52.2
Metacercarial Infection	7	218	225	3.1
Total	120	330	450	

Reject H_0; X^2 = 114.832; df = 1; $P < 0.001$

Table 11. Chi-square 2 X 2 contingency table of the type of infection to test the null hypothesis (H_0) that single infection, of 44%, was equal to dual infection, of 6.2%. Total chi-square value includes the Yates's correction for continuity.

	Infected newts	Uninfected newts	Total	Percent prevalence
Single Infection	99	206	225	44
Dual Infection	14	211	225	6.2
Total	113	337	450	

Reject H_0; X^2 =73.791; df = 1; $P < 0.001$

Table 12. Chi-square goodness-of-fit test for type of infection. Numbers in table indicate numbers of single infection, either *Plagitura parva* or *P. salamandra*, numbers of dual infection, both species together in the same host, and numbers of metacercariae (i.e. pre-adult) infections. The null hypothesis (H_0) is that there is uniform infection of single, dual or metacercarial infections.

Observed	Expected	(O-E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
99	40	+59	3481	**87.025**
14	40	-26	676	16.900
7	40	-33	1089	27.225
120	120	0		$X^2 = 131.150$

Reject H_0; $X^2 = 131.150$; $P < 0.001$

Table 13. Chi-square goodness-of-fit test for *Plagitura parva* in different sections (i.e. anterior, middle, and posterior small intestine) of the host's small intestines. Numbers in table indicate numbers of trematode individuals in each section of the small intestine. The null hypothesis (H_0) is that there is uniform distribution of *Plagitura parva* throughout the small intestine of the newt.

Observed	Expected	(O-E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
74	60.7	+13.3	176.9	2.914
66	60.7	+5.3	28.1	0.463
42	60.7	-18.7	349.7	**5.761**
182	182	0		$X^2 = 9.138$

Reject H_0; $X^2 = 9.138$; $P = 0.011$

Table 14. Chi-square goodness-of-fit test for *Plagitura salamandra* in different sections (i.e. anterior, middle, and posterior small intestine) of the host's small intestines. Numbers in table indicate numbers of trematode individuals in each section of the small intestine. The null hypothesis (H_0) is that there is uniform distribution of *Plagitura salamandra* throughout the small intestine of the newt.

Observed	Expected	(O-E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
22	83	-61	3721	44.831
33	83	-50	2500	30.120
194	83	+111	12321	**148.446**
249	249	0		$X^2 = 223.397$

Reject H_0; $X^2 = 223.397$; $P < 0.001$

Figure 1. Distribution of *Notopthalmus viridescens viridescens* in the United States and Canada. (Petranka 1998)

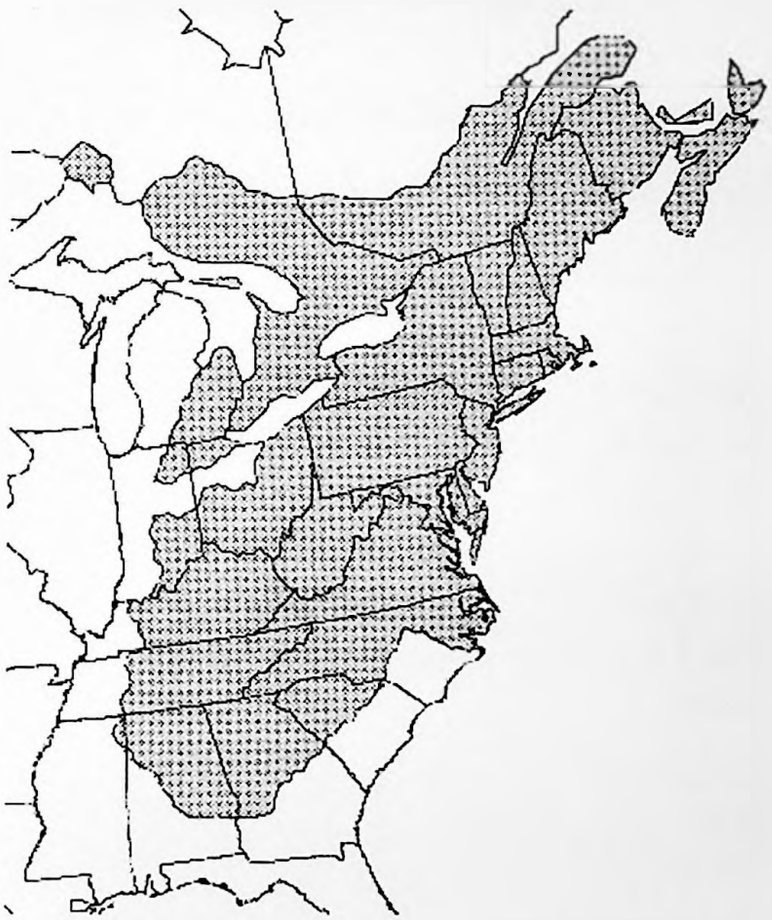


Figure 2A. Type specimen of *Plagitura salamandra* Holl 1928. Figure 2B. Type specimen of *Plagitura parva* Stunkard 1933. (Stunkard 1936).

g –gut

o – ovary

os – oral sucker

p – pharynx

te - testes

u - uterus

vs – ventral sucker

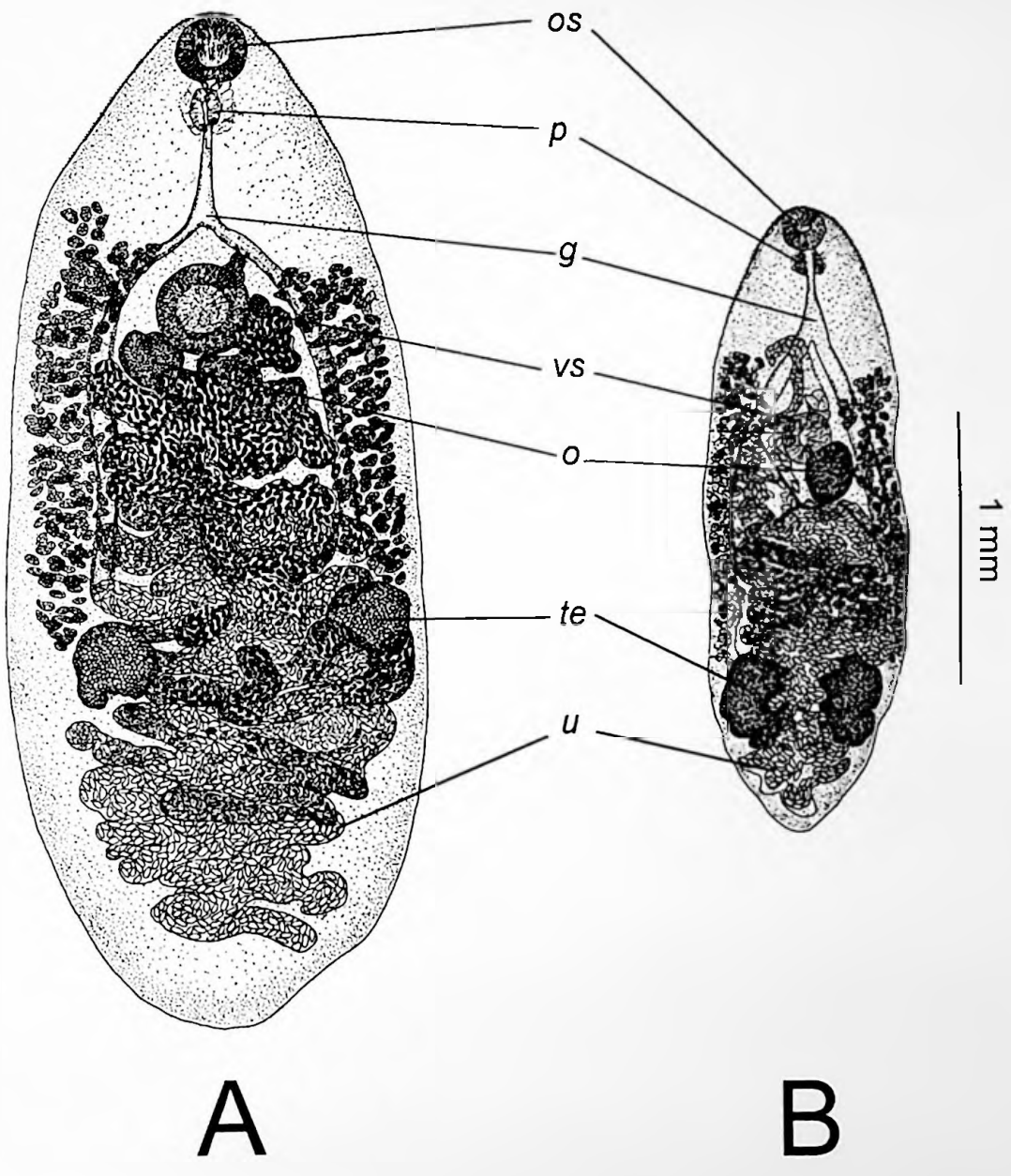

os
p
g
vs
o
te
u
1 mm
A
B

Figure 3A. Egg of *Plagitura parva.* Figure 3B. Primary sporocyst of *P. parva*. Figure 3C. Secondary sporocyst of *P. parva*. (Stunkard 1936).

c – cercaria

g*m* – germ mass

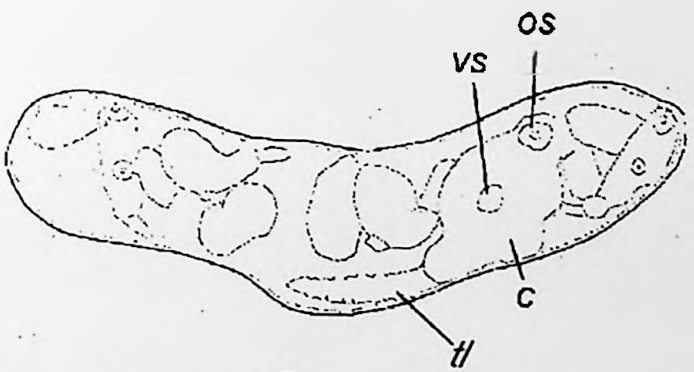
os – oral sucker

tl – tail

vs – ventral sucker

A

B



C

Figure 4. Drawing of a *Plagitura parva* cercaria. (Stunkard 1936).

cg – cystogenous gland

cgd – cystogenous gland duct

eb – excretory bladder

ed – excretory duct

g - rudimentary gut

os – oral sucker

p – pharynx

tl – tail

s – stylet

vs – ventral sucker

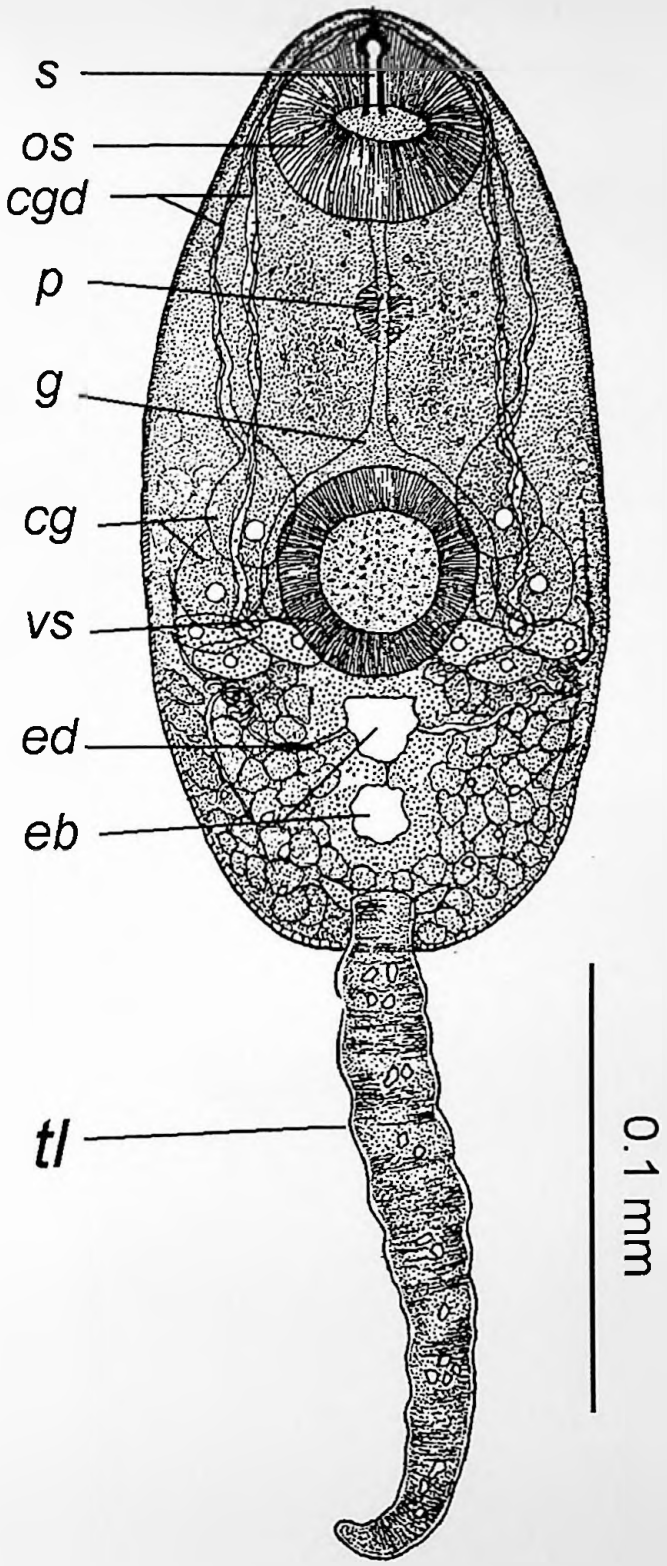

s
os
cgd
p
g
cg
vs
ed
eb
tl
0.1 mm

Figure 5. Drawing of *Plagitura parva* twelve days post-ingestion. (Stunkard 1936).

g – gut

os – oral sucker

p - pharynx

te – testes

vs – ventral sucker

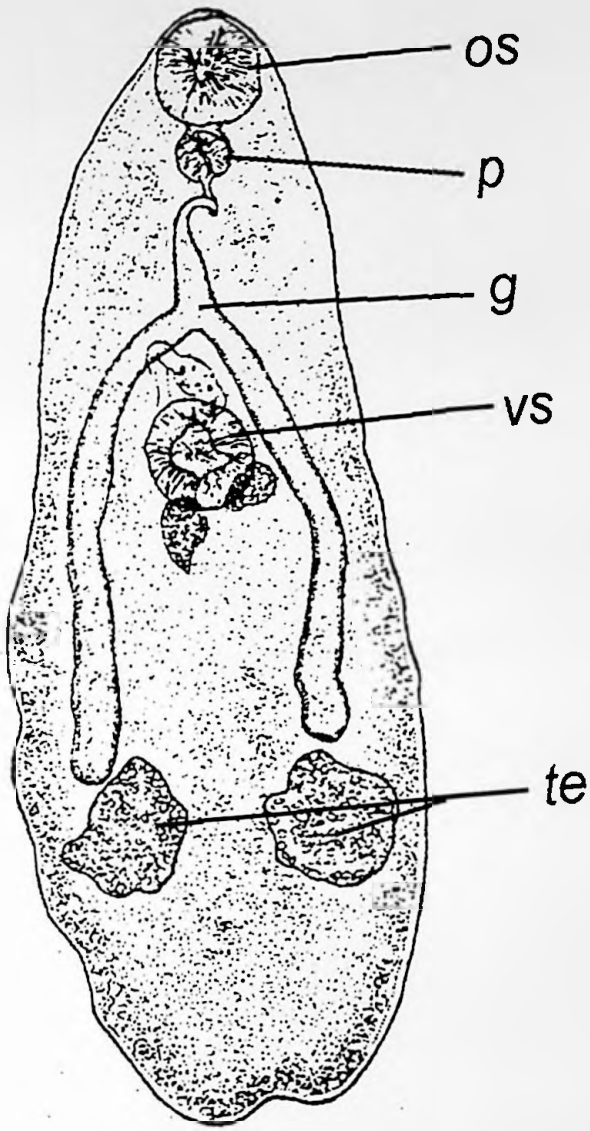

os
p
g
vs
te

Figure 6. A drawing of *Plagitura parva* eighteen days after ingestion of the secondary intermediate host (Stunkard 1936).

e – eggs in uterus

g – gut

os – oral sucker

p – pharynx

te – testes

v – vitteline

vs – ventral sucker

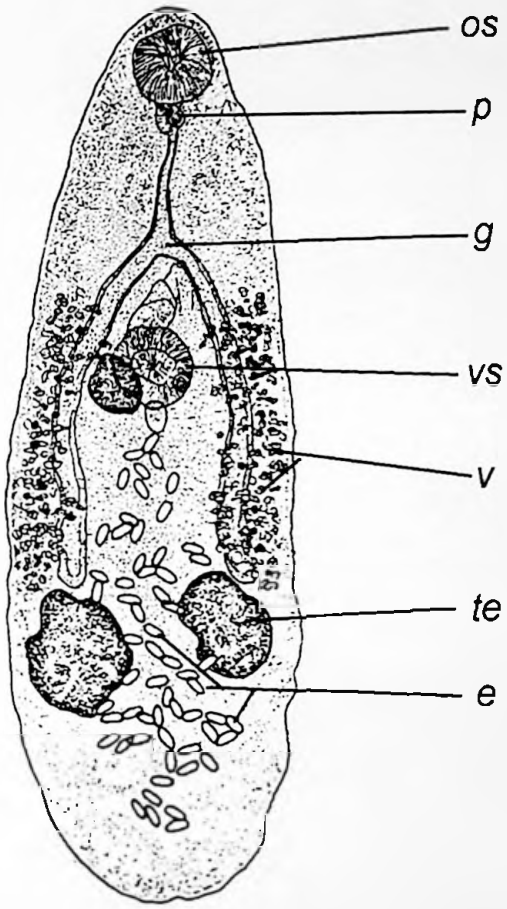

os
p
g
vs
v
te
e

Figure 7A. Primary sporocyst of *Plagitura salamandra*. Figure 7B. Secondary sporocyst of *Plagitura salamandra*. (Owens 1946).

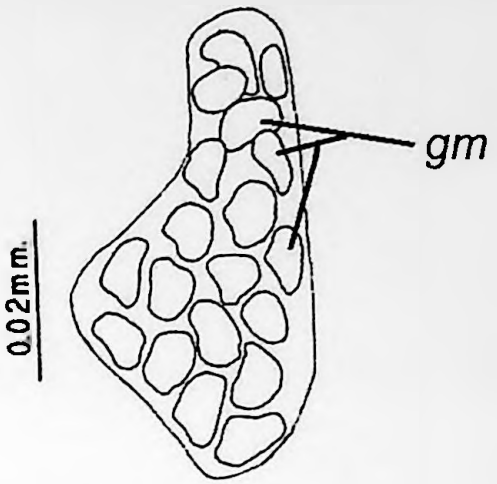
c – cercaria

gm – germ mass

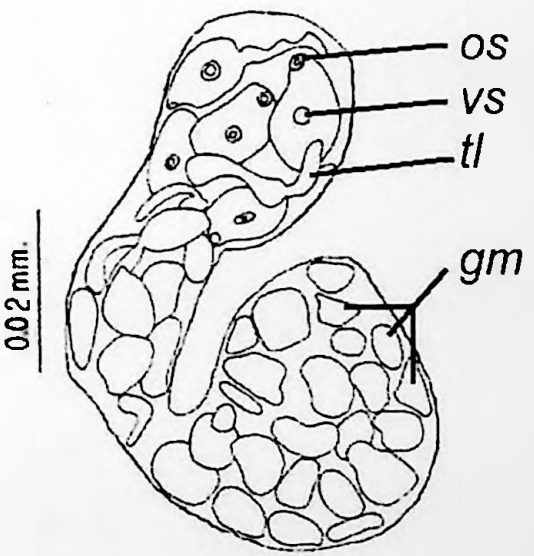
os – oral sucker

tl – tail

vs – ventral sucker



A



B

Figure 8A. Cercaria of *Plagitura salamandra*. Figure 8B. Cyst of *Plagitura salamandra*. (Owens 1946).

cg – cystogenous gland

ev – excretory vesicle

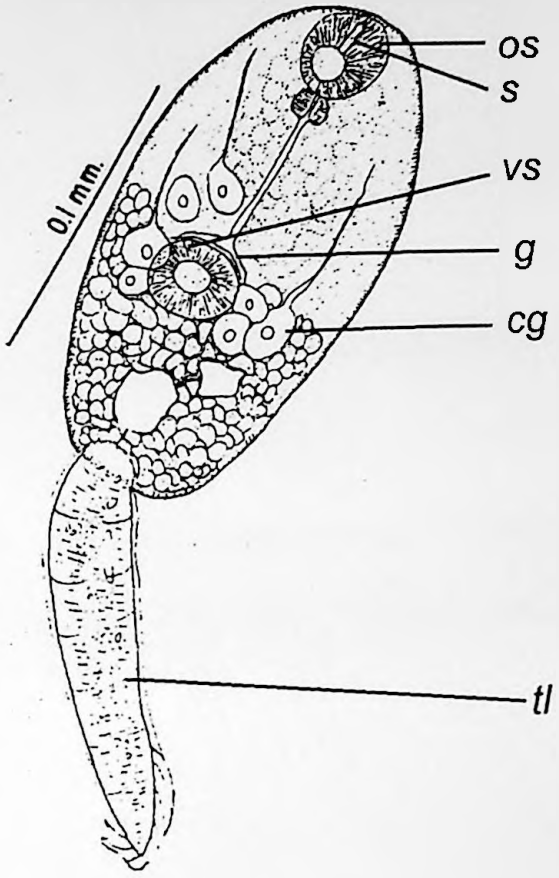
g – rudimentary gut

os – oral sucker

s – stylet

tl – tail

vs – ventral sucker



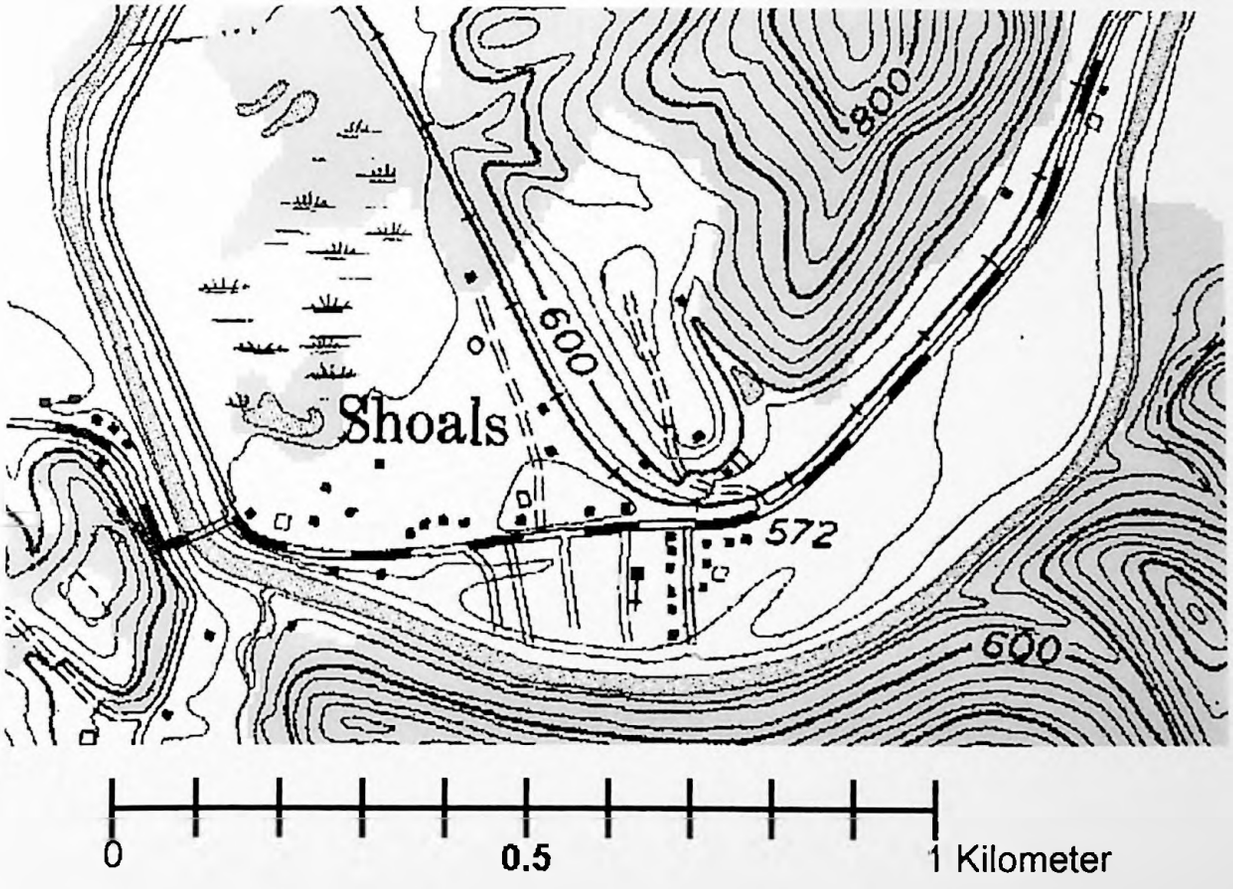
A

B

Figure 9A. Map of West Virginia showing location of USGS Topographic Quadrant – Lavalette. Figure 9B. Map of Shoals, WV, showing area of marsh used in the study.

Quadrangle location

A



B

Figure 10. A still photograph of Shoals Marsh, Shoals, WV. The photograph was taken in February 1995. Notice the layer of ice covering the water. (Photo taken by Dr. James Joy)

Figure 11. A still photograph taken of Shoals Marsh, Shoals, WV. This picture was taken in February 1995. Notice the layer of ice on the surface of the water. (Photo taken by Dr. James Joy)

Figure 12. A scatter diagram of host weight (g) as a function of host snout-vent length (SVL). Each point represents an individual newt. The Pearson's coefficient (r) is 0.664. This shows a correlation between the host SVL and weight. The regression line ($\hat{y}$ = -2.90 + 0.139X; $F_{0.05, 223}$ = 176.40) shows that the weight and SVL are related.

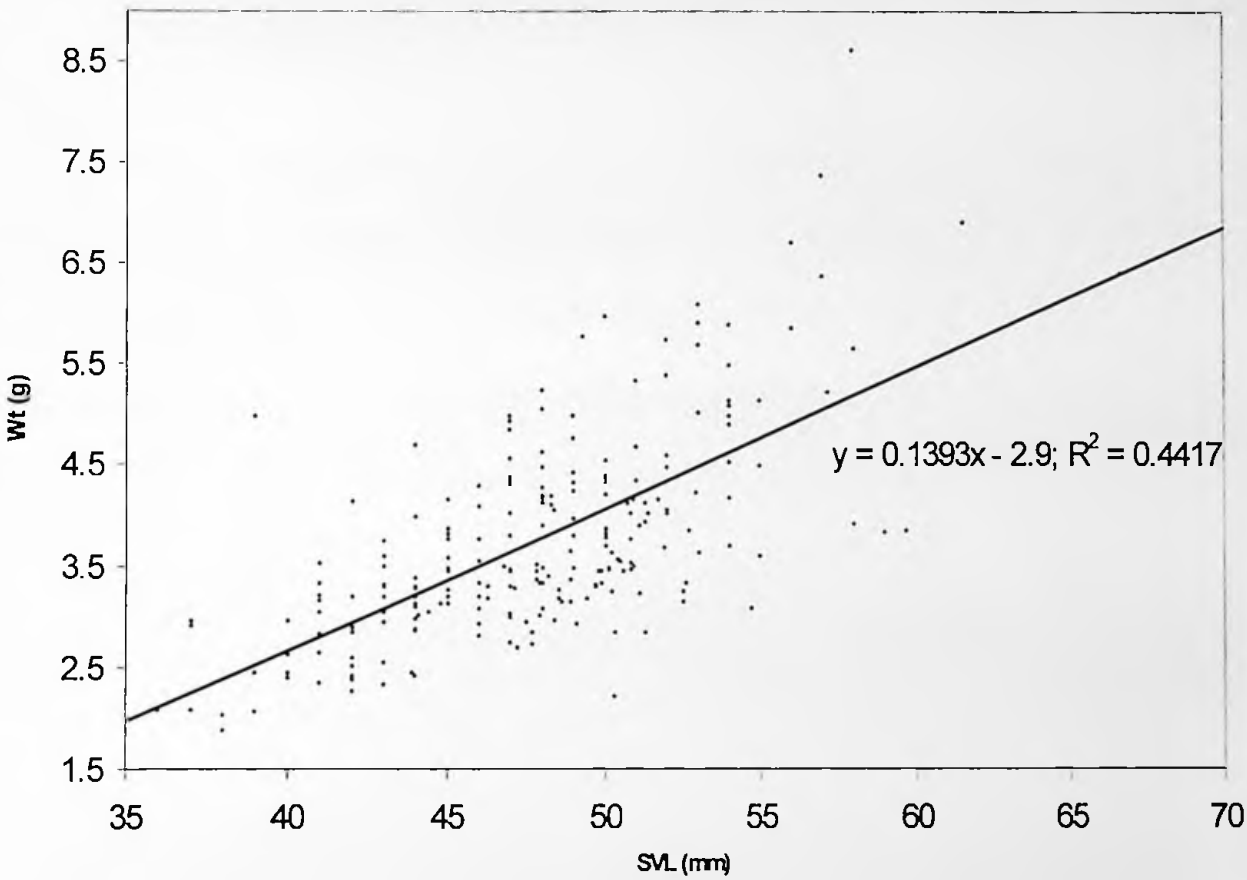

8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
Wt (g)
35
40
45
50
55
60
65
70
SVL (mm)
y = 0.1393x - 2.9; R2 = 0.4417

Figure 13. A scatter diagram of the number of individual *Plagitura parva* found in the small intestine as a function of host snout-vent length (SVL). The regression line ($\hat{y} = 6.83 - 0.069X$; $F_{0.05, 51} = 0.333$) shows that the number of individuals of *P. parva* in the small intestine is not a function of host SVL.

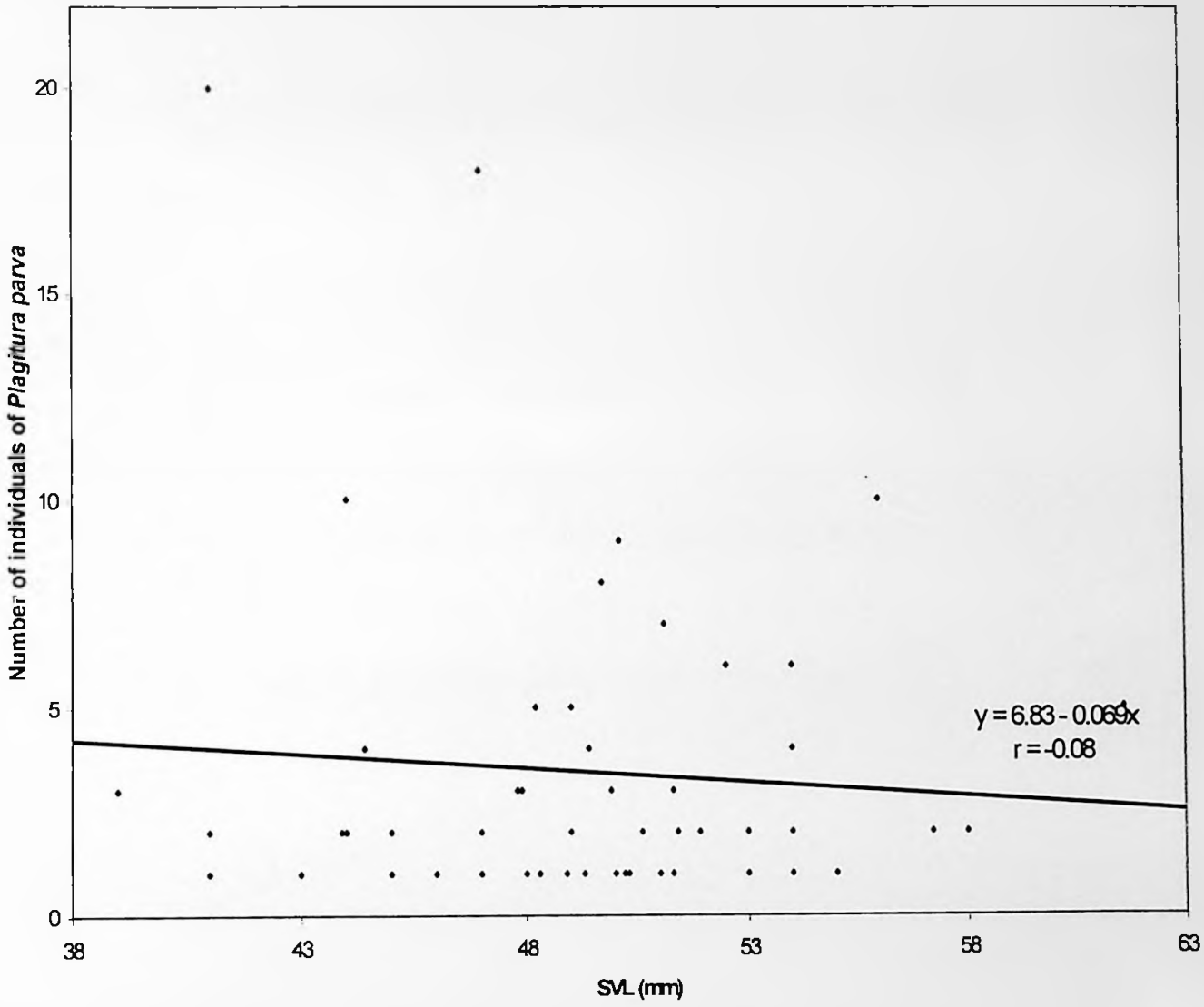
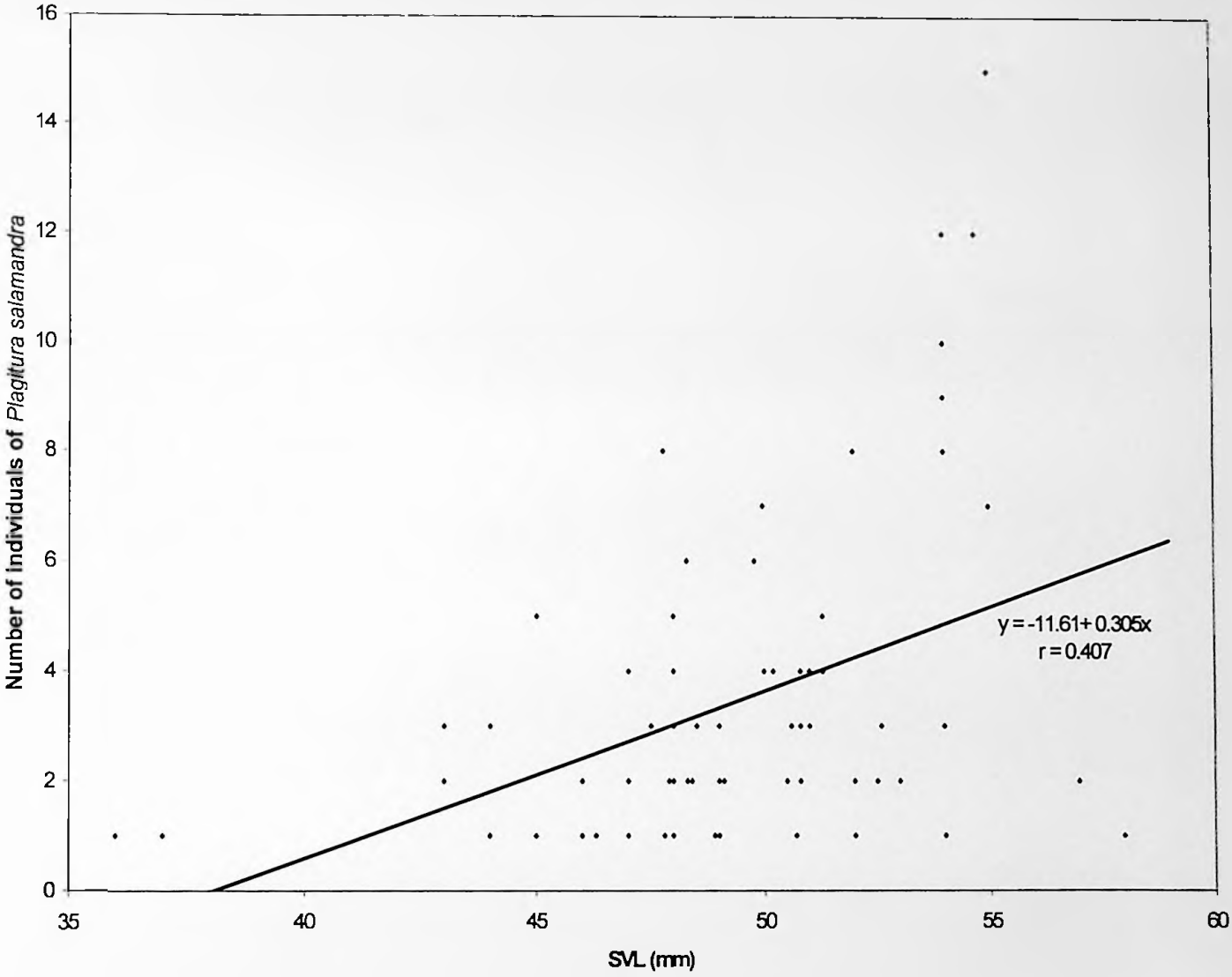

Number of individuals of Plagitura parva
20
15
10
5
0
38
43
48
53
58
63
SVL (mm)
y = 6.83 - 0.069x
r = -0.08

Figure 14. A scatter diagram of the number of individual *Plagitura salamandra* found in the small intestine as a function of host snout-vent length (SVL). The regression line ($\hat{y}$ = -11.61 + 0.305X; $F_{0.05, 71}$ = 14.13) shows that the number of individuals of *P. salamandra* in the small intestine is a function of host SVL.


Number of individuals of Plagitura salamandra
SVL (mm)
y = -11.61 + 0.305x
r = 0.407
0
2
4
6
8
10
12
14
16
35
40
45
50
55
60