Tabanid vectors of the arterial nematode, Elaeophora schneideri in southwestern Montana
by Rolando Humberto Espinosa

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University

Abstract:
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A total of 1122 tabanids was collected, representing thirteen species. Hybomitra osburni was the most abundant species, 50.0%, followed by H, tetrica, 25.3% , and H.

rupestris, 19.5%. These three species comprised 95.0% of the total tabanids collected. Hybomitra osburni emerged in late June with numbers peaking in late July. Hybomitra rupestris and H. tetrica peaked shortly after emergence in early July. The latter species were rarely trapped after mid July.

Elaeophora schneideri larvae; were present in 0.8% of the tabanids dissected. Three first stage larvae (L1) were recovered from H. osburni in 1984 and 51 larvae (L1 to L2) from H. rupestris and H. tetrica in 1985. Percent infection of infected flies was 50.0% for H. tetrica, 37.5% for H. rupestris, and 12.5% for H osburni. The latter two species. were new hosts records for Elaeophora schneideri.

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APPROVAL

of a thesis submitted by

Rolando Humberto Espinosa

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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INTRODUCTION

Elaeophora schneideri Wehr and Dikmans, 1935 (Nematoda: Onchocercidae) is a nematode that lives in the arterial system of native wild and domestic ruminants (elk, moose, mule deer, white-tailed deer, domestic sheep and goats) in North America (Adcock et al., 1965; Hibler et al., 1968, 1969, 1971; Hibler and Adcock, 1971; Anderson and Weinmann, 1972; Prestwood and Ridgeway, 1972; Worley et al., 1972, Worley, 1975). Other ruminant species (sika deer, barbary sheep and ibex) introduced to North America also serve as a definitive host for the arterial nematode (Robinson et al., 1978; Pence and Gray, 1981; Hibler and Prestwood, 1981).

Elaeophora schneideri was originally found in the mesenteric, iliac, and carotid arteries of sheep from Catron County, New Mexico in 1933 (Kemper, 1938). In 1935, Wehr and Dikmans of the Zoological Division of the Bureau of Animal Industry, U.S.D.A., named the nematode found by Kemper Elaeophora schneideri in honor of Dr. F. L. Schneider of the Field Inspection Division, Albuquerque, New Mexico.

Kemper's initial find was followed by Huffman's discovery of the arterial worm in mule deer in Utah (Wehr and Dikmans, 1935). Arizona Fish and Game Department personnel observed blindness of unknown etiology in elk

from 1944 to 1961. Eventually the arterial nematode was implicated as the causative agent (Adcock et al., 1965).

In New Mexico, the first record of the arterial worm in elk was in 1964 (Hibler et al., 1969). Elaeophora was not reported from mule deer in New Mexico until Hibler et al. found the worm in mature mule deer in 1968, although reports of the nematode in mule deer in other states and Canada were confirmed prior to 1968 (Hibler et al., 1969).

Flies (Diptera) belonging to the families Tabanidae (horse and deer flies) and Rhagionidae (snipe flies) were found infected with a nematode that resembled E. schneideri (Hibler et al., 1969). Dissection of horse flies captured in the Gila National Forest in New Mexico permitted collection of the infective stages of the arterial worm that were injected into mule deer and domestic sheep. Recovery of adult E. schneideri from the arterial system of these hosts confirmed the biological cycle of the arterial nematode and incriminated horse flies as the natural intermediate host (Hibler et al., 1970).

Hibler et al. (1971a) found two genera of the Tabanidae, Hybomitra sp. and Tabanus sp., acting as vectors of the arterial worm. Later studies in the Gila National Forest identified the following tabanid species as vectors of E. schneideri : Hybomitra laticornis, H. phaenops, H. tetrica rubilata, Tabanus abditus, T. eurycerus, and T. gilanus (Clark, 1972). At the same time H. procyon and T.

monoensis were incriminated as vectors of the arterial worm in black-tailed deer in California (Anderson and Weinmann, 1972).

Studies in Vermego Park, New Mexico showed four species of horse flies (H. aatos, T. punctifer, T. subsimilis subsimilis and T. stoni) were naturally infected with larval forms of E. schneideri (Davies, 1979).

Clinical elaeophorosis in white-tailed deer has been reported from Florida, Georgia, and South Carolina (Prestwood and Ridgeway, 1972; Hibler and Prestwood, 1981). The intermediate hosts of E. schneideri and the life cycle were reconfirmed in South Island, South Carolina (Couvillion et al., 1984). Tabanids found infected with the larval stages of the arterial worm in South Carolina included Tabanus lineola hinellus, and T. nigrovittatus (Couvillion et al., 1984).

The pathogenesis of elaeophorosis varies with the host infected. Kemper (1938) described "filarial dermatosis" on the poll of the head in sheep, and Dikmans (1948) reported lesions on domestic sheep caused by the arterial nematode. Similar lesions described as "scabbing" were reported on the face, muzzle, ears, and crown of Barbary sheep (Ammotragus lervia), an introduced species from North Africa (Pence and Gray, 1981). Wild ungulates in western states show marked muzzle necrosis, ear cropping, antler deformity, and blindness (Jensen and Seghetti, 1955; Hibler and Adcock,

1970). Reviews of the pathogenesis of elaeophorosis are present in the literature and will not be repeated here (Hibler and Adcock, 1971; Davies, 1979; Hibler and Prestwood, 1981)

Elaeophorosis in Montana

The first reported case of elaeophorosis in Montana was from Kalispell, (Wilkins, 1951). Three domestic sheep imported from Idaho were found with lesions in the poll area of the head. Laboratory examination of skin scrapings showed microfilariae present, and the lesions matched the description of elaeophorosis in sheep from Catron County, New Mexico (Kemper, 1938).

In 1971, a moose was observed staggering and moving in circles in the Boulder River drainage, in Sweetgrass County, Montana. The animal died shortly after Montana Fish and Wildlife biologists arrived at the scene (Worley et al., 1972). Necropsy revealed eight immature arterial worms in the right common carotid artery and numerous "fifth stage nematodes in the arteries of the optic nerve sheath and sclera" (Worley et al., 1972).

Three additional moose infected with E. schneideri were collected from other areas in southwestern Montana. A mature cow with 28 arterial worms was found in Park County in November of 1971, and a cow moose from the Bridger mountains had eleven adult worms. A young female moose found with the

previous cow was infected with one adult worm (Worley et al., 1972).

Later surveys in wild ruminants from Montana found additional moose and, for the first time, mule deer infected with E. schneideri (Worley, 1975). The data indicated that moose have a "lack of resistance" to the arterial nematode while mule deer showed lower worm burdens and presumably are asymptomatic (Worley, 1975; Worley et al., unpublished). These surveys indicate that the arterial worm exists in isolated areas in the foothills and mountaineous areas above 1950 meters in southwestern Montana (Worley, 1975).

To date, elaeophorosis in elk has not been reported in Montana (Worley, 1975; Worley et al.,unpublished). Efforts to survey the potential intermediate hosts and vectors of the arterial worm showed that a number of tabanids are sympatric with wild and domestic ruminant populations in southwestern Montana (Murray, 1972; Worley, 1975).

Although tabanid species have been incriminated as vectors of Elaeophora schneideri in the southeastern and southwestern United States (Hibler et al., 1969, 1971; Clark, 1972; Davies, 1979; Couvillion et al., 1984) the vectors of the arterial worm in Montana are unknown.

Because 82.4% of the mule deer found infected with E. schneideri were from the Bridger Range north of Bozeman, the study was concentrated on the west and east side of the Bridger Range. The purposes of this study were 1) to collect

and identify species of the family Tabanidae occurring in southwestern Montana; 2) to dissect tabanids collected to determine which species serve as intermediate host for the immature forms of *E. schneideri*; and 3) to collect data on the parity (oviposition history) of the female tabanids at intervals during the horse fly season to analyze the seasonal aspects of *Elaeophora* transmission and if possible clarify the restricted host range of the parasite.

MATERIALS AND METHODS

The Bridger Range is located in the northeastern corner of Gallatin county north of Bozeman, Montana (Figure 1). The range lies in a northwest-southeasterly direction and extends from approximately 8-9 km northeast of Bozeman, 40 km northwest to Blacktail Mountain (Figure 1). The range covers approximately 345.6 km^2. Study areas were located on the east and west slopes of the northern Bridger range (Figure 1), 40 km north of Bozeman, and comprised 12.8 km^2 of the total Bridger range area.

The study site on the west slope was located 3.2 km south of Flathead Pass in the North Cottonwood drainage northwest of the Armstrong ranch. Trap sites in 1984 and 1985 were located 7.2 km northeast from the ranch boundary along North Cottonwood Creek (Figure 2).

In 1984, trap locations on the east slope of the Bridger range included the middle fork of Brackett Creek and the Fairy Lake area . Additional trap sites were located on the Carrol Creek drainage and the Middle fork of Brackett Creek during the 1985 collecting season. Trapping was also done in the Gallatin mountains, approximately 25 km south of the main study area in the vicinity of Rat Lake (Figure 3).

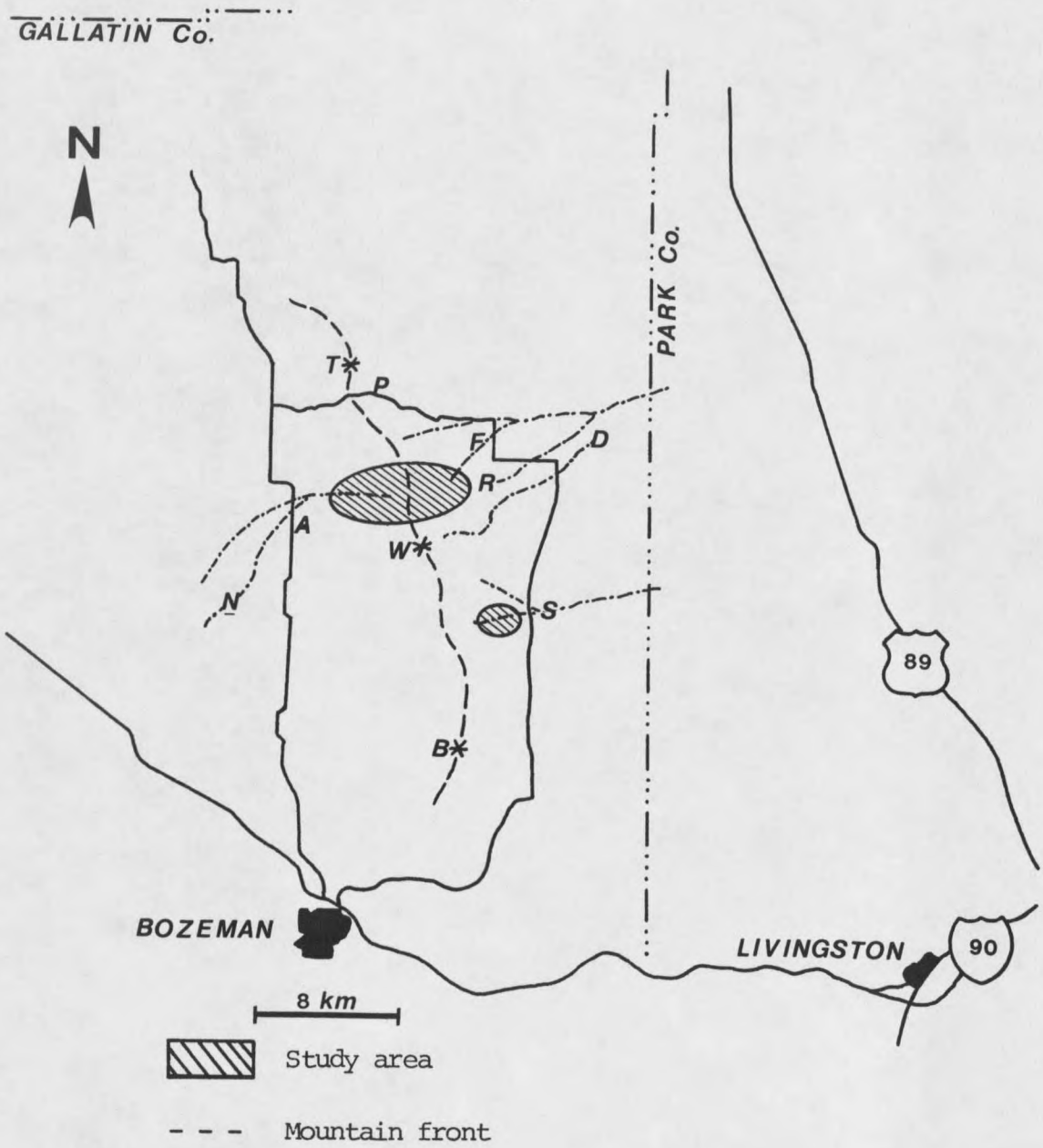
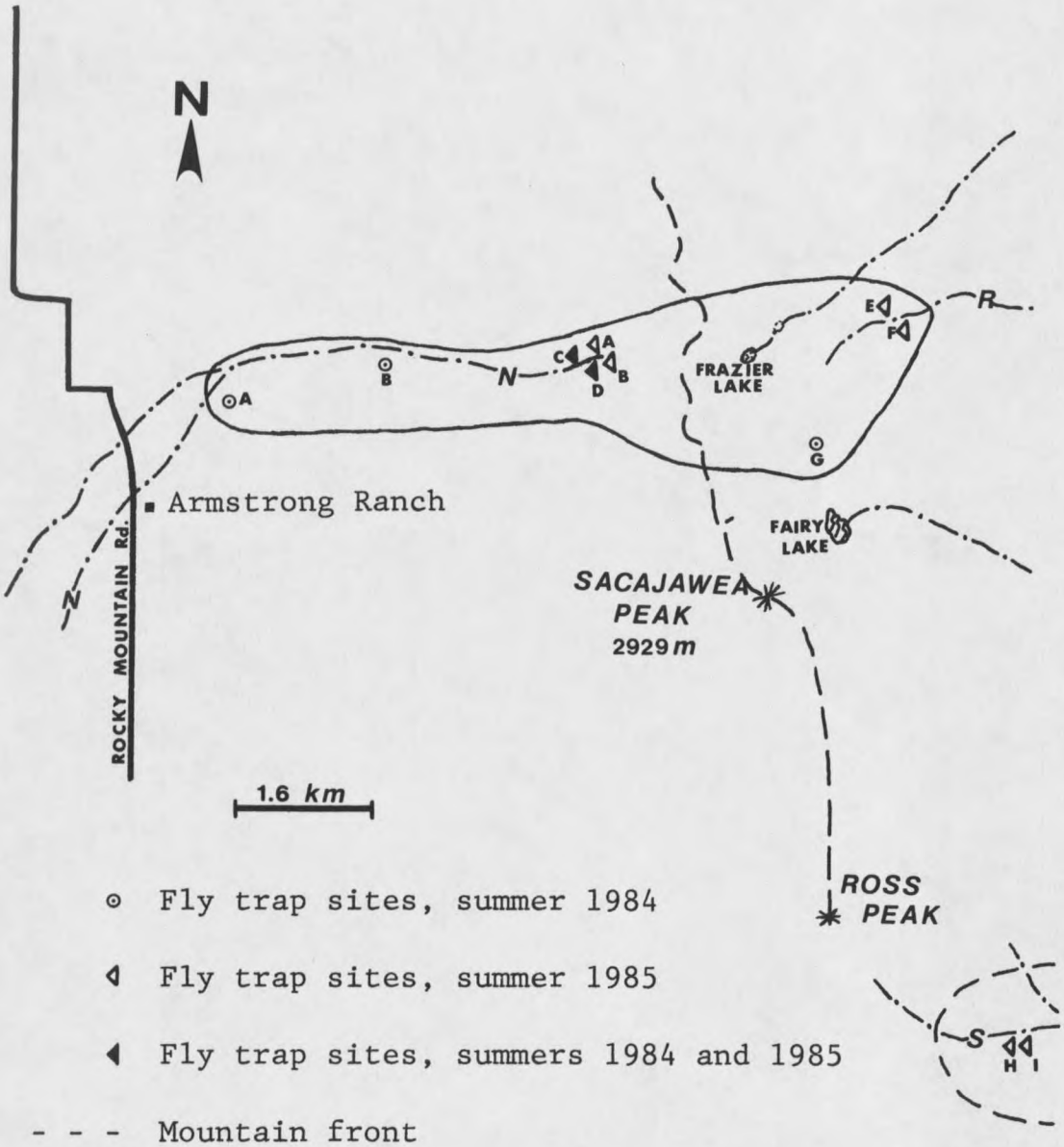


Figure 1. Study area in the Bridger Range, summers 1984 and 1985. A, Armstrong Ranch; B, Baldy Mt; R, Carrol Creek; D, Fairy Creek; S, Brackett Creek, F, Frazier Creek; N, North Cottonwood Creek; P, Flathead Pass road; T, Blacktail Mt; W, Sacajawea Peak.



⊙ Fly trap sites, summer 1984

◁ Fly trap sites, summer 1985

◀ Fly trap sites, summers 1984 and 1985

- - - Mountain front

Figure 2. Location of Manitoba fly traps in the S, Brackett Creek; R, Carrol Creek; Fairy Lake area, and North Cottonwood Creek drainages in the Bridger Range, summers 1984 and 1985.

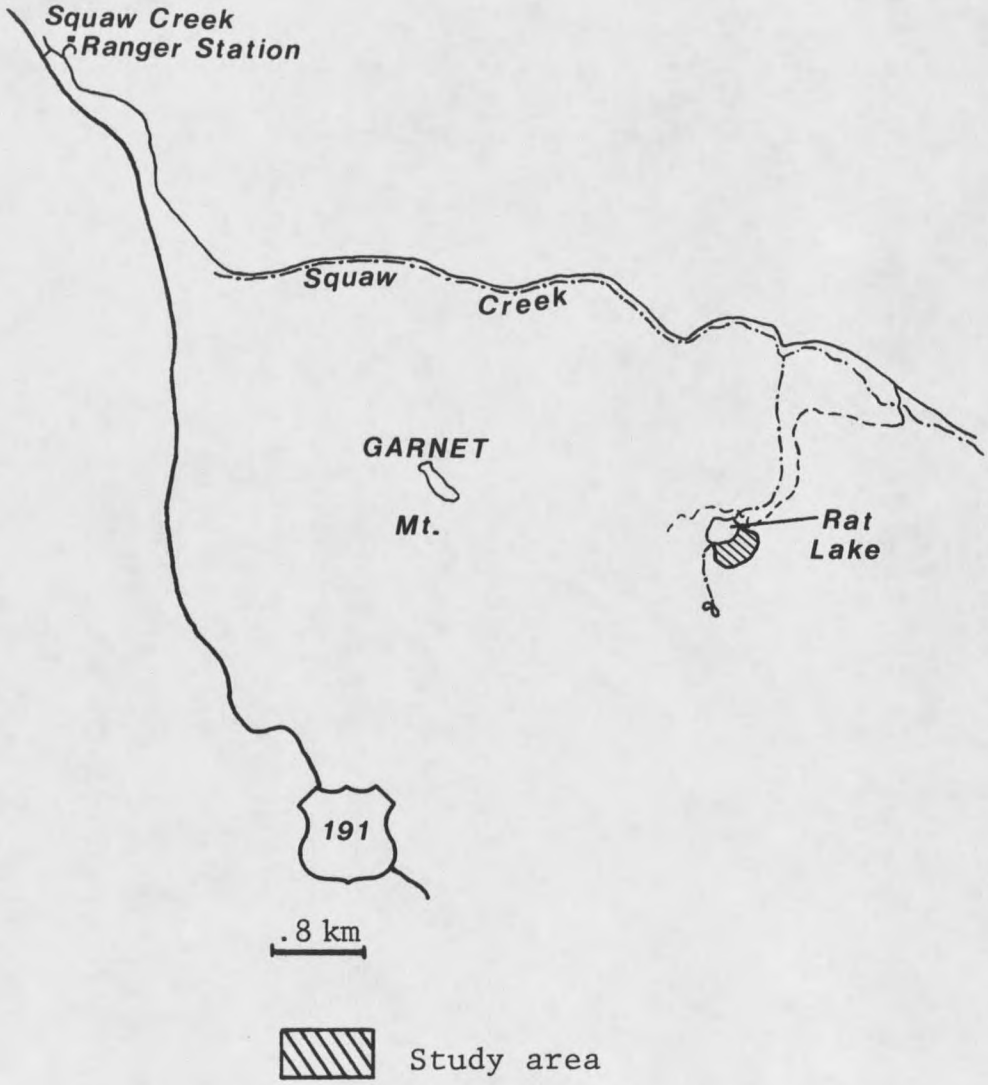


Figure 3. Collecting area in the Rat Lake campground in the Gallatin Range, summer 1985.

Tabanids were collected with modified Manitoba fly traps (Thompson 1969; Catts 1970, Adkins et al.,1972). The traps were made of fiberglass screening (CCS Hanover, Hanover, PA). Flat sheets of the material were cut into four identical trapezoids with the following dimensions : base, 175 cm, top edge, 12.5 cm, and sides of 187.5 cm. The sides of the individual trapezoids were sewn together to form a three dimensional structure (Figure 4) that had a 175 cm^2 bottom opening and a 12.5 cm^2 top opening.

Canvas webbing was sewn to the lower third of the trapezoid seams (Figure 4) and to the apex of the trapezoid for reinforcement (Figure 5,A).

The trap collar, located in the apex of the trapezoid served to hold an inverted acetate funnel, provide an attachment area for the top canister, the fiberglass trap body and the adjustable pole (Figures 5, A,B,C).

An inflatable vinyl beach ball (60 cm, diameter), painted with black glossy vinyl spray paint, was suspended from the pole with a nylon rope, so that 2/3 of the ball was below the edge of the canopy when viewed from a distance (Figure. 4). The black color served to concentrate heat on the surface of the ball and thus attract tabanids (Thorsteinson, 1958). The edge of the canopy was 60 cm from the ground.

Use of fiberglass screening to build the trap canopy and insect holding container eliminated weight and

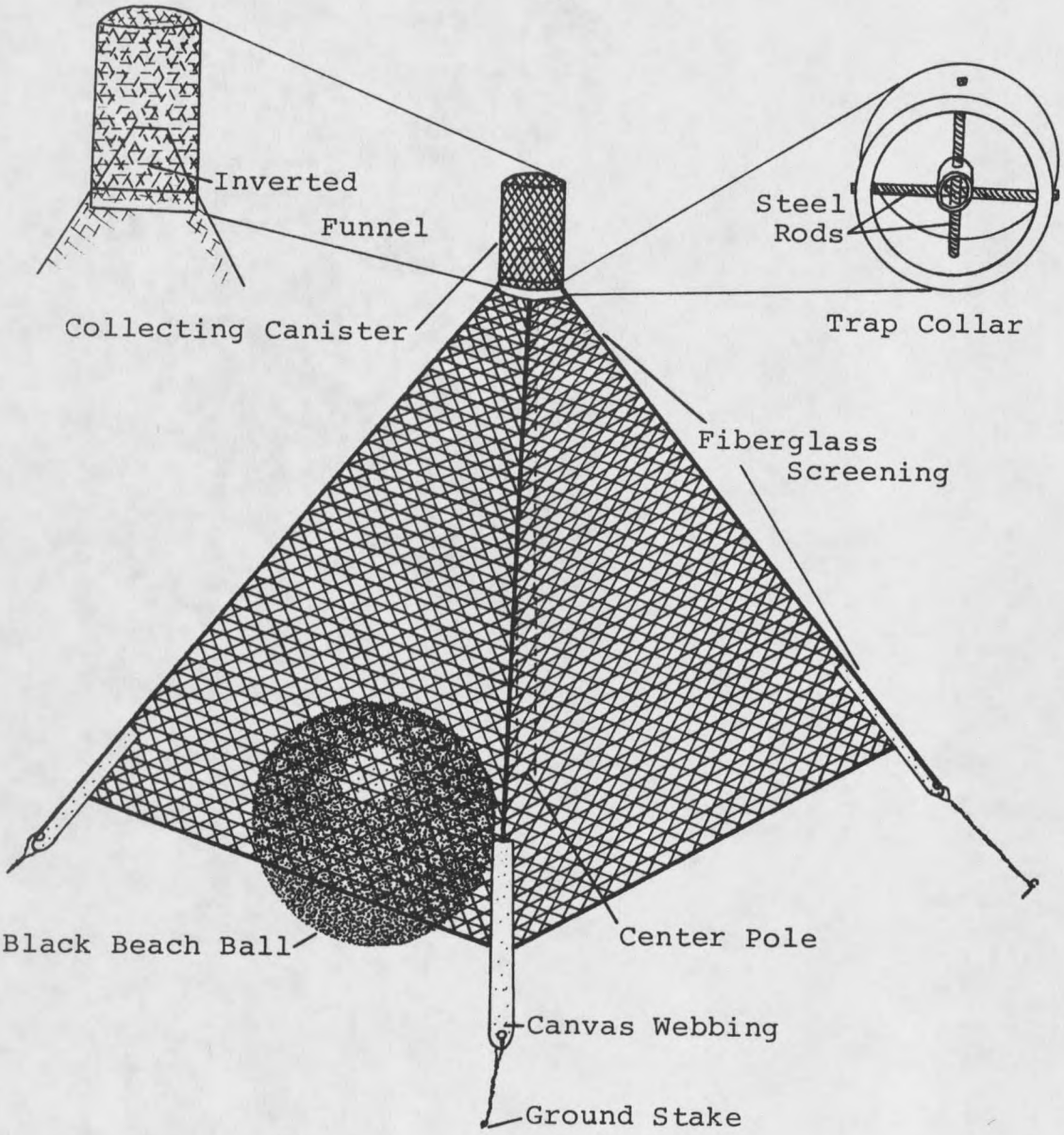


Figure 4. Modified Manitoba fly trap used to collect horse and deer flies, summers 1984 and 1985.

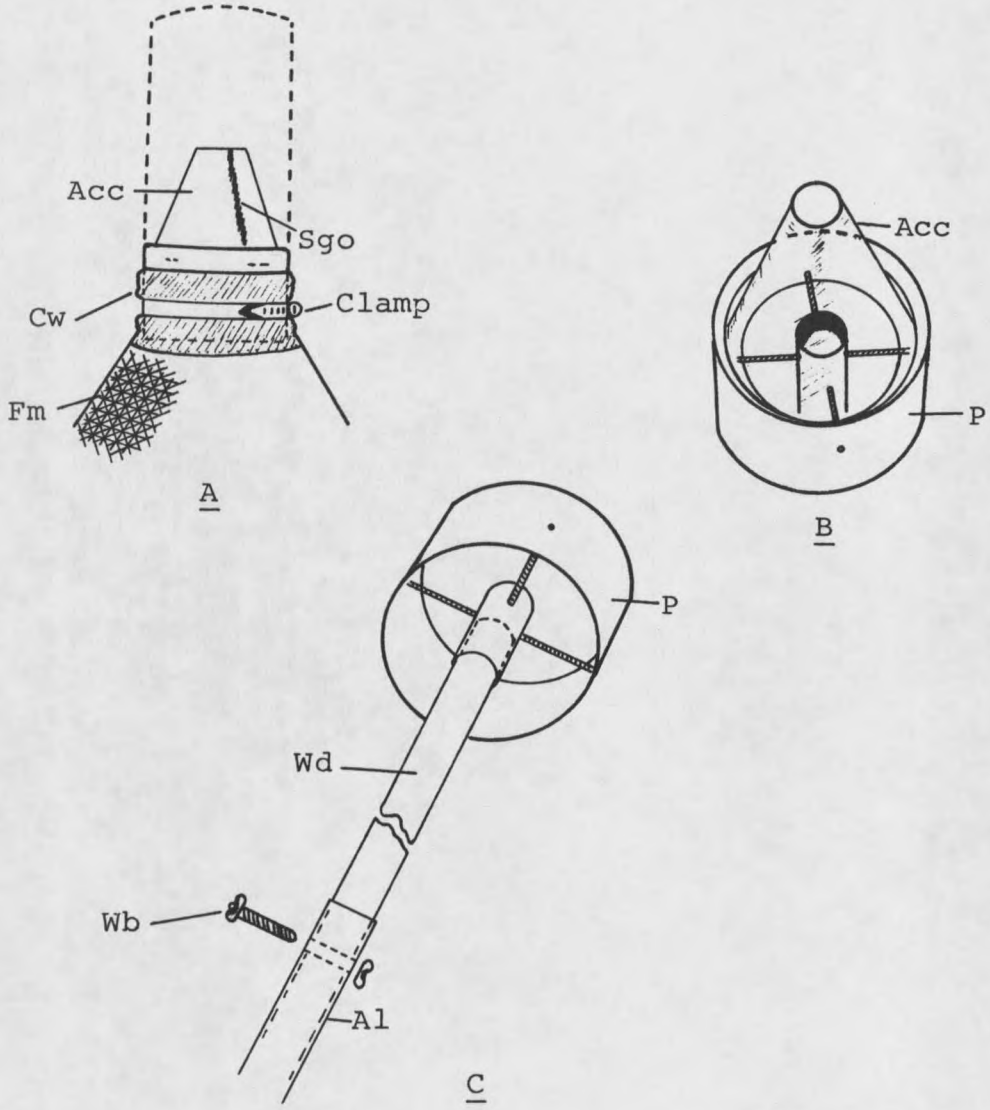


Figure 5. Detailed view of the (A) collar, (B) collecting funnel, (C) aluminum tube, wood dowel, and attachment to the trap collar. Fm, fiberglass mesh; Acc, inverted acetate funnel; Sgo, ShooGoo sealer; Cw, canvas webbing; P, collar with center support; Wd, wood dowel; Al, aluminum tube; Wb, wing bolt and nut.

compactability problems of the conventional Manitoba fly trap. This modification also eliminated the need to invert the collecting canister (to force swarming of the trapped flies at the apex of the trapezoid) as recommended by Davies (1979) to increase fly survival. Tabanids readily rested on the inside surface of the canisters. This reduced fly activity after capture, and minimized damage to anatomical characters important in identification.

Fly Survival

To ensure fly survival during collection and transportation to Bozeman, a styrofoam cooler 30 cm x 20 cm x 40 cm was modified to fit into a backpack. Three Blue Ice frozen bags were attached to the internal walls of the cooler at the beginning of each collecting period. Ice bags were frozen in a horizontal position for several days at -20.5 C before use, to ensure flatness of the ice bags, thus reduce space used in the cooler.

Collected flies were placed in pint size Ziplock bags tagged with the date, time, temperature, relative humidity (RH), trap site and trap number and placed in the cooler. This procedure maximized use of space in the cooler and allowed 10-15 bags to be transported with minimal damage to the flies. Air was removed from bags prior to placing them in the cooler.

The cooler maintained temperature between 1.1-4.4 C for periods of 12 to 36 hours, depending on external temperatures and location of the pack during the day (shade versus direct sunlight).

Trapping

Trapping was done from July 20 to August 30 in 1984 and from June 19 to August 10 in 1985. In the North Cottonwood Canyon, traps were placed at different altitudes during the 1984 season. Trap A was located at an elevation of 1800 m on a southwest slope, 1.9 km southwest of the canyon entrance. Trap B was at 1950 m, 1.9 km northeast from trap A on a rocky north facing slope. Trap C and D were located 7.3 km east of the canyon entrance at 2310 m and 2340 m respectively (Figure 2), on a flat wet meadow, covered with horsetail, sedges, grasses and forbs. On the east side of the collecting area, traps in 1984 were located in the Fairy Lake area (G) at 2040 m and in the Brackett Creek area (H,I) at 1980 m, 3.2 km southeast of Ross Peak (Figure 2).

In 1985, traps on the west side of the Bridgers were concentrated in the east end of the canyon. Traps A,B,C, and D were located between 2220 and 2340 m altitude zone (Figure 2). During the 1985 season the east side had two traps (E,F) in the Carrol Creek drainage and two traps (H,I,) in the Brackett Creek site south of Ross Peak. The latter traps were removed due to lack of fly activity.

Traps were checked for flies every 2-3 days on the east and west side of the study area during the 1985 season. In 1984, collection of flies was concentrated on the west side of the area because tabanid activity was minimal on the east side. During the collecting day, each trap was checked every 3-4 hours during both collecting seasons.

Tabanid Dissection

Tabanids were transported to the Veterinary Research Lab and stored at 4 C for 3-4 days before dissection. Each fly was identified to species prior to dissection. The protocol for examination was as follows: tabanids were immobilized by cutting through the surface of the prosternum (Figure 6) with microscissors to sever the ventral nerve chord.

The pleural membrane between the abdominal tergites and sternites was cut longitudinally with the microscissors, starting at the terminal abdominal segments and ending at the anterior abdomen-metapleuron margin (Figure 6). This was done on both sides of the abdomen and allowed complete separation of the dorsal and ventral segments.

The abdominal halves were placed in 0.86% physiological saline solution. The gastrointestinal tract, ovaries, and fat bodies were removed and placed in 0.86% physiological saline for observation under a dissecting microscope at 50X and 100X.

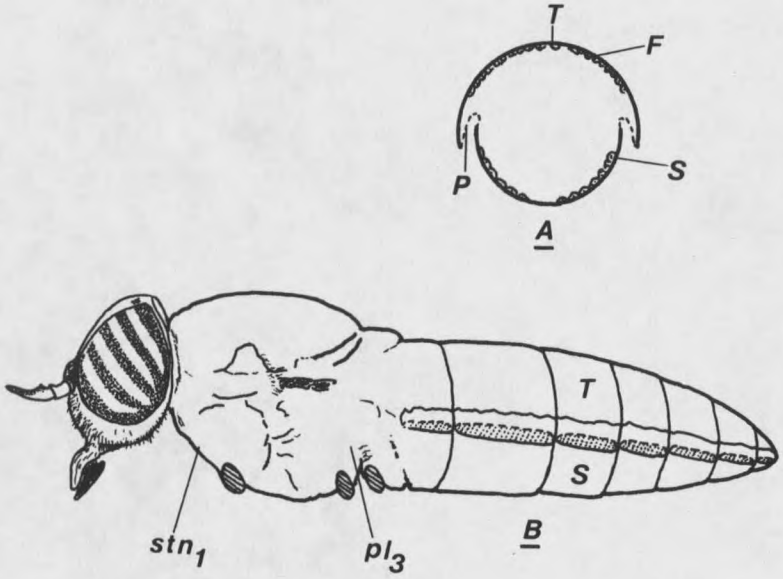


Figure 6. Cross section (A) and lateral view (B) of a horse fly showing the location of the pleural membrane. T, abdominal terga; S, abdominal sterna; P, pleural membrane; pl_3, metapleuron; stn_1, prosternum; F, fat cells lining the interior of the abdominal sclerites.

The thorax was opened at the midlateral line bilaterally and separated to expose the thoracic muscles and gastrointestinal tract components. While dissecting other body regions, the head was severed and left for five minutes in physiological saline. The cephalic end of the food canal was observed for emerging larval forms, then teased apart and viewed under a dissecting microscope.

Ovaries were teased apart while viewed under a dissecting microscope at 100X. The follicular tubes of individual ovarioles were observed and any dilatations present recorded. The number of dilatations in the

follicular tubes is used as an indicator of parity (oviposition history) of female flies (Detinova, 1962, Bertram, 1962). Parity is the completion of a gonotrophic cycle, where a gonotrophic cycle consists of a number of steps; search for a host, feeding on its blood, digestion of the blood meal, and oocyte maturation followed by ovipositon (Thomas, 1972). An absence of dilatations (nulliparity) indicates lack of prior oviposition, one dilatation (uniparity) represents one prior oviposition, two dilatations (biparity), two prior ovipositions, etc (Thomas, 1972; Magnarelli and Pechuman, 1975).

Parity of individual females together with the time of emergence is used to determine autogeny, egg maturation in the absence of a blood meal, or anautogeny, egg maturation dependent on a blood meal (Cameron, 1926; Spielman, 1971; Thomas, 1972). When nulliparity is rare or absent, that population is autogenous. A population with large numbers of nulliparous individuals is anautogenous (Thomas, 1969, 1972; Magnarelli and Anderson, 1981).

Observations of the fat body present in the abdomen of the tabanids was done. Although this was not an original goal, an arbritary scale (+++, fat body occupied a large portion of the abdominal space; ++, fat body occupied a moderate portion; +, fat body occupied a small portion) was used to measure the amount of abdominal space the fat body

occupied. Fat body data were used with parity data to determine autogeny or anautogeny of tabanids dissected.

Taxonomic identification of horse and deer flies was based on keys by Phillip (1936), Nowiersky and Gittins (1979), Teskey (1983) and Turner (1985). Identification of larval nematodes found in the tabanids was based on descriptions by Hibler and Metzger (1974), Poinar (1975, 1985), Poinar et al. (1976), and Sonin (1985). Identification of anatomical features of tabanids was based on descriptions by Borror et al. (1976), and Snodgrass (1935).

RESULTS

A total of 1122 flies representing three genera and thirteen species was collected during 1984 and 1985. The species and the percent composition for each season are given in Table 1. Of the 1122 flies collected, 992 (88.4%) were dissected and 130 (11.6%) were pinned for identification. Of the 992 flies dissected, 784 were dissected after immobilization as discussed previously and the remaining 208 flies were dissected after being frozen for nine months.

Flies collected on the east side of the Bridger Range totaled 356 for all species compared with 447 from the west side, and 319 from the Gallatin Range south of Bozeman, MT.

Specimens collected on the east side of the Bridger Range included *Atylotus insuetus*, *Chrysops exitans*, *C. furcatus*, *Hybomitra opaca*, *H. osburni*, *H. rupestris*, and *H tetrica* during both seasons. Tabanids collected during the same period on the west side of the range included *A. insuetus*, *C. fulvaster*, *H. captonis*, *H. melanorhina*, *H. lasiophthalma*, *H. osburni*, *H. rupestris*, and *H. tetrica*.

Additional trapping and collecting with an insect net (two day period) at Rat Lake in the Gallatin Mountains south of Bozeman yielded the following species: *A. insuetus*, *C. ater*, *C. furcatus*, *H. osburni*, *H. rupestris*, and *H. tetrica*.

Table 1. Horse fly and deer fly species collected in southwestern Montana, summers 1984 and 1985.

Species	1984		1985		Totals	
	No. of Flies	Species %	No. of Flies	Species %	No. of Flies	Species %
Atylotus insuetus Osten Sacken	4	1.5	12	1.4	16	1.4
Chrysops ater Macquart	-	-	1	0.1	1	0.1
Chrysops exitans Walker	-	-	2	0.2	2	0.1
Chrysops fulvaster Osten Sacken	-	-	4	0.5	4	0.4
Chrysops furcatus Walker	-	-	12	1.4	12	1.1
Chrysops noctifer Osten Sacken	3	1.1	8	0.9	11	1.0
Hybomitra captonis (Marten)	2	0.8	4	0.5	6	0.5
Hybomitra lasiophthalma (Marquart)	-	-	1	0.1	1	0.1
Hybomitra melanorhina (Bigot)	1	0.4	-	-	1	0.1
Hybomitra opaca (Coquillett)	1	0.4	-	-	1	0.1
Hybomitra osburni (Hine)	216	81.2	348	40.7	564	50.3
Hybomitra rupestris (McDunnough)	23	8.6	196	22.9	219	19.5
Hybomitra tetrica (Marten)	16	6.0	269	32.3	284	25.3
Totals	266	100.0	856	100.0	1122	100.0

Seasonal Distribution of Tabanids

Seasonal distribution of three of the tabanid species collected is given in Figures 7 and 8. *Hybomitra osburni* peaked in numbers the latter part of July in 1985. The peak in 1984 was not as large but also occurred in late July and early August (Figure 8). *Hybomitra rupestris* and *H. tetrica* were present in early summer (late June to early July) in 1985. The last two species were rarely collected after mid-July during both seasons.

Fly Dissection and Recovery of Elaeophora schneideri Larvae

Two Tabanid species, *H. rupestris*, and *H. tetrica* were positive for first (L_1) (Figures 9, 10) and second (L_2) stage *Elaeophora schneideri* larvae (Figure 11). One species, *H. osburni*, was positive for L_1 larvae. Infected tabanids were found only in the study area west of the Bridger Range front. *Hybomitra osburni* and *H. rupestris* represent new host records for this filariid (Table 2). A subspecies of *H. tetrica*, *H. tetrica rubrilata* is an active vector of the arterial worm in southwestern United States (Clark, 1972; Hibler et al., 1971b).

Eight of 992 flies (0.8%) were positive for *E. schneideri* larvae in 1984 and 1985 (Table 2). The percent infection was 0.5% in 1984, and 0.9% in 1985 (Table 2). The total number of larvae recovered was 54 (Table 3). Measurements of the L_1 and L_2 stages are given in Table 4.

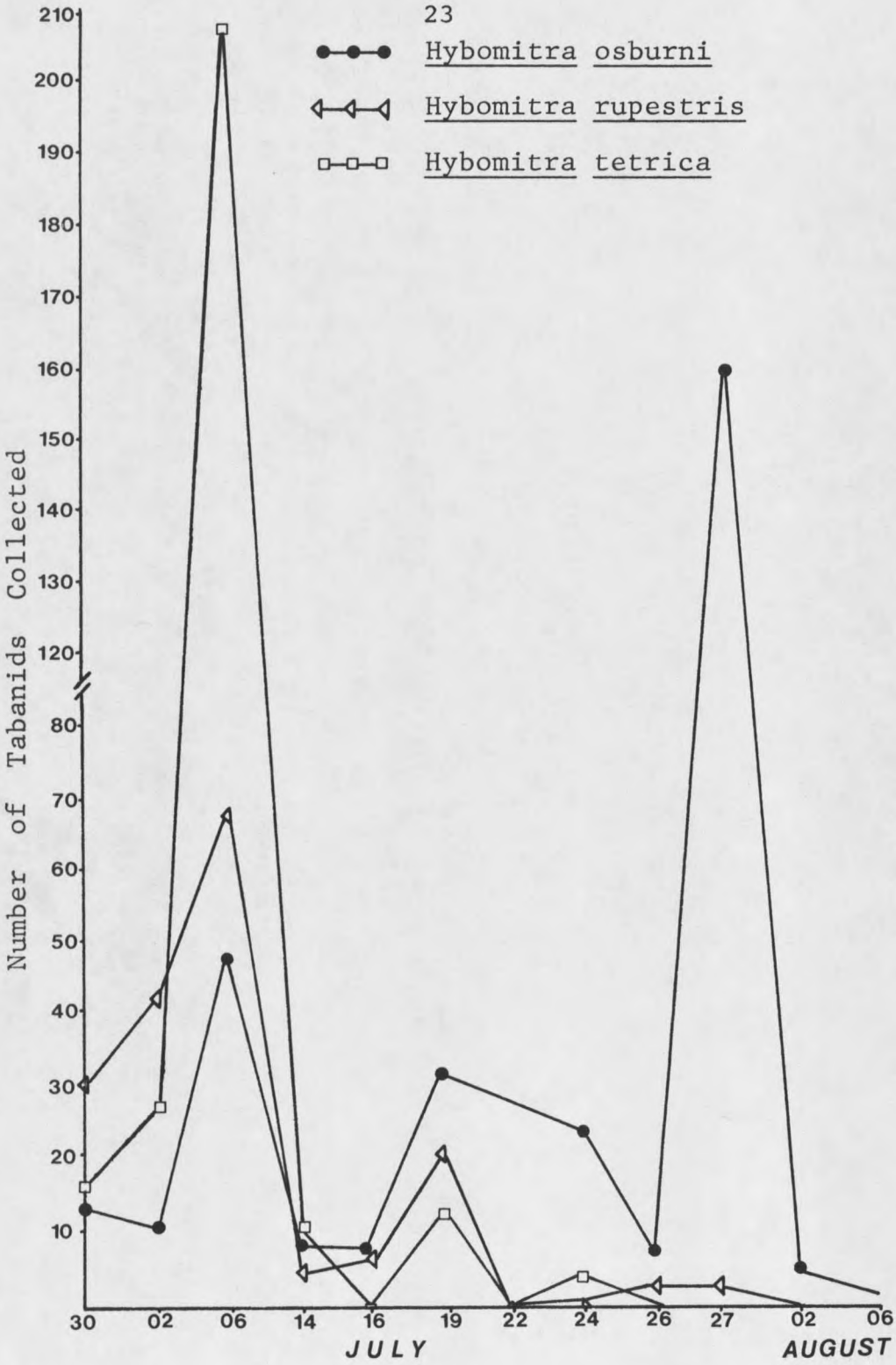


Figure 7. Seasonal distribution of three horse fly species collected in the Bridger Range, summer 1985.

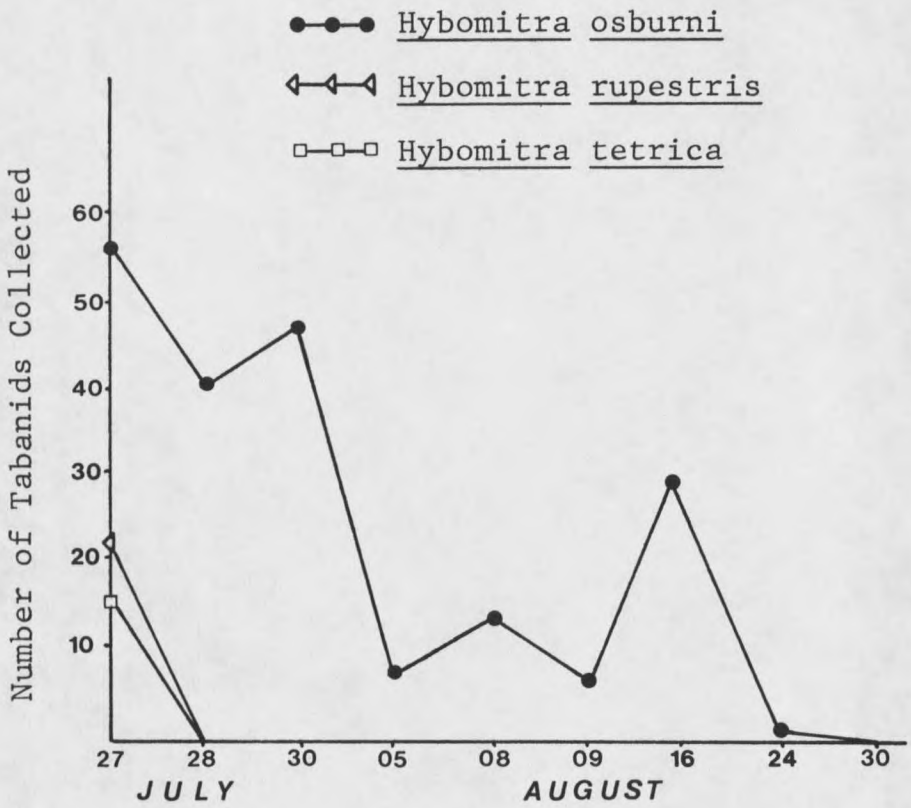


Figure 8. Seasonal distribution of three horse fly species collected in the Bridger Range, summer 1984.

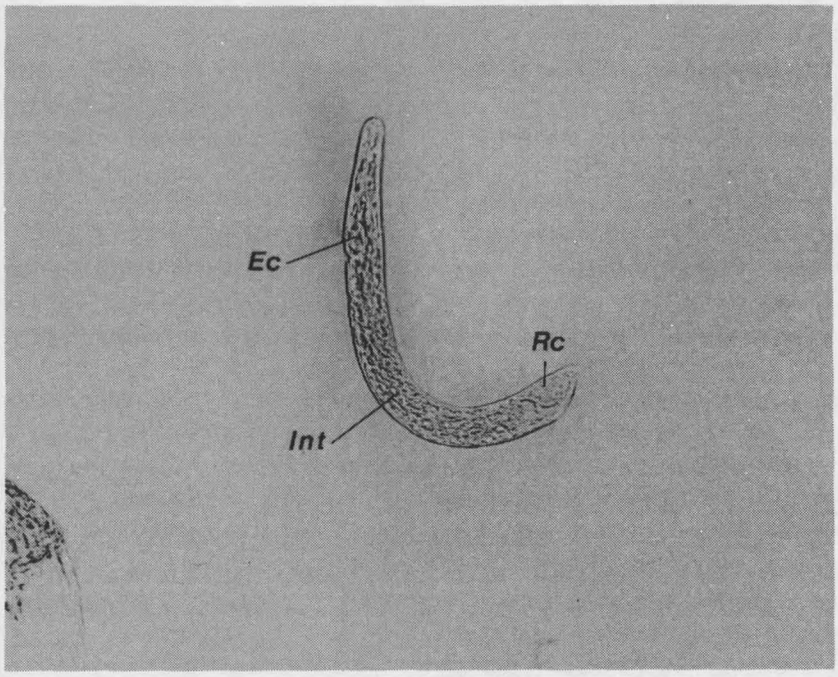


Figure 9. Early first stage larva of Elaeophora schneideri from a female Hybomitra osburni, summer 1984. Ec, excretory cell; Int, intestine; Rc, rectal cell. Magnification 200X.

Figure 10. First stage larva of Elaeophora schneideri from a female Hybomitra rupestris, summer 1985. Magnification 125X.

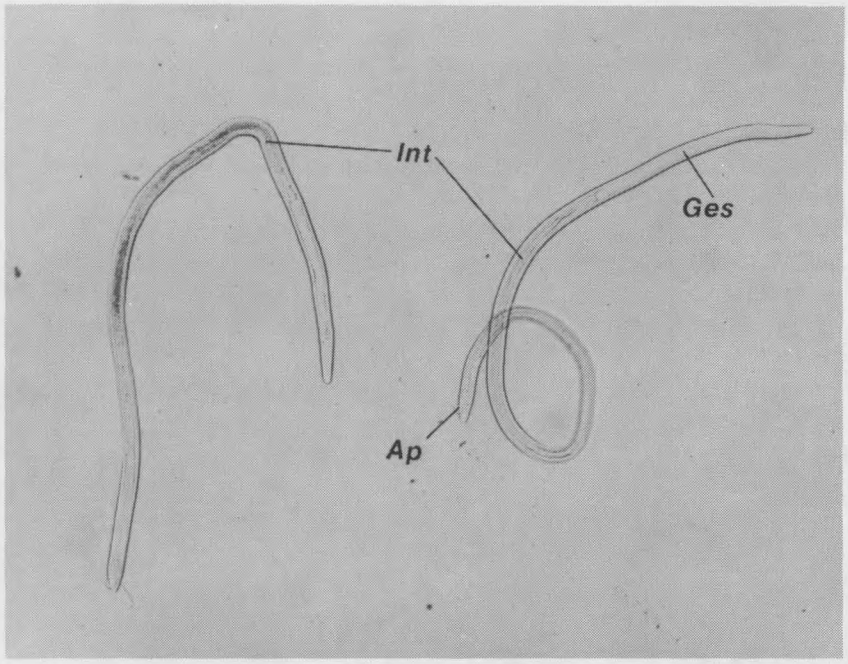


Figure 11. Second stage larvae of Elaeophora schneideri from a female Hybomitra tetrica, summer 1985. Ap, anal plug; Ges, glandular esophagus; Int, intestine. Magnification 60X.

Table 2. Horse and deer fly species examined for *Elaeophora schneideri* larvae, summers 1984 and 1985.

Species	1984		1985		Totals	
	No. Dissected	% Infected	No. Dissected	% Infected	No. Dissected	% Infected
Atylotus insuetus	1	0.0	10	0.0	11	0.0
Chrysops exitans	-	-	1	0.0	1	0.0
Chrysops furcatus	-	-	7	0.0	7	0.0
Chrysops noctifer	-	-	5	0.0	5	0.0
Hybomitra captonis	-	-	2	0.0	2	0.0
Hybomitra osburni	203	0.5	334	0.0	537	0.2
Hybomitra rupestris	3	-	169	1.8	172	1.7
Hybomitra tetrica	2	-	255	1.6	257	1.6
Totals	209	0.5	783	0.9	992	0.8

Table 3. Horse fly species infected with *Elaeophora schneideri*, summers 1984 and 1985.

Species	Loc.	Flies Infected	No. of Larvae Recovered
Hybomitra osburni	NC	1	3
Hybomitra rupestris	NC	3	10
Hybomitra tetrica	NC	4	41
Totals		8	54

NC-North Cottonwood Canyon, Bridger Mts. Gallatin Co. MT.

Table 4. Measurements of *Elaeophora schneideri* larvae from horse flies in southwestern Montana, summers 1984 and 1985. All measurements in micrometers (μm).

Larval Stage	n	Length $\bar{x}$(range)	Width $\bar{x}$(range)
L_1	32	975(357-1190)	40.2(31.6-49.7)
L_2	22	1767(1454-2825)	39.3(33.3-49.7)

Recovery of Mermithidae

Specimens of an unidentified mermithid nematode were recovered from the fat bodies, tracheoles, and internal organ surface of some tabanids, including a specimen from the lumen of the uterus. Twelve percent of the flies dissected were carrying this nematode during 1984 and 1985. The infection percent for each collecting period is given in Table 5.

Table 5. Horse and deer fly species examined for mermithid nematodes, summers 1984 and 1985.

Species	1984		1985		Totals	
	No. Dissected	% Infected	No. Dissected	% Infected	No. Dissected	% Infected
Atylotus insuetus	1	0.0	10	0.0	11	0.0
Chrysops exitans	-	-	1	0.0	1	0.0
Chrysops furcatus	-	-	7	0.0	7	0.0
Chrysops noctifer	-	-	5	0.0	5	0.0
Hybomitra captonis	-	-	2	0.0	2	0.0
Hybomitra osburni	203	8.8	334	18.8	537	15.0
Hybomitra rupestris	3	0.0	169	14.7	172	14.5
Hybomitra tetrica	2	0.0	255	5.4	257	5.4
Totals	209	8.6	783	13.0	992	12.0

The nematode was found in 12.1% (120/992) of thefemale tabanids, and 16.7% (2/12) of the males examined. The organism was always found dead, melanized and coiled (Figures 12, 13, 14). The melanization differed in pigment concentration from specimen to specimen. The significance of melanization will be discussed later.

Parity Data

Parity data were collected for Atylotus insuetus in 1984 only, and for H. osburni, H. rupestris, and H. tetrica in 1984 and 1985. Table 6 shows that 95.6% (194/203) of H. osburni dissected were uniparous, 3.4% (7/203) were nulliparous, and 1.0% (2/203) were biparous in 1984. All individuals of A. insuetus (1/1), H. rupestris (3/3) and H tetrica (2/2) examined in 1984 were nulliparous.

In 1985 (Table 7), 59.6% (199/334) of H. osburni were uniparous, and 13.2% (44/334) nulliparous. The parity of 27.2% (91/334) flies could not be determined. Hybomitra rupestris had 39.6% (67/169) specimens uniparous, 31.4% (53/169) nulliparous, and in 29.0% (49/169) the parity was unknown. Hybomitra tetrica had 20.4% (52/255) uniparous females, 28.6% (73/255) nulliparous, and 51.0% (130/255) were unknown.

Data relevant to fat body development (Tables 6 and 7) showed that the majority of H. osburni (71.1% in 1984, and 63.1% in 1985) were uniparous and had a well developed fat

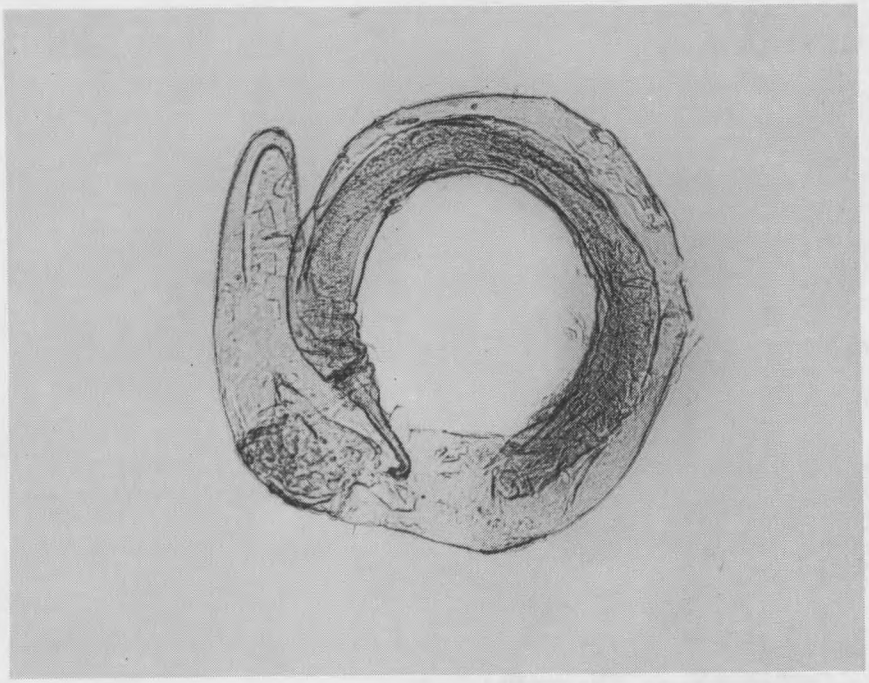


Figure 12. Mermithid nematode from a female Hybomitra osburni, summer 1984. Magnification 175X.

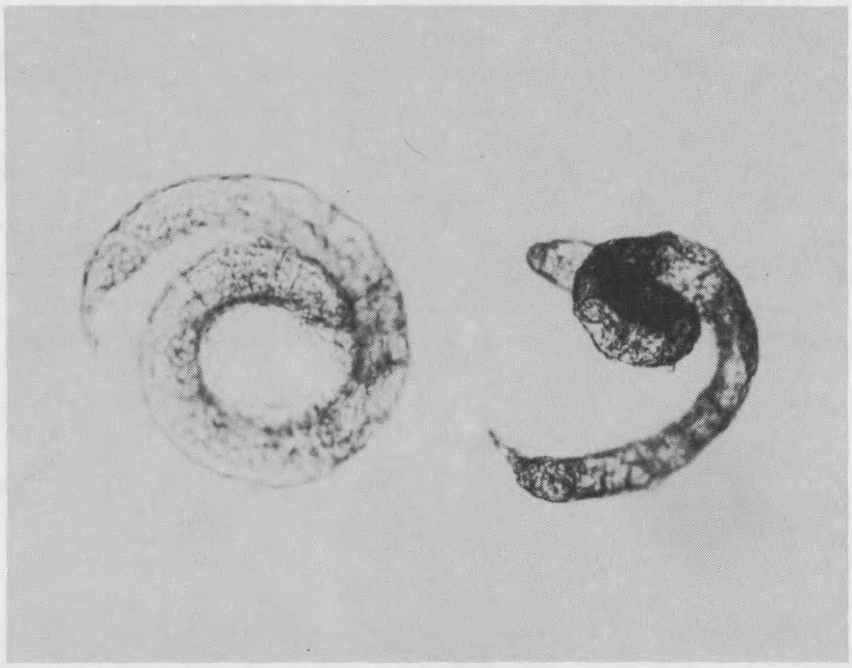


Figure 13. Mermithid nematode from a male Hybomitra osburni, summer 1985. Magnification 145X.

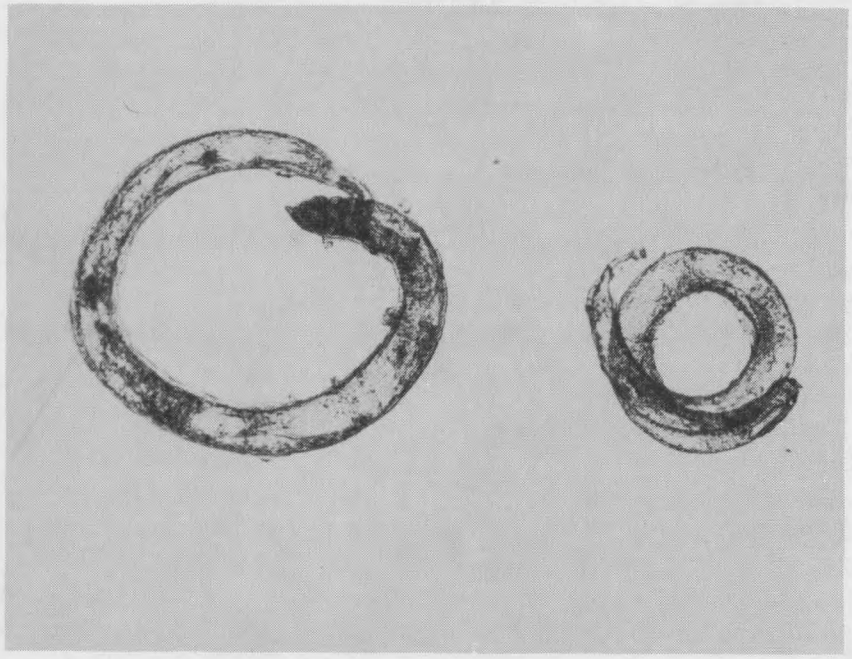


Figure 14. Mermithid nematode from a female Hybomitra tetrica, summer 1985. Magnification 145X.

Table 6. Parity of three horse fly species dissected, summer of 1984.

Species	Parity(n)	Fat Body[a] +++	++	+
Atylotus insuetus	U1[b]	1	0	0
Hybomitra osburni	U(194)	138	50	6
	N(7)	6	1	0
	B(2)	2	0	0
Hybomitra rupestris	N(3)	2	1	0
Hybomitra tetrica	N(2)	2	0	0

a Fat body/cells development, +++= well developed, ++= medium development, += poorly developed.
b N= nulliparous, U= Uniparous, B= Biparous

body in the abdomen. Uniparous and nulliparous individuals of H. rupestris and H. tetrica had a high percent of well developed fat bodies.

A comparison of the number of flies found to be uniparous and nulliparous with the date when collected is given in Figure 15. This was done for each of the three common species. Hybomitra osburni showed a low number of nulliparous individuals, while uniparous individuals were present in large numbers during the summer of 1985. Hybomitra rupestris initially showed a larger number of nulliparous individuals but uniparity appears to increase after July 2nd and probably stays level until July 24th. The low number of uniparous members during July 14 to 16 may reflect low numbers collected during that period (Figure

7). Nulliparity and uniparity were similar in number throughout the summer for *H. tetrica*.

Table 7. Parity of three horse fly species dissected, summer of 1985.

Species	Parity(n)	Fat Body[a] +++	++	+
Hybomitra osburni	U(190)[b]	120	59	11
	U(9)	-	-	-[c]
	N(37)	25	12	0
	N(7)	-	-	-
	-(91)	-	-	-
Hybomitra rupestris	U(59)	47	7	5
	U(8)	-	-	-
	N(33)	19	1	13
	N(20)	-	-	-
	-(49)	-	-	-
Hybomitra tetrica	U(35)	35	0	0
	U(17)	-	-	-
	N(41)	31	0	10
	N(32)	-	-	-
	-(130)	-	-	-

a Fat body/cells development, +++= well developed, ++= medium development, += poorly developed.
b N= nulliparous, U= Uniparous, B= Biparous
c No data available.

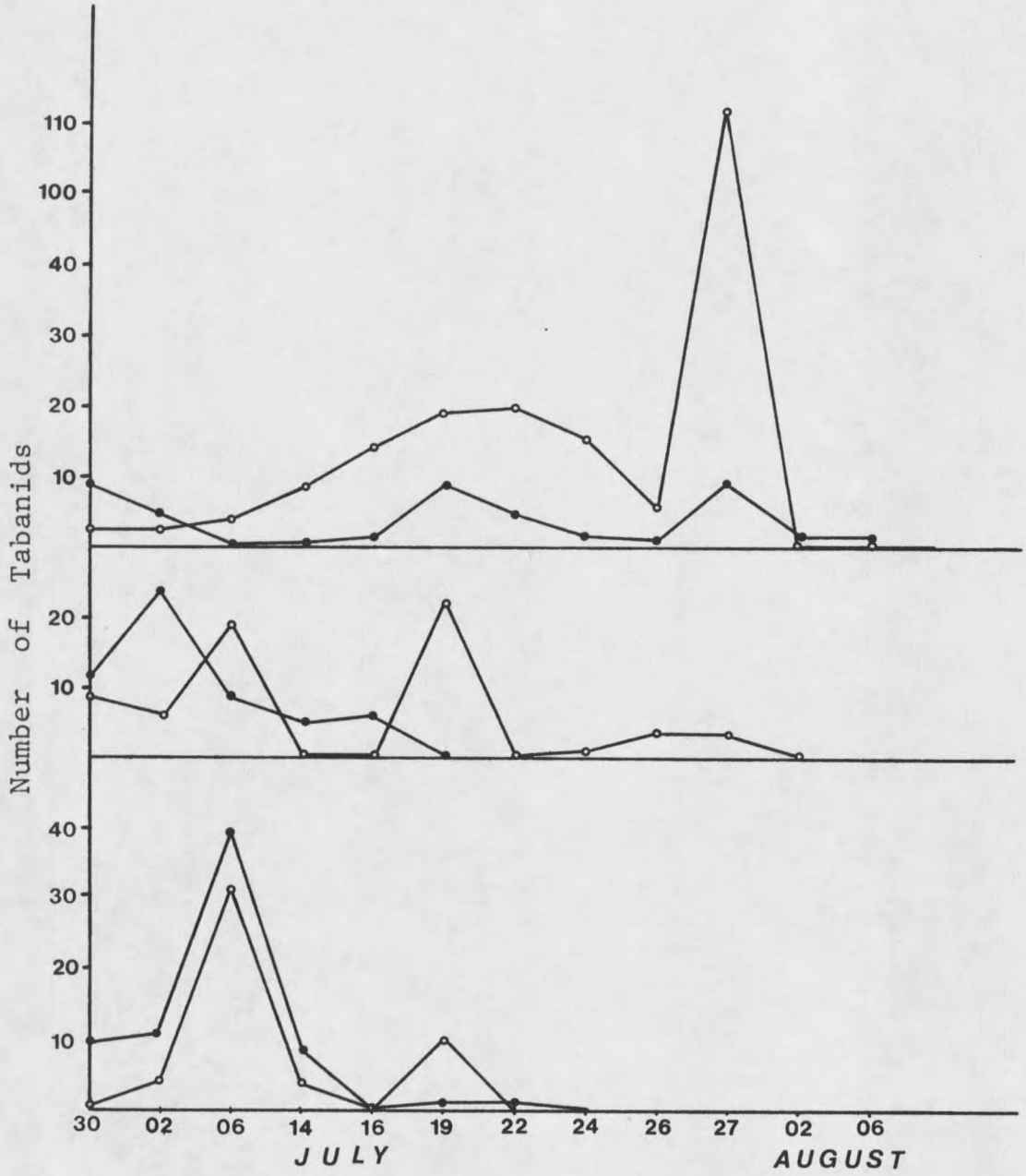


Figure 15. Number of nulliparous and uniparous individuals of three horse fly species trapped in the summer of 1985.

DISCUSSION

Species Composition of Tabanidae

Differences in tabanid species collected on both sides of the Bridger Range and in the Gallatin Range probably reflect the habitat selection during trap placement, behavioral aspects of individual species present, and trap efficiency. I suspect that all species collected are present throughout the Bridger and Gallatin Mountains.

Comparative studies of fly traps show that canopy traps are efficient for horse flies while attracting few species of deer flies (Roberts, 1976; Thomas, 1970). Thompson (1969) reported that the Manitoba trap collects more specimens of deer flies than any other method. Results of this study are similar to collections by Thomas (1970). The Manitoba traps collected more species of horse flies than deer flies.

Male tabanids are not often caught using Manitoba fly traps (Thomas, 1970; Roberts, 1976). The male flies hover in a fixed area or lie on vegetation waiting for passing females, then engage in active pursuit (Leprince et al., 1983). This behavior may explain how the males, which are exclusively nectar feeders (Kniepert, 1980; Leprince et al., 1983) were captured in the Manitoba traps.

The seasonal occurrence of tabanids was determined for three of the thirteen species collected (*Hybomitra*

osburni, H. rupestris, and H. tetrica). In the Bridger Range, H. osburni was abundant throughout the summer in 1984 and 1985. Numbers of this species decreased from July 14 to July 16, 1985, then increased and dropped again on July 16, 1985. I believe the decrease in numbers is the result of windy conditions, cloudiness and temperature drops during the collecting periods mentioned, rather than two separate emergence peaks. An increase in numbers was noticed from late July to early August (Figures 7 and 8). In contrast, this species peaks in numbers in early July in Alberta but remains present from mid-June to mid-August (Thomas, 1970).

Numbers of Hybomitra rupestris and H. tetrica peaked in early July in 1985 (Figure 7) and appeared to be dropping from a similar peak in 1984 (Figure 8). Thomas (1970) reported that H. rupestris emerged in late July with peak numbers in early August. This may be attributed to latitude differences between Alberta and southwestern Montana. However, emergence of H. tetrica was similar to emergence patterns of H. tetrica hirtula in Alberta with peak numbers present during the first ten days of July.

Horseflies and Elaeophora schneideri

Clark (1972) found six horse fly and one deer fly species in northern New Mexico infected with larval stages of E. schneideri. Hybomitra laticornis made up 90% ofall infected flies. Similar studies in Vermejo park, New Mexico,

showed 98% of the vectors of E. schneideri to be Hybomitra aatos (Davies, 1979).

Three tabanid species were found infected with the larval stages of the arterial worm in the present study (Table 3). One species, H. tetrica made up 50% of the individual vectors collected (Table 3). The infective L_3 stage of E. schneideri was not recovered from dissected flies whereas L_1 and L_2 stages were found (Table 3).

Hybomitra osburni was the most common fly collected, but its importance as a vector of E. schneideri may be minimal. Hybomita rupestris and Hybomitra tetrica probably have a greater role in the transmission of the arterial worm in southwestern Montana. The latter species had a higher infection percentage than H. osburni.

Why Hybomitra rupestris and H. tetrica have the highest prevalence of infection is not known. The fact that Hybomitra species act as vectors in southwestern Montana is not surprising. Hybomitra species are the most common vector of E. schneideri in the southwestern United States (Hibler and Adcock, 1971).

Members of the genus Tabanus have been implicated as vectors (Clark, 1972; Davies, 1979) but the prevalence of infection is low. Specimens of this species were not collected during this study. The absence of Tabanus species may indicate the lack of this genus in the areas sampled, since Tabanus species are readily collected with Manitoba

traps (Thorsteinson, 1958; Thompson, 1969; Thomas, 1970; Davies, 1979; Pechuman et al., 1983). Chapman (1954) showed that Tabanus sequax was the only member of this genus observed at Squaw peak, Missoula Co., Montana at an elevation of 2423 m.

The larval stage of E. schneideri recovered from the three tabanid species may depend on the longevity of the vector species involved. Olkowski (1966) stated that the mean survival after emergence of Tabanus nigrovittatus was 12.3 days. Thompson and Krauter (1980) showed that for T. nigrovittatus and T. l. hinellus only 50% survived six days, 18% for 14 days, and 5-7% for 21 days. Fifty percent of Tabanus acutus survived for 9 days, 15-20% for 14 days, and 5-6% for 21 days.

If these longevity data are a fair representation of tabanid populations, and two weeks are required for E. schneideri to develop to the infective L_3 stage after initial ingestion by a fly, only 5-6% of any given tabanid population would be available to allow development to the infective stage. These flies would have 4-5 days of survival after acquiring E. schneideri larvae. Because of these time limitations, such a fly population would be a dead end for the arterial nematode larvae.

Longevity of tabanids appears to vary, and no comparative work is available for species of North America. Chvala et al.,(1972) reported that adult horse flies live

for six weeks. This differs significantly from the work by Olskowski (1966) and Thompson and Krauter (1980) previously cited.

Autogenous species may acquire Elaeophora larvae at the beginning of the gonotrophic cycle (actually their second gonotrophic cycle) that requires blood from a definitive host as the energy source. The latter egg development cycle may begin at a point when only 6-7 days are left in the vector's lifespan. This could be the case for H. osburni and would allow development of E. schneideri larvae to the L_1 stage. Anautogenous species, if they became infected shortly after emergence, would have enough time for the development of E. schneideri larvae to the infective stage. This scenario may be present in H. rupestris and H. tetrica in southwestern Montana.

The prevalence of the arterial worm in tabanids in southwestern Montana was low (0.8%), compared to Arizona and New Mexico, where the average prevalence is 14.5% (Clark, 1972) and 19.1% (Hibler et al., 1971). Davies (1979) found 10% of H. aatos infected and studies in South Carolina showed a prevalence of 0.3% in Tabanus l. hinellus surveyed (Couvillion et al., 1984).

Specimens of a nematode believed to belong to the family Mermithidae were present in the fat bodies and other internal organs of flies during present study. Similar

organisms were identified as dead L_1 stages of the arterial worm by Davies (1979).

Identification of the mermithid species was impossible due to melanization, a cellular-humoral defense mechanism that involves a number of insect blood cells (amoebocytes, leucocytes, lymphocytes, microneuclocytes, thrombocytoids) and precipitation of components from the non-cellular portion of the haemolymph (Ratcliffe and Rowley, 1979). Coordinated or individual actions by these systems results in the formation or release of precursors needed for the formation of the pigment melanin (Poinar et al., 1979). A successful response by the humoral system results in the deposition of melanin layers around a foreign object entering the haemocoel of an insect (Poinar et al., 1979). All mermithids collected were melanized, but the degree of melanin present varied. General anatomy of invading nematodes remain constant through the melanization process (Poinar and Leutenegger, 1971, Poinar, personal communication). The nematodes with low amounts of melanin present on the surface of the cuticle did not fit descriptions of larval stages of E. schneideri (Hibler and Metzger, 1974).

The mermithids were probably obtained during the larval stage of the tabanids and died as a result of melanization 3-5 days after infection (Poinar, personnal communication). Comparison of melanized nematodes collected from female

horse flies and specimens from male horse flies showed morphologic features similar to the larvae identified as mermithids in this study (Figures 13, 14). Since males are strictly nectar feeders (Kniepert, 1980; Leprince et al., 1983) and E. schneideri larvae can only be acquired through active blood feeding, I believe the nematode belongs to the Mermithidae, a family that parasitizes a number of insect orders (Welch, 1965; Nickle, 1972; Poinar, 1972). Melanized nematodes found were not included in the E. schneideri infection prevalence data.

Horse Fly Parity and Host Restriction

Information collected on parity and fat body deposits during this study points to H. osburni as autogenous. This agrees with Thomas (1972) who concluded that H. osburni is autogenous and present throughout the summer. This species was also present throughout the summer in southwestern Montana with peak numbers present between July 27 and August 6, 1984 and 1985.

The number of uniparous and nulliparous individuals was similar for H. rupestris although the nulliparous numbers preceed the uniparous individuals. This may indicate anautogeny of this species. Hybomitra tetrica showed a difference between the numbers of uniparous and nulliparous flies collected, but there was no difference in the date at which each group appeared. No clear decision can be made,

but the larger numbers of nulliparous H. tetrica, the early emergence of H. rupestris and H. tetrica, and the presence of peak numbers before mid July, suggest that these species are anautogenous. Similar results were obtained for anautogenous species by Thomas (1972).

The amount of fat bodies present in the abdominal cavity is indicative of the ability of the species in question to be autogenous or anautogenous (Lake and Burger, 1980). Autogenous individuals have a greater volume of fat bodies present in the abdominal cavity than do anautogenous flies (Rockel, 1969). The present study showed that H. osburni specimens had well developed fat bodies and suggests that this species is autogenous. No decision could be made on autogeny or anautogeny of Hybomitra rupestris and H. tetrica based on fat body development.

A hindrance to properly determining the parity and fat body levels of H. rupestris and H. tetrica was that a large number of these species were frozen prior to dissection. The freezing damaged cellular detail and caused irreversible dehydration of body tissues. This is in contrast to work by Thomas (1972, 1973) that showed no difference between ovarioles of frozen specimens and fresh specimens. However detecting mermithid and filarial nematodes was not hindered by freezing.

The restricted host range of E. schneideri in Montana is not well understood and will require further research

before an adequate explanation can be offered. This study has shown that H. tetrica and H. rupestris are the vectors of the arterial worm in southwestern Montana. Hybomitra osburni, although involved, may play a smaller role due to its autogenous egg production.

The Bridger mountains provide habitat for moose, elk, and mule deer. All three species are infected with E. schneideri in different areas in the western United States (Hibler and Adcock, 1971; Worley et al., 1972, Worley, 1975). In Montana, only mule deer and moose have been found infected.

Why elk are not infected with the arterial worm, although sharing the same geographic areas where the parasite, vector, and other definitive hosts are present, is not known. However, a few speculations can be made based on the results of this study and ecological studies of mule deer and elk in southwestern Montana. Mule deer spend the winter on the southwestern slopes of the Bridger Range and summer in the eastern slopes (Pac, 1976), and adult females select forest habitat and avoid prairie and subalpine areas (Pac, et al., 1984). The horse flies were found concentrated in the higher altitudes, in open fields adjacent to forested areas.

This difference in habitat selection may decrease the encounters between mule deer and horse flies, possibly explaining the low prevalence of the infection in Montana.

It is equally likely that the parasite is present in low numbers in this state. Worley et al.(unpublished) believe that Montana is located at the northern limit of the geographic range of the arterial worm, since there are no reports of its occurrence in wild ruminants in northern Montana or adjacent portions of Canada.

Elk exposure to infected tabanids may be minimal to none. Although elk share winter grounds with mule deer in the northwestern slopes of the Bridger range, they move from the wintering grounds 4-6 weeks prior to the emergence of the horse flies (Pac, personal communication). Brazda (1953) observed elk moving from wintering grounds (at lower elevations) to higher windy elevations, thus reducing exposure to infested geographic areas in the Gallatin drainage in southwestern Montana.

Red deer in Scotland move during early spring to avoid emergence of tabanids and occupy "well winded areas" for the remainer of the season (Darling, 1937). The elk population in the Bridger mountains numbers about 200 animals (Pac, personal communication) and movement away from the west slopes, a focus of infected tabanids, would minimize exposure.

The role of moose in elaeophorosis is not known, but Worley (1975) reported infections in moose from the Bridger Range area and Absaroka Mountains. These large cervids show clinical symptoms indicative of greater susceptibility or

exposure to the arterial nematode (Worley et al., unpublished). Further information about habitat selection of resident moose or transient individuals is needed to understand their role in *Elaeophora* transmission in the Bridger Range.

Before the ecology of *E. schneideri* is understood in southwestern Montana, variables not addressed during this study (climate, habitat diversity, horse fly and deer population movement, and habitat selectivity by vectors and hosts), need to be studied and evaluated. Species of tabanids implicated as important vectors of the arterial worm need to be subjected to parity, feeding behavior, longevity, and life cycle studies before the subtle relationship between vectors and primary hosts is understood.

SUMMARY

A study was undertaken during June, July, and August, 1984 and 1985, to identify possible tabanid vectors of the arterial nematode Elaeophora schneideri Wehr and Dikmans, 1935 in southwestern Montana. Thirteen species (Diptera: Tabanidae) were collected: Atylotus insuetus Osten Sacken, Chrysops ater Macquart, Chrysops exitans Walker, Chrysops fulvaster Osten Sacken, Chrysops furcatus Walker, Chrysops noctifer Osten Sacken, Hybomitra captonis (Marten), Hybomitra lasiophthalma (Macquart), Hybomitra melanorhina (Bigot), Hybomitra opaca (Coquillett), Hybomitra osburni (Hine), Hybomitra rupestris (McDunnough), and Hybomitra tetrica (Marten).

Three Hybomitra species, (H. osburni, H. rupestris, and H. tetrica) comprised 95% of the 1122 tabanids collected. Hybomitra osburni was most abundant during both years of the study, followed by H. tetrica, and H. rupestris.

Three tabanid species were found infected with larval stages of E. schneideri. One female H. osburni was found infected with three first stage larvae in 1984. Hybomitra rupestris and H. tetrica were infected with first and second larval stages. Hybomitra osburni and H. rupestris are a new host records for E. schneideri.

Prevalence of infection was 0.5% for H. osburni, 1.8% for H. rupestris, and 1.6% for H. tetrica. Percent infection for all species dissected was 0.5% in 1984, 0.9% in 1985, and 0.8% for both years.

Seasonal distribution data were obtained for the three common species. Emergence of H. osburni occurred in late June and persisted until late July when a peak in numbers was observed. In contrast, H. rupestris and H. tetrica peaked between June 30 and July 19. The latter species were present in low numbers the rest of the summer (1984, 1985).

Elaeophora schneideri larvae were recovered from eight horse flies. Fifty percent (4/8) of the infected flies were H. tetrica, 37.5% (3/8) H. rupestris, and 12.5% (1/8) were H. osburni.

An unknown nematode species (Nematoda: Mermithidae) was found in 12.1% of all flies dissected during both years of the study. As with the arterial nematode larvae, this nematode was found in the fat bodies and free in the haemocoel of horse flies dissected. Proper identification was hindered by the hosts immune response that resulted in the deposition of the pigment melanin around the invading nematodes.

Observations of the fat body depots and ovariole dilatations of dissected tabanids allowed determination of parity (oviposition history). Based on these data, it appears that H. osburni was autogenous; i.e, (haemotophagous female

does not require a blood meal for development of the first egg batch) whereas H. rupestris and H. tetrica appear to be anautogenous.

Hybomitra rupestris and H. tetrica, were the important vectors of E schneideri in the Bridger Mountain study area. The most common species, H. osburni was not considered to be important, based on percent of infection and autogenous egg production.

More studies are needed to determine conclusively the parity, areas of emergence, tabanid population movements together with ungulate behavior during the horse fly season to understand the lack of the arterial nematode in elk populations in southwestern Montana.

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