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The diurnal host-seeking and carbohydrate feeding pattern of *Tabanus nigrovittatus* (Macquart) and *Tabanus conterminus* (Walker).

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THE DIURNAL HOST-SEEKING AND CARBOHYDRATE FEEDING
PATTERN OF TABANUS NIGROVITTATUS (MACQUART)
AND TABANUS CONTERMINUS (WALKER)

A Thesis Presented

by

GABRIELLE ELIZABETH SAKOLSKY

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
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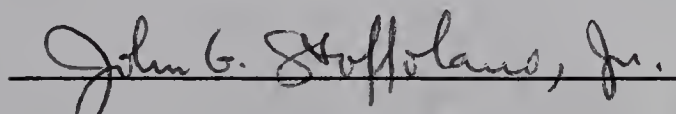
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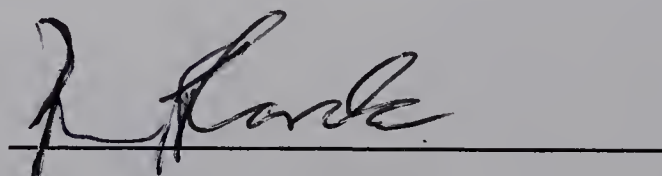
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CHAPTER 1 INTRODUCTION

Literature Review

The Pest Status of Tabanidae

The salt marsh greenhead fly is a complex of two species Tabanus nigrovittatus Macquart and Tabanus conterminus Walker (Diptera: Tabanidae). Both species are found sharing marshes on the Atlantic coast from Nova Scotia to southeastern Georgia (Sofield et al. 1984a). Emergence traps set out on the salt marsh areas in New Jersey catch greenhead flies from late June to late August (Rockel and Hansens 1970). Adults emerge and are found on Cape Cod, Massachusetts, marshes from the last week of June through the middle of August.

Some species of tabanids are anautogenous. This means that they require a blood meal for oocyte maturation. Blood feeding behavior by the deer fly, Chrysops univittatus Macquart, was studied by Troutbridge and Davies (1975) and Leprince and Lewis (1983) who concluded that this species is anautogenous because nulliparous flies were host-seeking. Many studies on the species Hybromita lasiophthalma Macquart have shown this species to be anautogenous (Morris and Defoliart 1971, Thomas 1972, Magnarelli and Anderson 1981, and Leprince and Bigras-Poulin 1990). Studies conducted on Tabanus quinquevittatus Wiedmann have determined that this species also is anautogenous (Magnarelli 1976, Magnarelli and Anderson 1981, Leprince and Lewis 1986). Wilson (1967) showed that Tabanus lineola Fabricius and Tabanus fuscicostatus Hine females blood meals not only to lay their first batch of eggs, but also continue to host-see and obtain blood meals for subsequent batches of eggs.

Other species of tabanids have been shown to be autogenous for the first gonotrophic cycle. This means that these flies can complete oocyte maturation and lay their first batch of eggs without ingesting a blood meal. The deer fly species, Chrysops

atlanticus Pechuman and *Chrysops fuliginosis* Wiedman, both have been shown to be autogenous in the first gonotrophic cycle (Rockel 1969, Anderson 1971, and Magnarelli and Anderson 1977). It has also been determined that *Tabanus nigrovittatus* does not require a blood meal for oocyte maturation in the first gonotrophic cycle (Anderson 1971, Bosler and Hansens 1974, Magnarelli and Stoffolano 1980). It is the physiological requirement of further nutrients for the maturation of any subsequent batches of eggs that causes these flies to seek a protein meal in the form of vertebrate blood (Bosler and Hansens 1974). The activity of obtaining a blood meal causes greenhead flies to become a nuisance to the public. The bite is not only painful, but can facilitate the spread of disease.

An article published by Foil (1989) on the role of tabanids as vectors of disease causing organisms describes a good vector as one whose feeding on an infected host can be interrupted so that the vector then moves to a susceptible host. The pain inflicted by a tabanid bite causes a host to try to remove the fly before its feeding has been completed. Krinsky (1976) wrote a review article on the disease organisms vectored by species of Tabanidae. This article examines 32 disease agents that are transmitted by tabanids. *Tabanus nigrovittatus* is listed as being capable of mechanically transmitting western equine encephalitis virus, *Anaplasma marginale*, and *Bacillus anthracis*. More recently, tabanids have been shown to carry *Borrelia burgdorferi*, the pathogen that causes Lyme disease (Magnarelli et al. 1986). Whether this pathogen is successfully transmitted to vertebrates by tabanids remains to be proven. Tabanids are thought to be a potential secondary vector of this disease, but may increase the risk of humans being infected with Lyme disease in tick-infested areas by spreading the disease among medium sized mammals (e.g raccoons).

Increases in population levels of the *Tabanus nigrovittatus* complex in the middle of the summer causes increased contact of host-seeking females with people. The increased contact means increased nuisance levels and increased potential for disease transmission.

Carbohydrate Feeding of Tabanidae

Diptera that require a blood meal for oocyte maturation also require carbohydrate intake for energy. Many studies have been conducted on carbohydrate feeding in mosquitoes. Some of these studies examined the natural carbohydrate feeding behavior of different species of mosquitoes in the field (McCrae et al. 1976, Riesen et al. 1986, Andersson 1990). A laboratory study determined that carbohydrate feeding has an effect on follicular development (Magnarelli 1978). Sugar type and concentration has been shown to affect the amount of food source consumed, as well as the destination of a meal (Downes 1958, Friend et al. 1988, Friend et al. 1989). In some species of mosquitoes, sugar solutions (i.e. nectar, etc.) are diverted to the crop while blood meals are directly processed in the midgut (Tremblay 1952, Friend 1981). The carbohydrate is stored and used slowly for energy as needed.

Although less significant as vectors of disease agents than mosquitoes, the nuisance caused by the tabanid blood feeding makes it important to understand the biology of these flies. The better the biology of these flies is understood the easier it is to find a point in their life cycle where control measures would be most successful. Like mosquitoes, tabanids also require carbohydrates for general metabolism and for flight energy (Hocking 1953). There have been observations of these flies obtaining carbohydrates from plant sources in the form of aphid honeydew, nectar, or both (Roberts 1967, Pratt and Pratt 1972). Many studies have tested for fructose in wild tabanids as an indicator of nectar feeding (Magnarelli and Anderson 1977, Magnarelli et al. 1979, Kniepert 1980, Magnarelli and Anderson 1981, Leprince et al. 1983, Leprince and Lewis 1986, Hoppe 1989, Leprince and Bigras-Poulin 1990). A few pollen grains have been found in the guts of individual tabanids (Wilson and Lieux 1972).

Schutz and Gaugler (1989) observed that male greenhead flies were feeding on honeydew as a source of carbohydrates. A study designed to identify not only the diurnal pattern of carbohydrate feeding, but also the source of carbohydrates for tabanids showed that no *T. nigrovittatus* of either sex were seen feeding on flowers near a salt marsh (Magnarelli et al. 1979). Where the females obtain carbohydrates for flight and egg

maturation has not been demonstrated. In a laboratory study, the effect of the sugar concentration on the amount of a food source consumed and the destination of the meal were also examined in the greenhead fly (Friend and Stoffolano 1991). Female *T. nigrovittatus* were shown to have a mechanism of crop diversion similar to that found in the mosquito (Stoffolano 1983). In contrast to mosquitoes, no one has examined the significance of carbohydrate feeding on flight, survival, and/or reproductive potential for tabanids. Before these aspects of tabanid biology can be addressed, it is important to develop a method for analyzing the carbohydrate stores in field caught flies. Thus, one objective of this thesis was to determine the quality and quantity of carbohydrates stored in the crop of female greenhead flies.

Flight Activity of Tabanidae

The daily flight activity of tabanids, in general, has been studied extensively. The flight activity pattern is important to understanding what is affecting the flies host-seeking behavior. Hollander and Wright (1980) studied the daily host-seeking activity cycles of many species of tabanids being attracted to a tethered cow and carbon dioxide traps. They showed that most flies were most active from 0900-2100 h. Later, work was done to determine the dispersal range of host-seeking *Tabanus abactor* Philip (Cooksey and Wright 1987). They found that most of the flies released stayed within 0.4 km of the release site. The effect of different environmental factors on the flight activity of freshwater female horse flies was studied by Burnett and Hayes (1974). They concluded that barometric pressure was the most influential factor. Studies of male tabanid activity focused on the timing of when male hovering behavior was occurring. Male *T. lineola* were shown to be most active between 0200-0600 h central standard time (Richardson and Wilson 1969).

Past studies have shown that weather has an important effect on the flight activity of greenhead flies. Schulze et al. (1975) examined the flight activity of females moving on to and off of the marsh. The greatest flight activity occurred between 0800-1600 h and was directly related to the temperature and percentage of cloud cover. An earlier study of the flight activity of flies on the marsh concluded that the flies were most active between 0800-

2000 h (Joyce and Hansens 1968). This activity was also directly related to temperature and percentage cloud cover. A more recent study examined the environmental influence on hovering behavior of male *T. nigrovittatus* and *T. conterminus* (Gaugler and Schutz 1989). That study concluded that each species has a specific hovering period that corresponds to time of day and temperature. No one, however, has determined whether female *T. nigrovittatus* and *T. conterminus* have different diurnal host-seeking activity patterns. Thus, the second objective of this thesis is to examine the diurnal host-seeking activity of each species of greenhead fly in relation to time of day.

Study Area

This study was conducted in Provincetown, Massachusetts, at Hatches Harbor salt marsh in the Cape Cod National Seashore (Figure 1.1). Observations at the site have shown that greenhead flies are present and breeding there (pers. obs.). Larval *T. nigrovittatus* and adults were found on sites within 5 miles of this marsh (pers. com. J. Freeman). The Hatches Harbor marsh is composed of *Spartina alterniflora* (Loisel) and *Spartina patens* (Loisel) grasses. Both of these grasses have been shown to occur in association with the breeding sites of *T. nigrovittatus* (Dale and Axtell 1976). Four traps were placed where these grasses come together at the back of the marsh (Figure 1.1). This site was chosen because of the lack of tabanid management (i.e., no trapping program for these flies) in the area and because of the diversity of possible carbohydrate sources found within around 0.5 km (Table 1.2). A dike extends along the side of the marsh and adjacent to the dike is a brackish marsh. The brackish marsh area provides a suitable environment for fresh water flowers that could possibly act as additional carbohydrate sources.

The marsh is partially flooded on a daily basis by a tidal gut that bisects the marsh. The marsh is completely flooded three to five days every month. On these days the grasses remain under water for a maximum of four hours. Islands with grass were still found in the marsh during this flooded period, but most of the marsh grass is completely submerged.

The site is also isolated from human use. The nearest tourist beach is over one mile away but is visible from the study site. Deer were sighted on the marsh only two times during both seasons. Large vertebrate hosts are not common on this marsh.

Daily Collection

Collection of *T. nigrovittatus* and *T. conterminus* were made during two summer seasons (1991 & 1992). Four box traps were placed in a line (North to South) across the marsh in the middle of June (Figure 1.1). The traps were placed about twenty meters apart with the last trap separated from the dike by about 90 meters. Collections were made twice a week, the first week of July through mid-August, during the first field season and from the last week in June through the end of August during the second field season. On collection days, flies were removed from traps hourly and environmental conditions (stage of tide, temperature, wind speed, wind direction, and percentage of cloud cover) were recorded between 0700 and 1700 h.

Blue box traps, provided by the Cape Cod Mosquito Control Project, were used to trap female flies. The dimensions of the traps were 80 cm x 82 cm x 60 cm. The trap bottoms were set about 70 cm above the marsh. The trap tops were modified into a pyramid shape, each with a collection jar on top (Dale and Axtell 1976) (Figure 1.2). At the highest point, these tops were about 40 cm deep. Each collection jar contained a piece of pesticide strip (active agent 2,2-dichlorovinyl-dimethyl-phosphate) to kill the flies as they entered the jar.

Active flies were removed from the trap by placing two bath towels over the pyramid top of the trap. This caused the entire trap to become dark except for the region having the collection jar at the top. Flies that had not already entered the jar were drawn toward the light and into the jar. The jar was then removed from the trap and closed with a lid. A new jar was placed on the top to replace the jar that had been removed. Flies died within five minutes of closing the jar. Flies which entered the trap at the beginning of a collection period would remain in the jar for at most an hour, but would die within the first

ten minutes of entering the jar. The flies were removed from the jar and placed into a container labeled with date, time, and trap number. Containers with flies were immediately placed on ice in a cooler after removal of all flies from the traps. Flies were returned to the laboratory at the end of the day and placed into a freezer at -20° C.

Table 1.1 Plants found on the marsh at Hatches Harbor, Provincetown,
Massachusetts.¹

Common name	Latin name	Bloom color ²	Bloom period
Salt Marsh Grass	<i>Spartina alterniflora</i>		
Salt Meadow Grass	<i>Spartina patens</i>		
Spike Grass	<i>Distichlis spicata</i>		
Black Grass ³	<i>Juncus gerardi</i>		
Woody Glasswort	<i>Salicornia virginica</i>		
Seaside Plantain ³	<i>Plantago</i> sp.		
Sea Lavender	<i>Limonium carolinianum</i>	l	Jul.-Oct.
Goldenrod	<i>Solidago tenuifolia</i>	y	Jul.-Oct.
Salt Marsh Aster ³	<i>Aster tenuifolia</i>	l/y	Aug.-Oct.
Groundsel Tree ³	<i>Baccharis halifolia</i>	y/w	Aug.-Oct.
Rush ³	<i>Scirpus</i> sp.		
Common Reed	<i>Phragmites communis</i>		
Panic Grass	<i>Panicum longifolium</i>		

¹ based on census conducted by Gabrielle Sakolsky and Edwin Hoopes during the 1991 season

² indicates plant has been observed at this site in the past, but not observed in 1991

³ l=lavender, y=yellow, w=white

Table 1.2 Plants found growing within one mile of the marsh at Hatches Harbor,
Provincetown, Massachusetts.¹

Common name	Latin name	Bloom color ²	Bloom period
Panic Grass	<u>Panicum longifolium</u>		
Poison Ivy	<u>Toxicodendron radicans</u>	y/g	May-Jul.
American Beachgrass	<u>Ammophilabreviliguta</u>		
Wormwood	<u>Artemisiacaudata</u>	y/g	Jul.-Aug.
Dusty Miller	<u>Artemisiastelleriana</u>	y	Jul.-Sep.
Beach Pea ³	<u>Lathyrus japonicus</u>	v/p	Jun.-Sep.
Salt-Spray Rose	<u>Rosa rugosa</u>	p/w	May-Oct.
Virginia Rose ³	<u>Rosa virginica</u>	p	Jun.-Aug.
Seaside Spurge	<u>Euphoria polygonifolia</u>	g	Jul.-Oct.
Sea Rocket	<u>Cakile edentula</u>	l	Jul.-Sep.
Seabeach Sandwort ³	<u>Arenaria peploides</u>	g	Jun.-Aug.
Sickel-leaved Golden Aster ³	<u>Chrysopsis falcata</u>	y	Jul.-Sep.
Beach Heath	<u>Hudsonia tomentosa</u>	y	May-Jul.
Seaside Goldenrod	<u>Solidago sempervirens</u>	y	Aug.-Oct.
Bearberry	<u>Arctostaphylos uva-ursi</u>	w	May-Jun.

continued, next page

Table 1.2 (cont.)

Common name	Latin name	Bloom color ²	Bloom period
Pitch Pine	<i>Pinus rigida</i>		
Saltwort	<i>Salsola kali</i>	w	Jul.-Aug.

¹ based on census conducted by Gabrielle Sakolsky and Edwin Hoopes during the 1991 season

² indicates plant has been observed at this site in the past, but not observed in 1991

³ l=lavender, y=yellow, w=white, p=pink, g=green

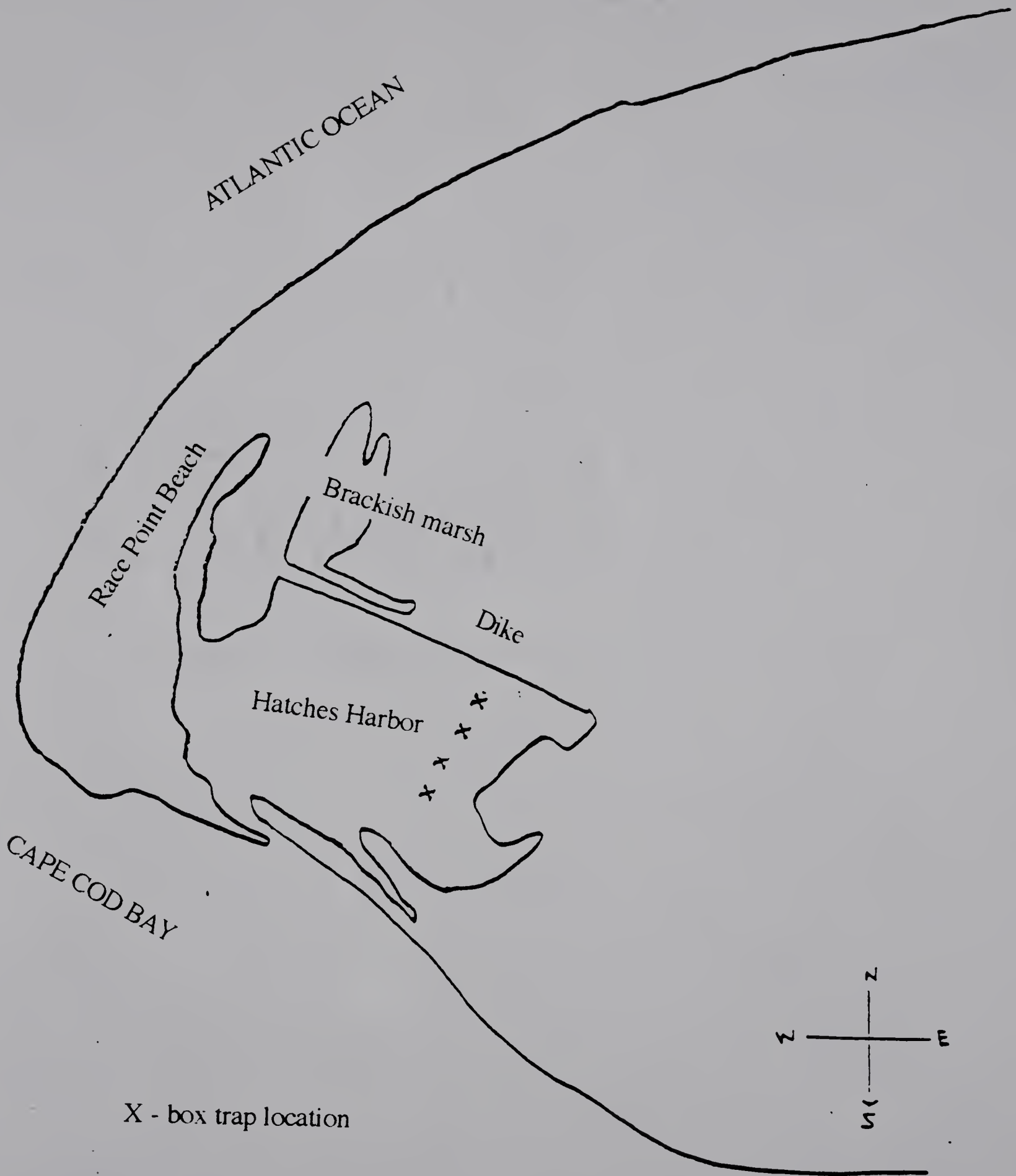


Figure 1.1 Map of Hatches Harbor, Provincetown, Massachusetts.

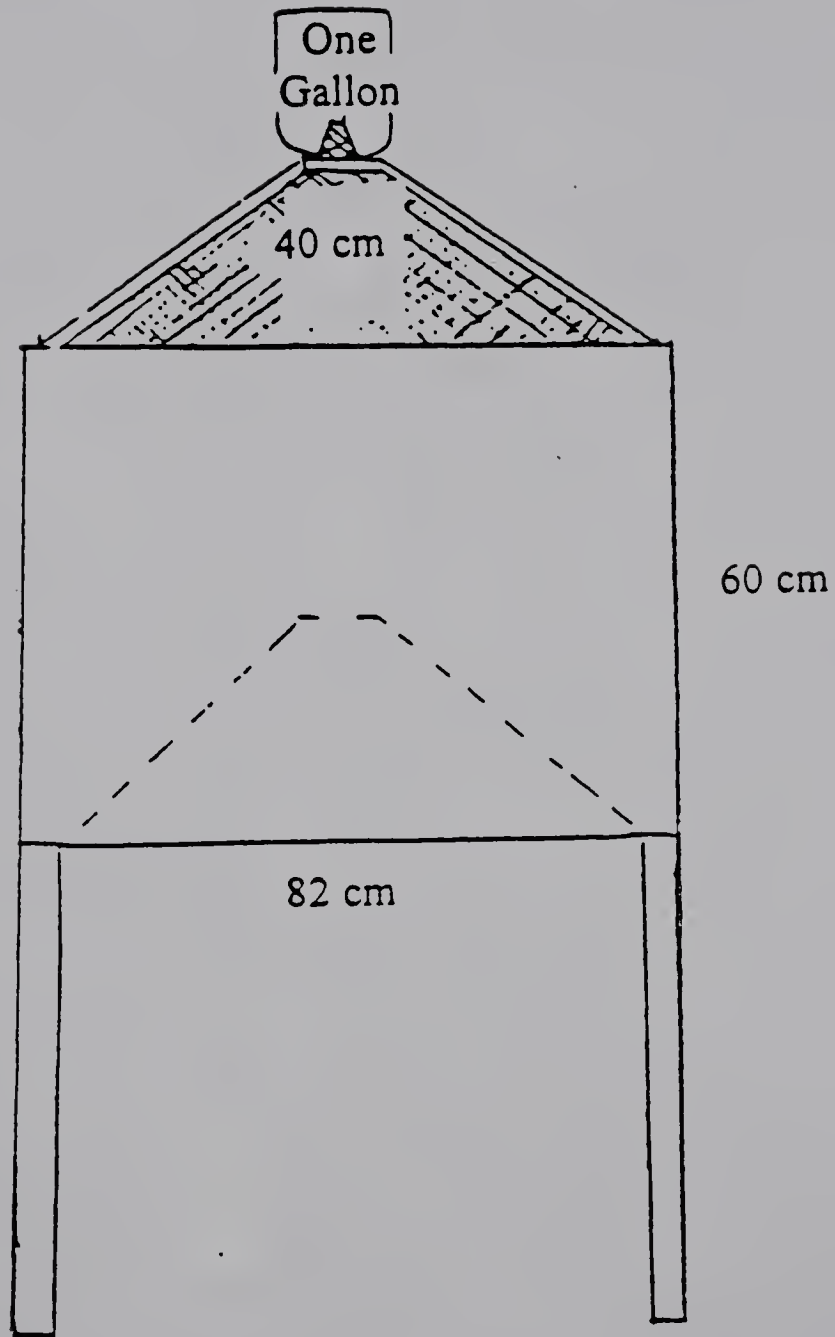


Figure 1.2 Diagram of a modified box trap.

CHAPTER II

DIURNAL HOST-SEEKING ACTIVITY OF GREENHEAD FLIES

Introduction

There have been many problems in identifying flies in the T. nigrovittatus complex to species (Burger et al. 1985). Previously, regardless of their size, greenhead flies were considered to be T. nigrovittatus. Genetic studies have identified that different sized greenhead flies are actually the cryptic species T. nigrovittatus and T. conterminus (Jacobson et al. 1981). Graham and Stoffolano (1983a, b) observed differences in the oviposition behavior of these two species. Further, a morphometric test to distinguish between these two species has been developed using discriminant function analysis (Sofield et al. 1984b). Many different studies centering on one or both of these species have been conducted, however, investigators did not attempt to separate these species. Three previous studies have been conducted to investigate factors affecting their flight activity (Burnett and Hayes 1968, Schulze et al. 1975). Because correct identification of these two species was not possible until recently, no work was done to examine a possible species/size effect on flight activity. Further, these studies did not examine any diurnal patterns of host-seeking females coming to box traps. These box traps are the only management strategy currently being used to control these flies (Wall and Doane 1980). This study was designed to examine the diurnal and seasonal host-seeking patterns of these two species coming to box traps. As there are other observed differences in the behavior of the two species, I believed that there would also be differences between the two species' host-seeking patterns.

Materials and Methods

Collection Methods

Collections of *T. nigrovittatus* and *T. conterminus* were made during two summer seasons (1991&1992) at Hatches Harbor salt marsh in Provincetown, Massachusetts (Figure 1.1). Collections were made twice a week starting in the last week of June and running through mid-August. Flies were removed from traps hourly and environmental conditions (stage of tide, temperature, wind speed, wind direction, and percentage of cloud cover) were recorded between 0700 and 1700 h. Flies were trapped in box traps provided by the Cape Cod Mosquito Control Project. The trap tops were modified into a pyramid shape each with a collection jar on top (Figure 1.2) (Dale and Axtell 1976). Each collection jar contained a piece of pesticide strip (active agent 2,2-dichlorovinyl-dimethyl-phosphate) to kill flies as they enter the jar. Flies were placed on ice after removal from traps and returned to the laboratory at the end of the day.

Species Identification

Morphological measurements were taken to identify each fly to species. Sofield et al. (1984b) developed a method for identifying a fly as *T. nigrovittatus* or *T. conterminus* using the following formula:

$$\begin{aligned} \text{score} = & 17.09 - (28.84 \times \text{width of frons dorsally in mm}) \\ & - (22.38 \times \text{width of scape in mm}) + (5.88 \times \text{width of head in mm}) \\ & - (1.45 \times \text{total body length in mm}) \end{aligned}$$

The score determined the species. If the score is greater than +0.30, the fly is *T. nigrovittatus* and if the score is less than +0.30, the fly *T. conterminus*. Sofield found that the distribution of this score was bimodal for the populations he studied. Few flies had scores close to +0.30.

In my study, the body of the fly was measured using a metric measuring stick. The fly was placed on its side on the ruler and its length was recorded. A ruler was used for two reasons: 1) the flies were too long to use the ocular micrometer and 2) the formula does not require the same degree of accuracy for the body length as the other measurements. Measurements were taken using a digital, filar micrometer (Boekeler Instruments; Tucson, Arizona). The head was removed from the body and was placed flat with the face up on a microscope slide. The head width and frons width were measured from this position. The antenna was removed and the width of the scape was measured.

Difficulties were experienced when attempting to identify flies utilizing Sofield's method. In an attempt to more accurately separate flies to species, the middle third of the canonical scores (describing around 3000 flies) were not used in the analysis of the data acquired in this study. The majority of flies with the bottom third canonical scores (i.e., larger flies) were assumed to be the species *T. conterminus*, while the majority of flies with the top third canonical scores (i.e., smaller flies) were assumed to be *T. nigrovittatus*.

Results

Species Identification

Morphometric analysis of the data from two seasons showed that the formula derived by Sofield et al. (1984b) does not definitively identify flies at our study site as being either *T. nigrovittatus* or *T. conterminus*. A histogram of the canonical scores of the flies reveals a unimodal distribution rather than the bimodal distribution that would describe the identification of two species (Figures 2.1a & b). The measurements of the different body parts were graphed and the flies head width, frons width, and scape width were also unimodal distributions (Figures 2.2a-e).

Principle component analysis was performed on the data. The relationship between head width, width of frons dorsally, and width of scape was examined. The data from both the first and second season were determined to have one principle component (Table

2.1a & b). This principal component analysis determined that the only thing affecting the relationship between head width, width of frons dorsally, and the width of the scape was the size of the fly. No other relationships, such as the species relationship determined by Sofield et al. (1984b) were found.

Daily Host-seeking Activity

Because these results did not positively identify the flies to species using the morphometric analysis developed by Sofield, the top third of canonical scores ($x > +1.20$) and flies with a body length less than 10.7 mm were used to represent the activity of *T. nigrovittatus*. Whereas, the bottom third of canonical scores ($x < -0.20$) were used to represent the activity of *T. conterminus*. Thus, using this technique, 33.3% of the flies collected were not identified to species because their scores were in a range of uncertainty (Figures 2.1a & b). Data was divided into early season (6/30-7/14), middle season (7/15-7/31), and late season (8/1-8/14). The greatest number of flies was collected during the middle season Table 2.2a & b).

In the 1991 season, both species of flies had a peak percent of flies attracted to box traps during early afternoon hours (Figures 2.3a & b). In the 1992 early and late seasons, the peak percent of *T. nigrovittatus* were collected during the morning hours (0800-0859 and 1000-1059 h, respectively), while the peak percent of *T. nigrovittatus* in the middle season occurred between 1400-1459 h. On the other hand, the peak percent of *T. conterminus* were collected between 1300-1359 h in the middle season, between 1000-1059 h and 1300-1359 h in the early season, and between 0800-0859 h in the late season (Figures 2.3 c & d). The number of flies trapped in the middle and late seasons of the 1992 season peaked between 1400-1459 h and 0800-0859 h. The percent of flies attracted hourly to box traps was also examined by fly size (Figures 2.4 a & b). Flies were divided into small sized (width of head < 3.50 mm), medium sized (3.49 mm < width of head < 3.85 mm), and large sized (width of head > 3.84). There was no real difference in the trends or patterns of the percent small, medium, or large size flies caught hourly.

The number of flies attracted to box traps daily was examined for any seasonal trends that would show different activity patterns for the different species (Figure 2.5a & b). Although there were no noticeable differences between species within a season, major differences did occur between all of the flies between seasons. The 1991 season was warmer and had less precipitation than the 1992 and the peak number of flies occurred earlier in the season (around 7/16). The peak number of flies trapped in 1992 were caught later in the season (around 7/28).

Discussion

The results of this study showed that current morphometric analysis developed by Sofield et al. (1984b) to identify greenhead flies to species does not work definitively at this study site. The morphometric species identification was developed by Sofield et al. (1984b) by running discriminant function analysis with a variety of different body measurements of flies from both species. This analysis determined the measurements that best differentiated between the species and weights that could be given to these measurements to make the differences more extreme. The weights and measurements could then be put into a formula to obtain the canonical score. This score is what is then used to identify flies to species. Due to small sample size ($n = 60$), Sofield's discriminant function analysis could not possibly take into account the wide range of body size within one species. Past studies of dipteran species have linked adult size not only to genetics (Coyne and Beecham 1987), but also to larval nutrition level (Black and Krafur 1987) and temperature (Leprince and Bigras-Poulin 1988). This could explain the size difference between flies at different study sites and the size difference between flies at the same study site, but during different years. The large amount of variation in the size amongst greenhead flies of the same species could be causing the canonical scores of the two species to overlap.

Due to the length of time it took to measure each fly, the flies trapped during the 1991 season were not all identified until the summer of 1992. Unfortunately, the identification problem was not discovered until the end of the 1992 season, as the problem was not evident with the fly measurements done during the first field season (i.e. very few flies in the first few hundred flies measured had a canonical score of 0.30 ± 0.10).

The only morphological difference found between these species of flies is their size. Past studies have determined that fly size can affect the host-seeking activity of tabanid flies (Mullens and Gerhardt 1979). In this study, the activity patterns of both species of flies during the 1991 season had no noticeable differences. Further, during the 1992 season, while some slight differences did occur between the activity of the two species, no significant species dependent activity patterns were noticeable. There was a major difference in the daily host-seeking patterns of flies identified to be the same species and size for both years making it impossible to conclude whether or not species or size is affecting the activity patterns of flies.

Gaugler and Schutz (1989) found a difference in the hovering behavior of male *T. nigrovittatus* and *T. conterminus*. However, the species identification for the flies in the field in their study was done through observation with binoculars and could be questioned as to its accuracy. Thus, based on this current study, there is no difference in the host-seeking behavior of these two species. Further studies of these flies should examine other means of identifying these flies to species that would be more universal in application, but still easy to use on dead or living flies. Perhaps under further examination host-seeking activity patterns that have been overlooked in this study could be found by positive species identification which would have included the 33% of flies that were considered unidentifiable in this study.

Table 2.1 Results from principal component analysis of the morphometric data, during
a. 1991.*

Body Measurement	Factor (1)	Factor (2)	Factor (3)
Head width	0.691	0.629	0.356
Width of frons	0.834	0.017	-0.552
Width of scape	0.669	-0.671	0.320
Variance explained by components	1.620	0.847	0.534

* Computed using the Factor function in the statistical program SYSTAT.

Table 2.1 continued, b. 1992.*

Body Measurement	Factor (1)	Factor (2)	Factor (3)
Head width	0.854	0.153	-0.497
Width of frons	0.798	0.449	0.401
Width of scape	0.699	-0.699	0.149

*Computed using the Factor function in the statistical program SYSTAT.

Table 2.2 Number of flies trapped daily, during a. 1991.

Date	Number of <u>T. nigrovittatus</u> ¹	Number of <u>T. conterminus</u> ²	Total ³
July 5	13	14	51
July 9	171	276	728
July 12	274	235	846
July 16	304	284	914
July 19	244	243	774
July 29	164	115	437
July 31	164	136	456
August 2	95	51	216

¹T. nigrovittatus are classified as flies with a canonical score ≤ -0.20 .

²T. conterminus are classified as flies with a canonical score ≥ 1.20 or body length < 10.5 mm.

³Total flies includes flies which could not be classified to species based on their canonical score.

Table 2.2 continued, b. 1992.

Date	Number of <u>T. nigrovittatus</u> ¹	Number of <u>T. conterminus</u> ²	Total ³
June 30	12	3	15
July 2	4	12	16
July 7	29	15	49
July 9	26	12	46
July 16	99	22	132
July 21	237	54	367
July 28	381	177	752
July 30	142	49	259
August 3	83	40	155
August 11	132	65	275

¹T. nigrovittatus are classified as flies with a canonical score ≤ -0.20 .

²T. conterminus are classified as flies with a canonical score ≥ 1.20 or body length < 10.5 mm.

³Total flies includes flies which could not be classified to species based on their canonical score.

Figure 2.1 Distribution of the canonical scores of flies trapped, during a. 1991.

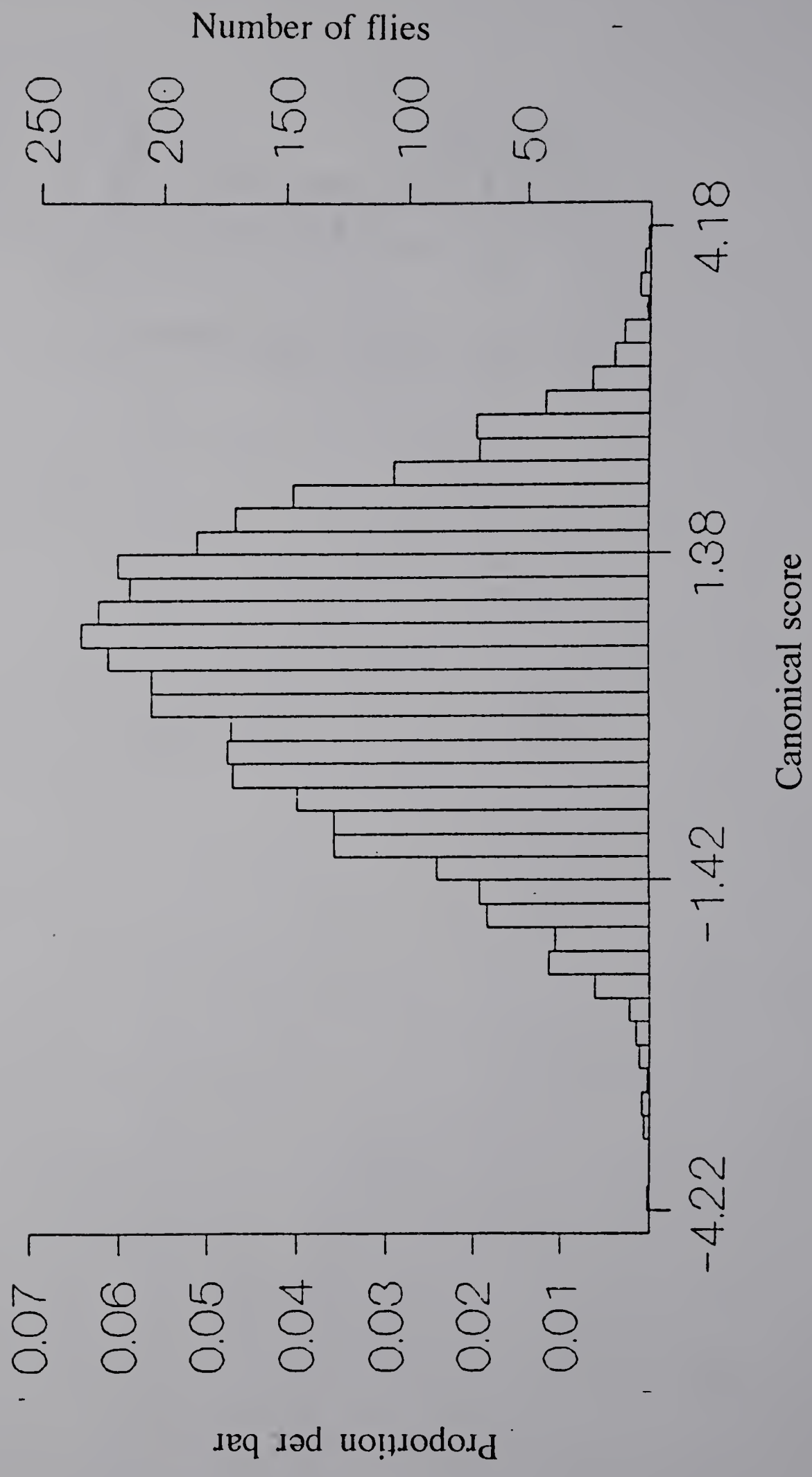


Figure 2.1 continued, b. 1992.

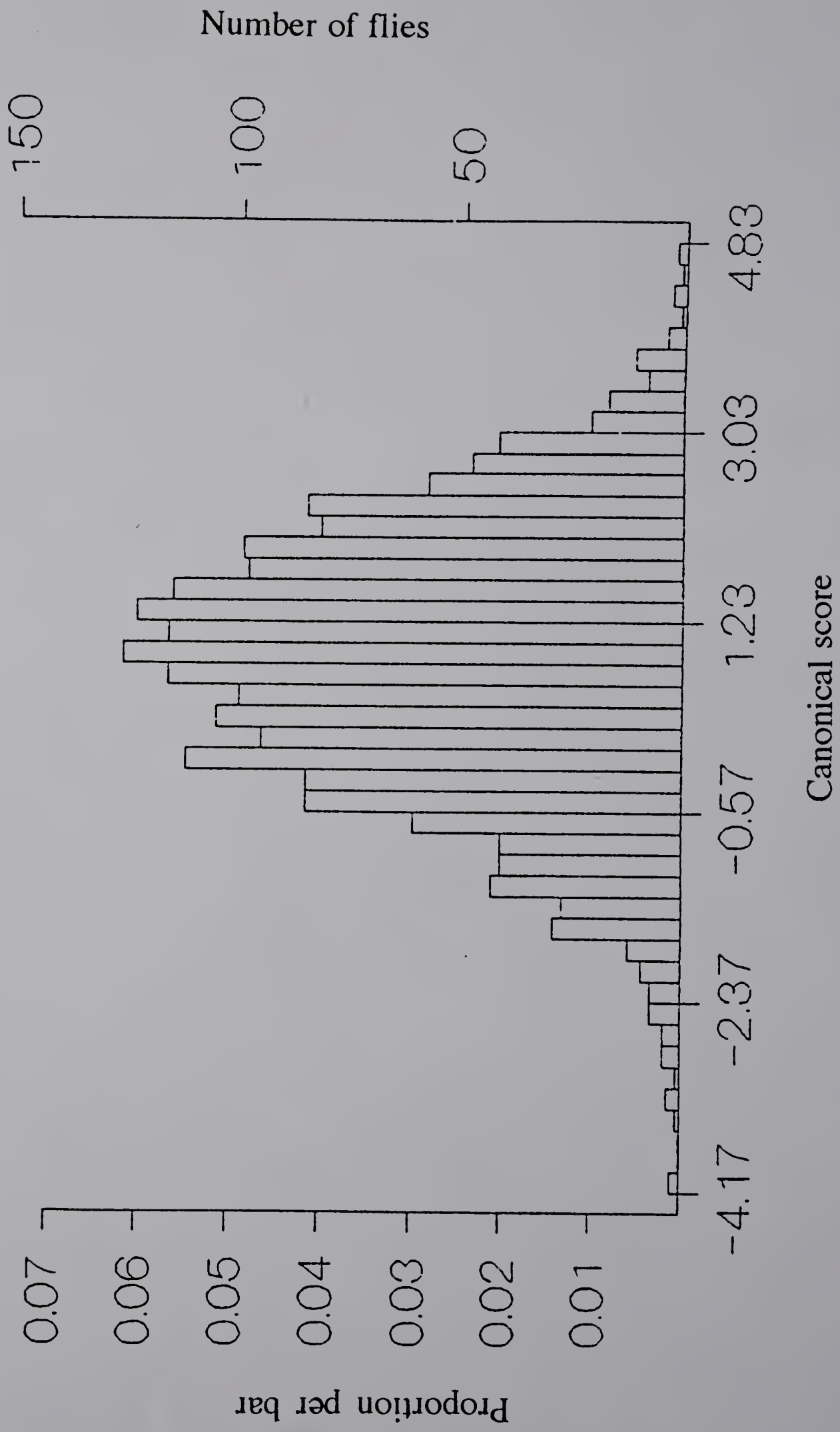


Figure 2.2 Distribution of the widths of the head measurements of flies trapped during each season,
a. width of head for flies trapped during the 1991 season.

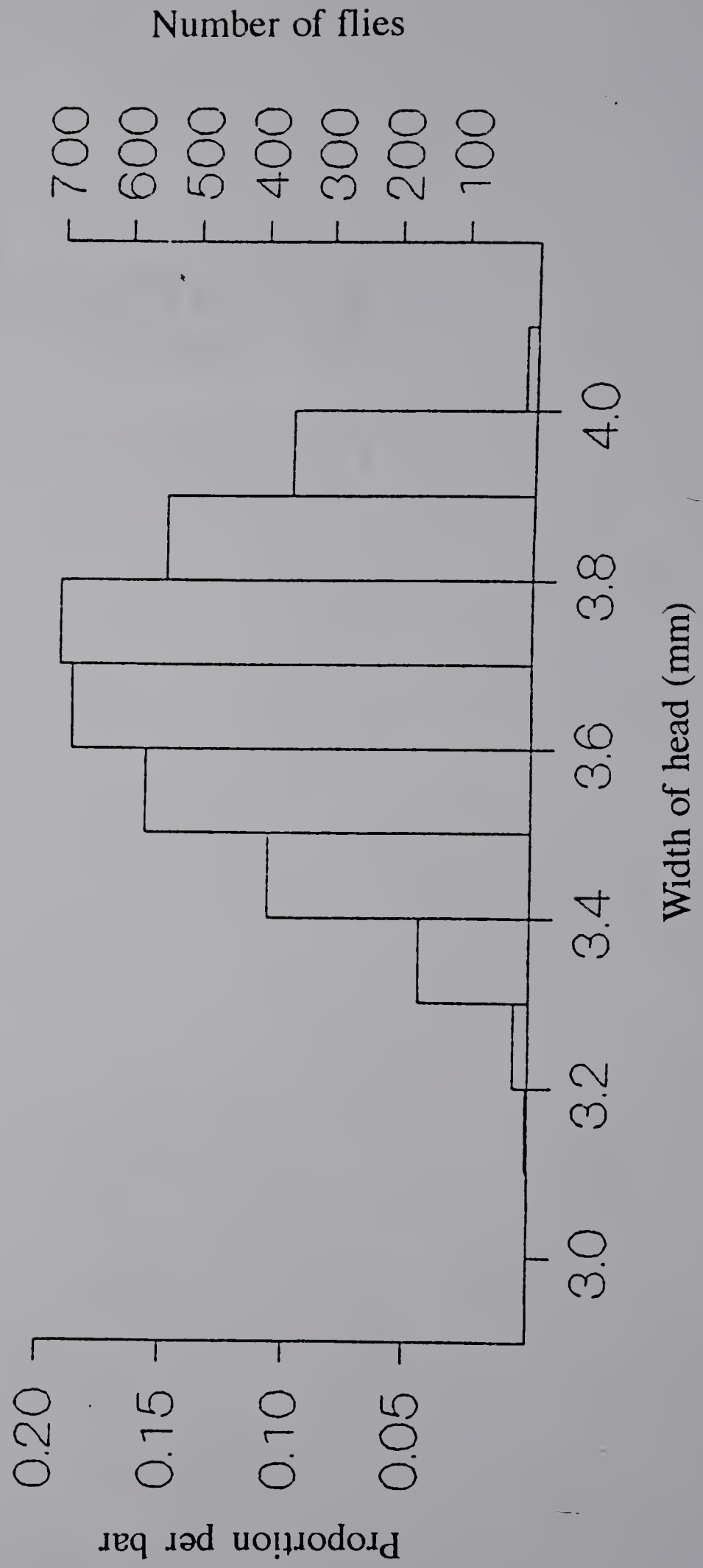


Figure 2.2 continued, b. width of head for flies trapped during the 1992 season.

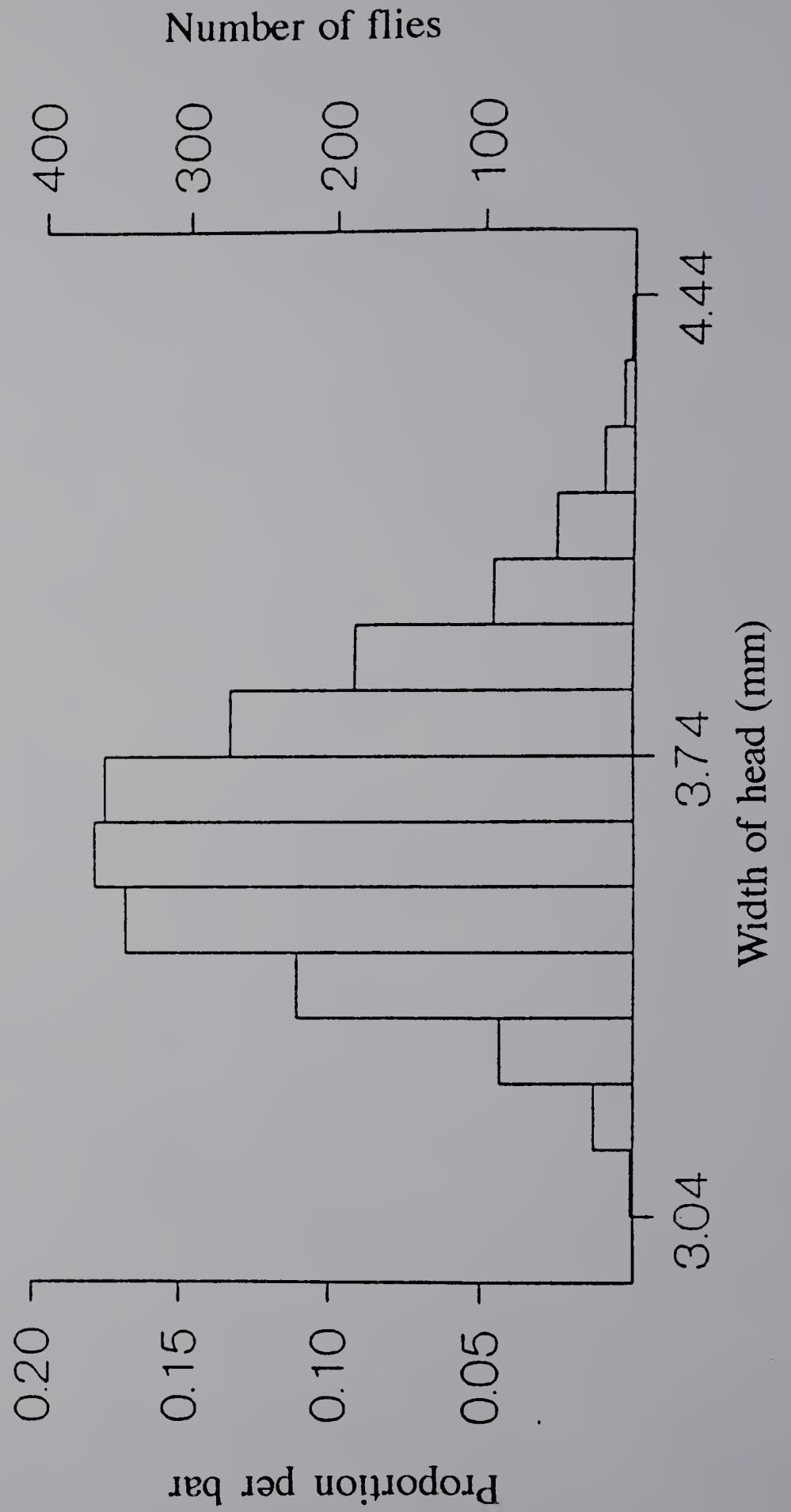


Figure 2.2 continued, c. width of frons for flies trapped during the 1991 season.

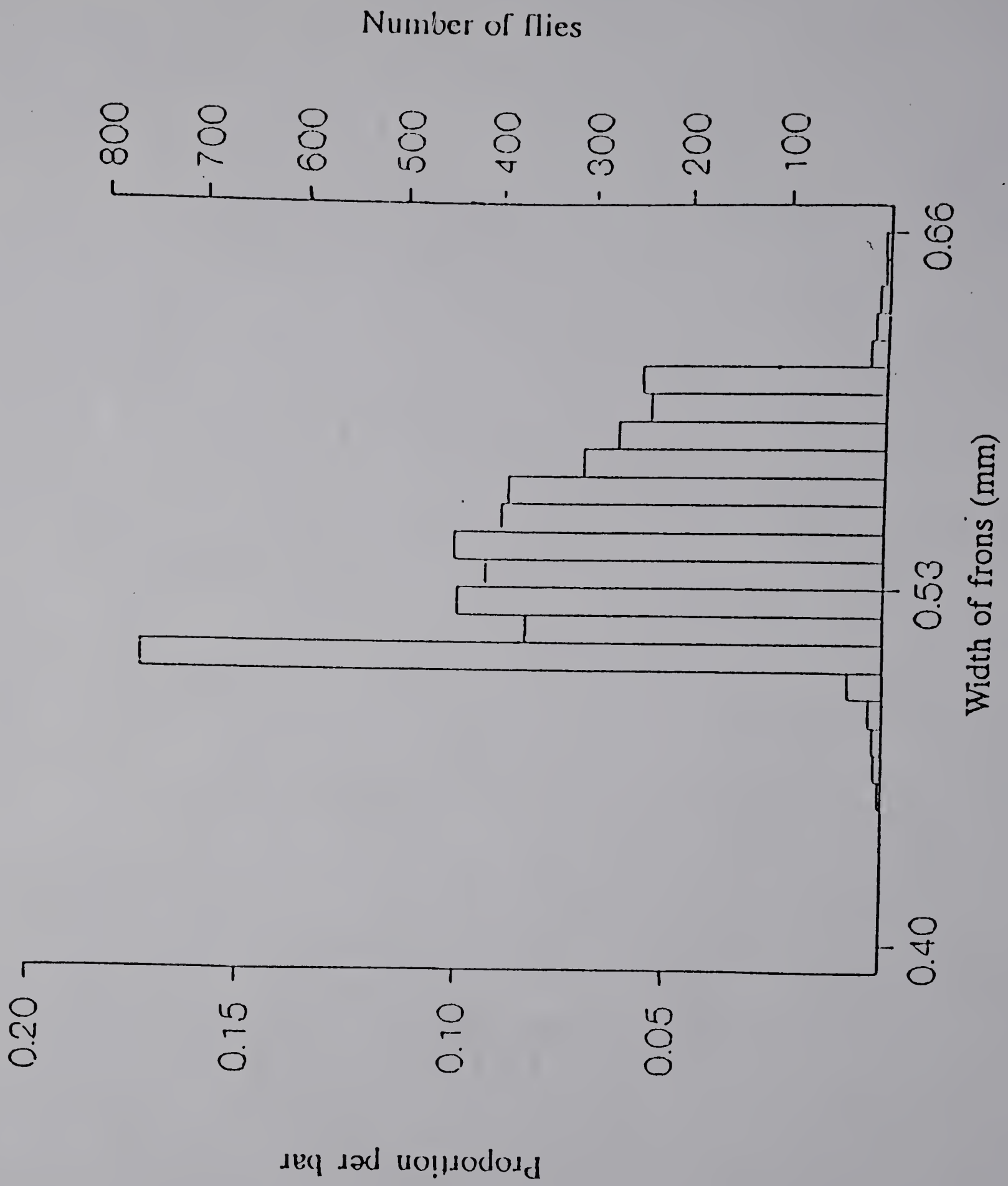


Figure 2.2 continued, d. width of frons of flies trapped during the 1992 season.

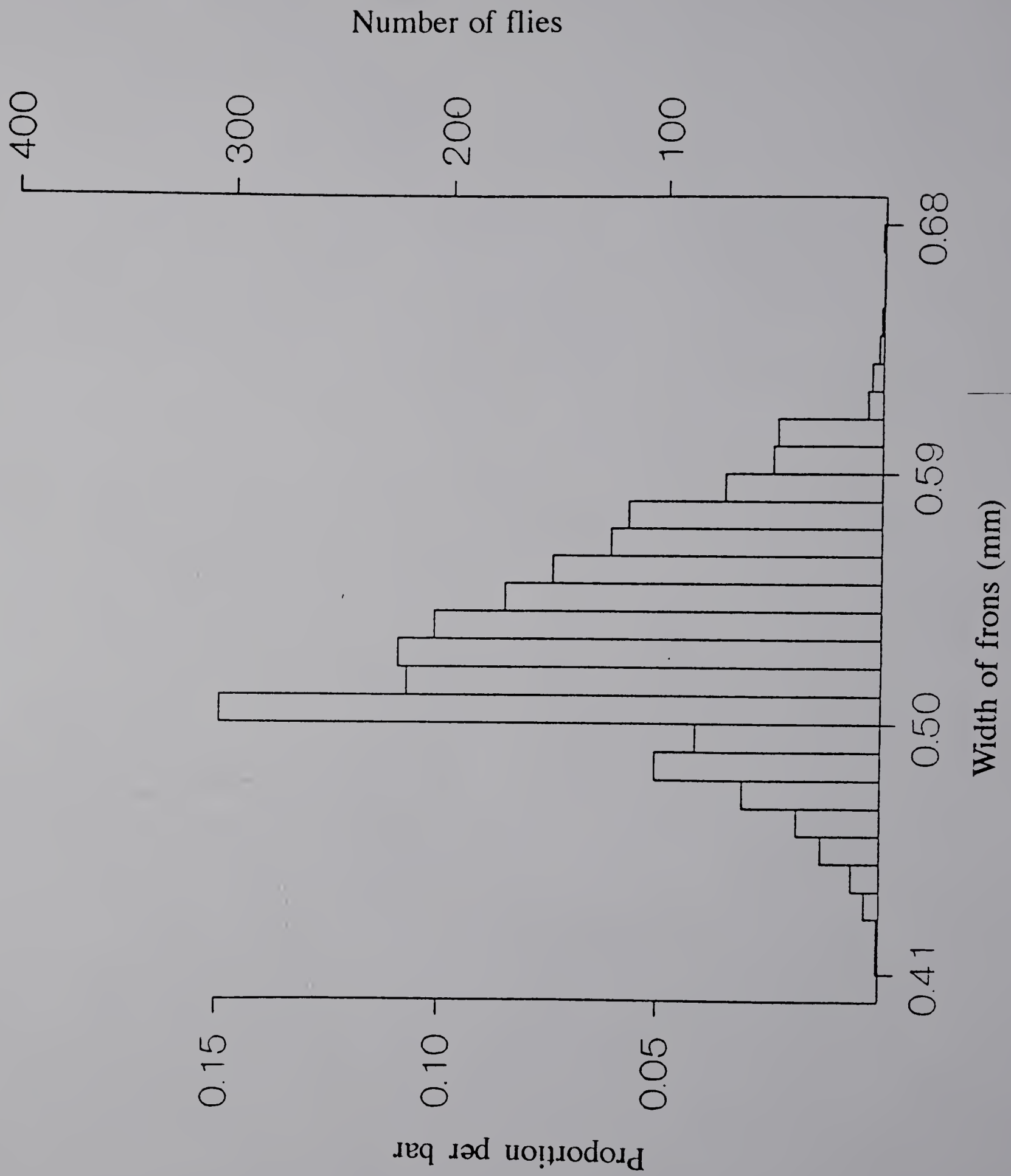


Figure 2.2 continued, e. width of scape of flies trapped during the 1991 season.

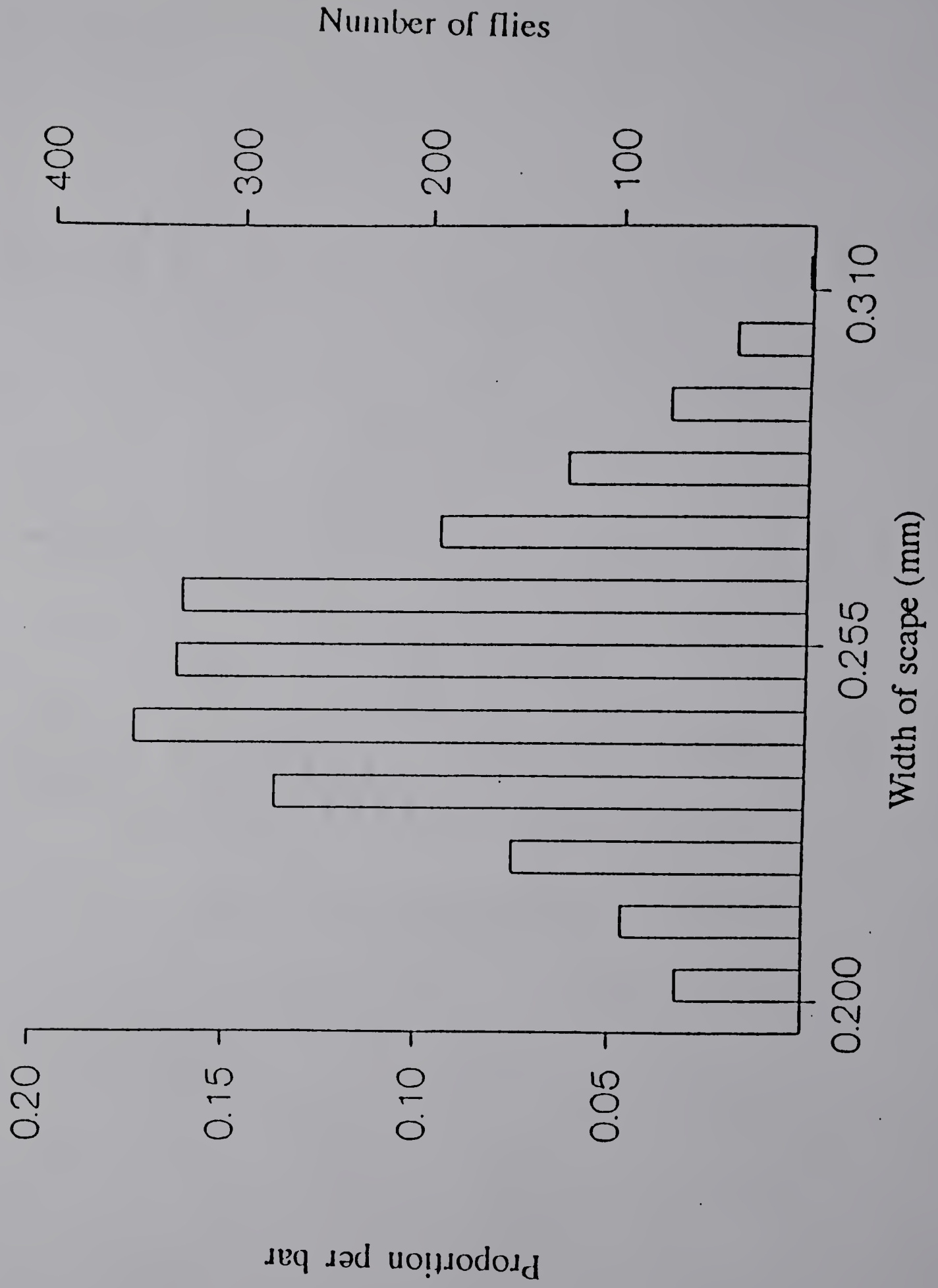


Figure 2.3 Percent of flies trapped hourly, a. classified as Tabanus nigrovittatus caught during the 1991 season.

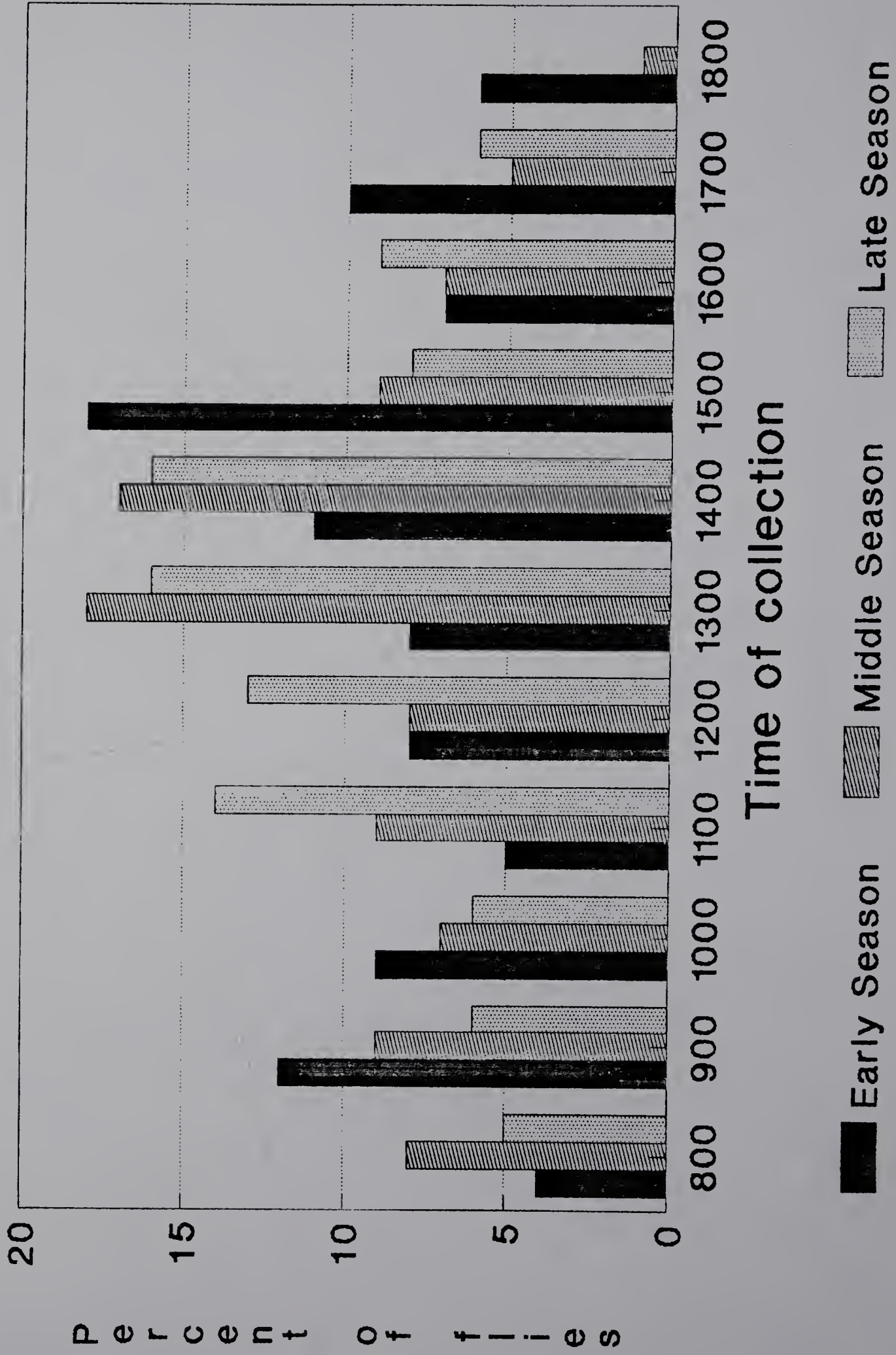


Figure 2.3 continued, b. classified as Tabanus conterminus caught during the 1991 season.

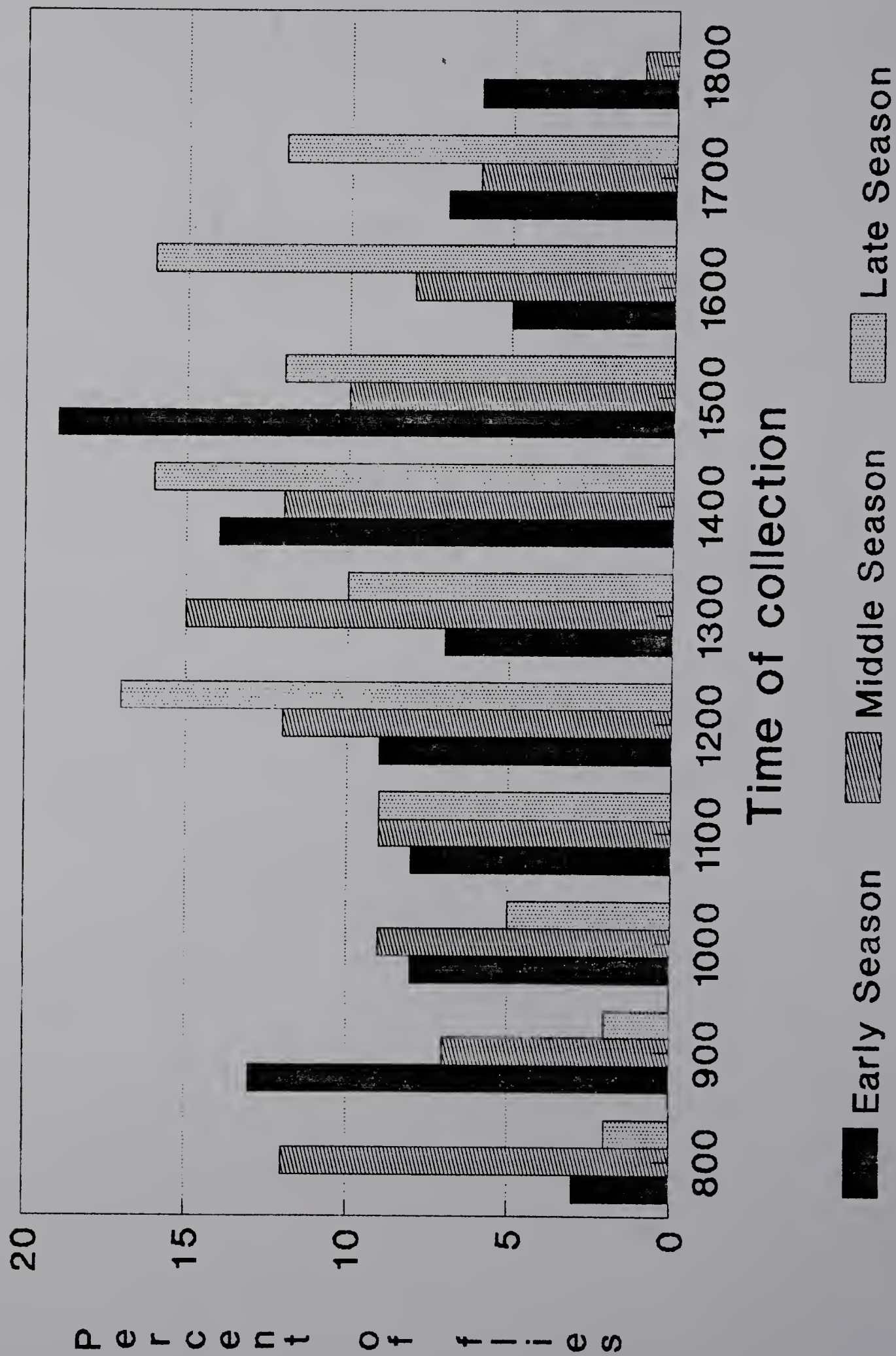


Figure 2.3 continued, c. classified as Tabanus nigrovittatus caught during the 1992 season.

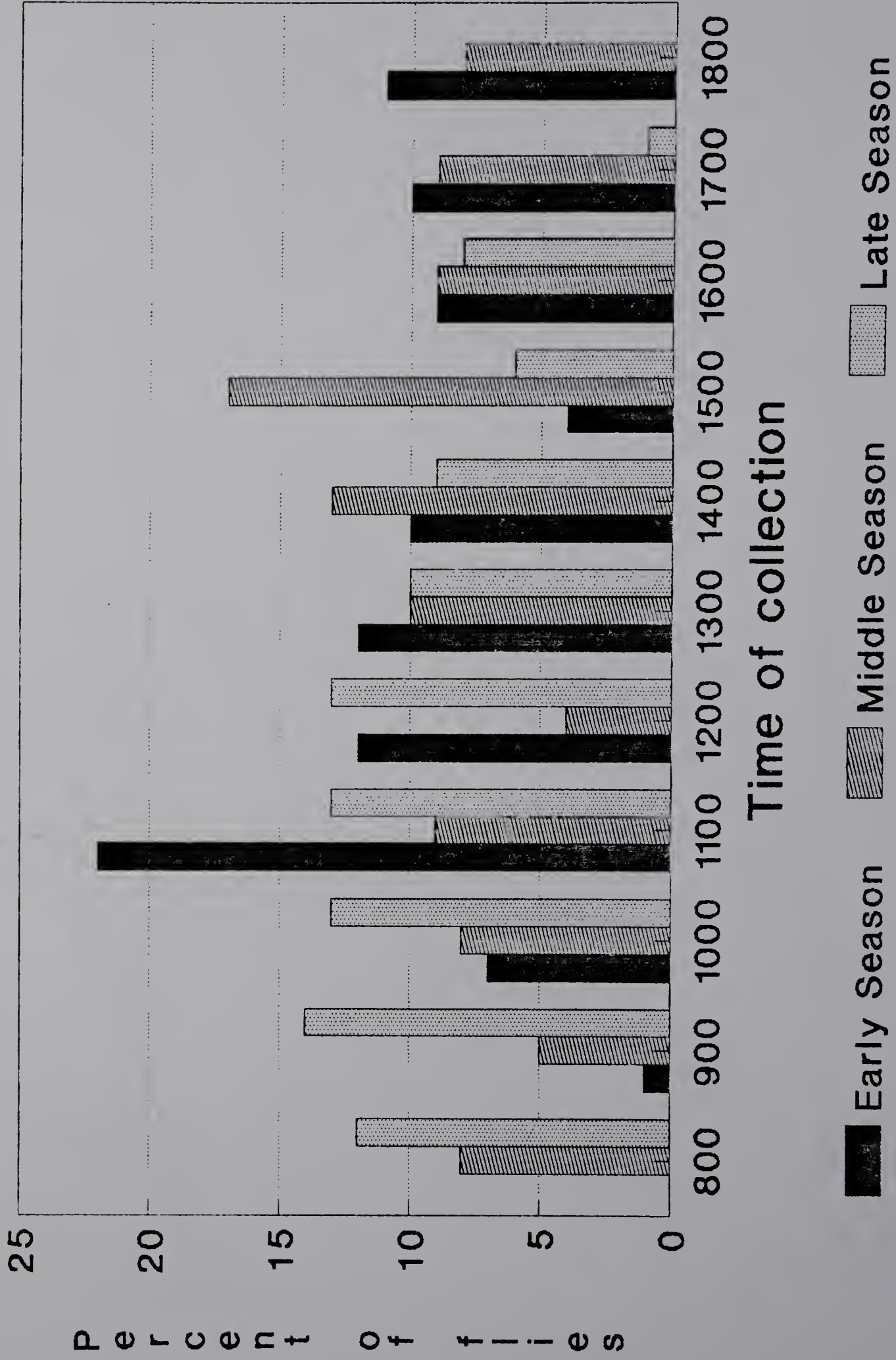


Figure 2.3 continued, d. classified as Tabanus conterminus caught during the 1992 season.

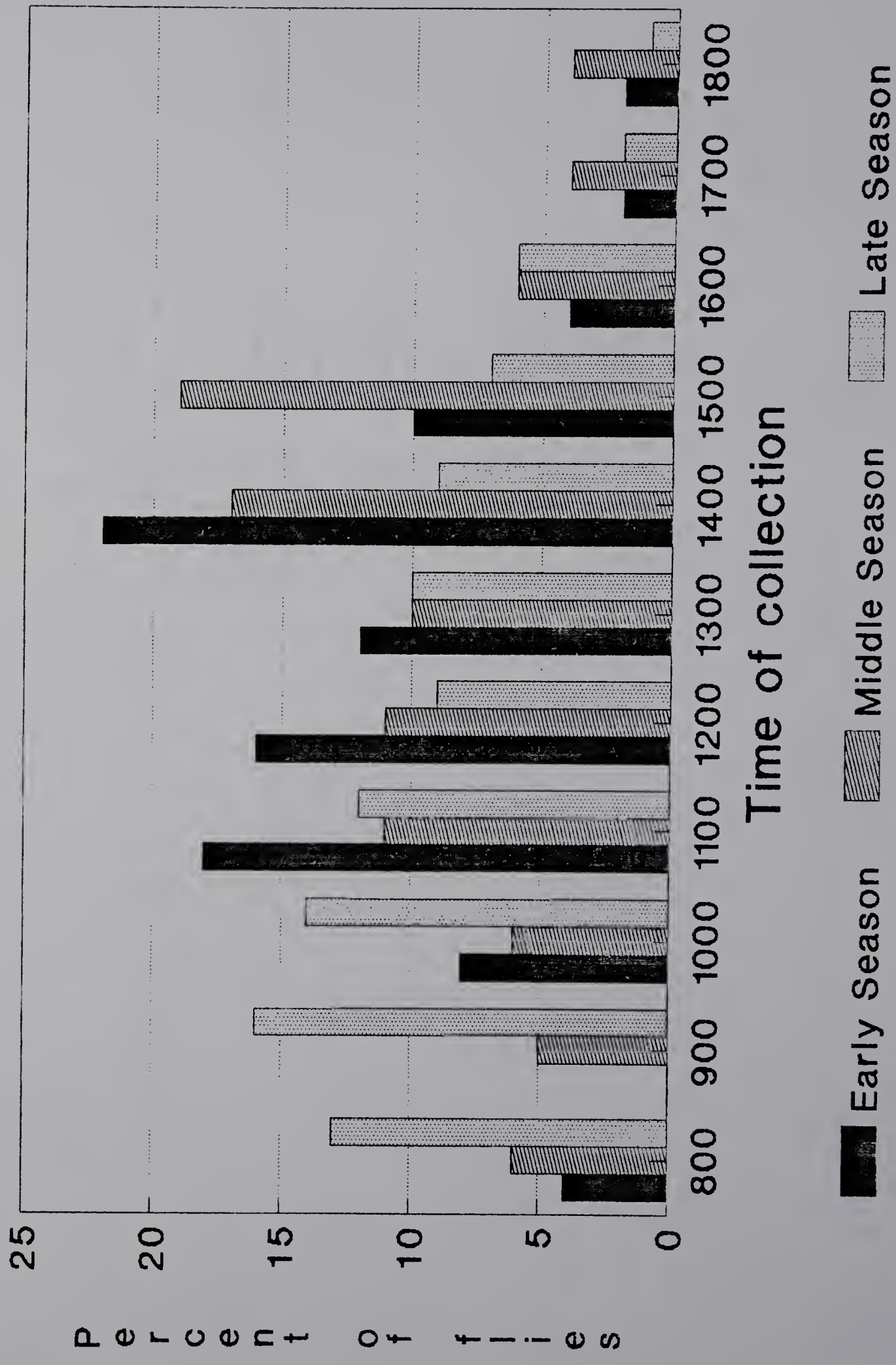


Figure 2.4 Percent of flies that are classified as small sized, medium sized, or large sized trapped hourly, during
a. 1991.

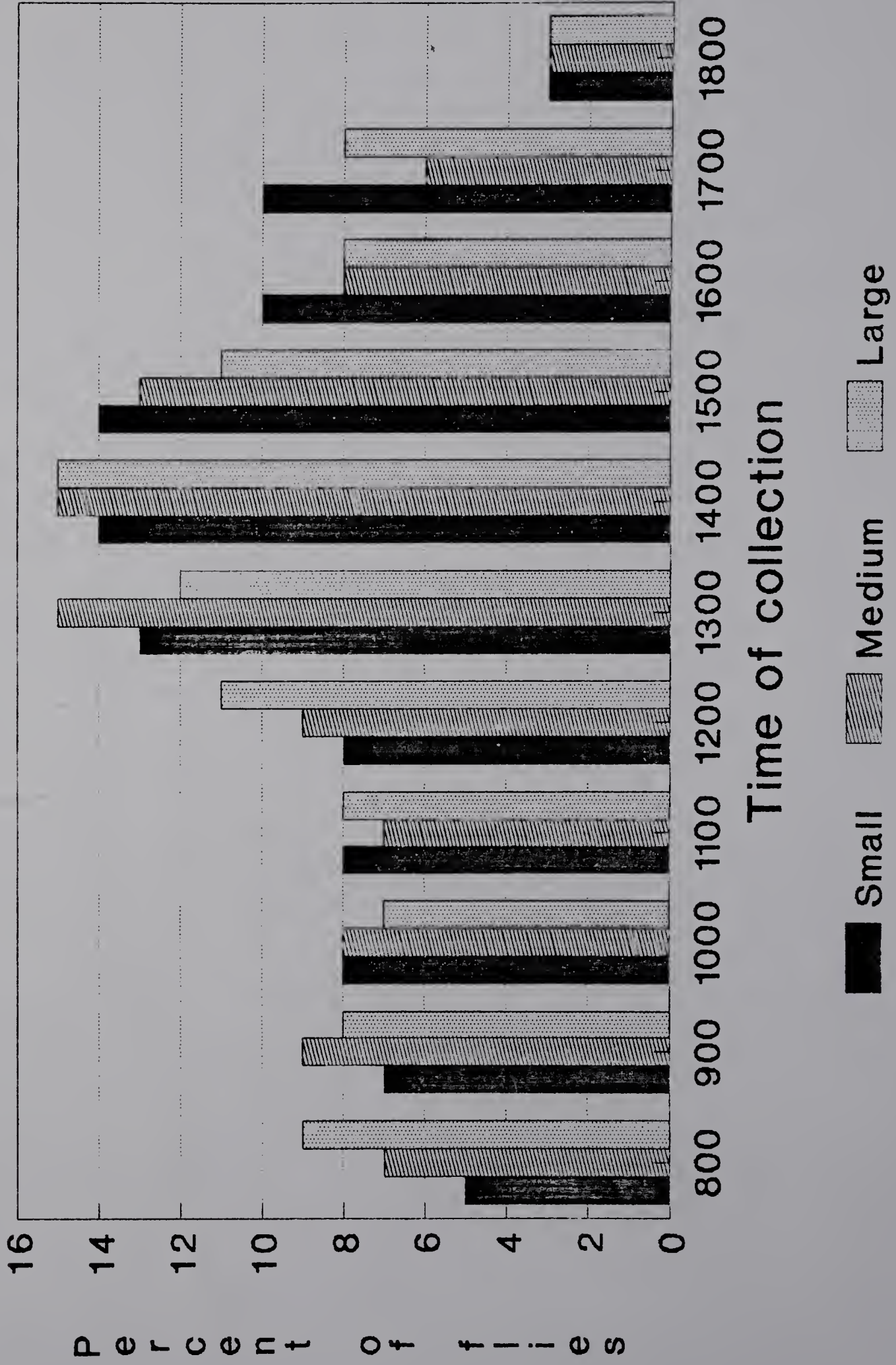


Figure 2.4 continued, b. 1992.

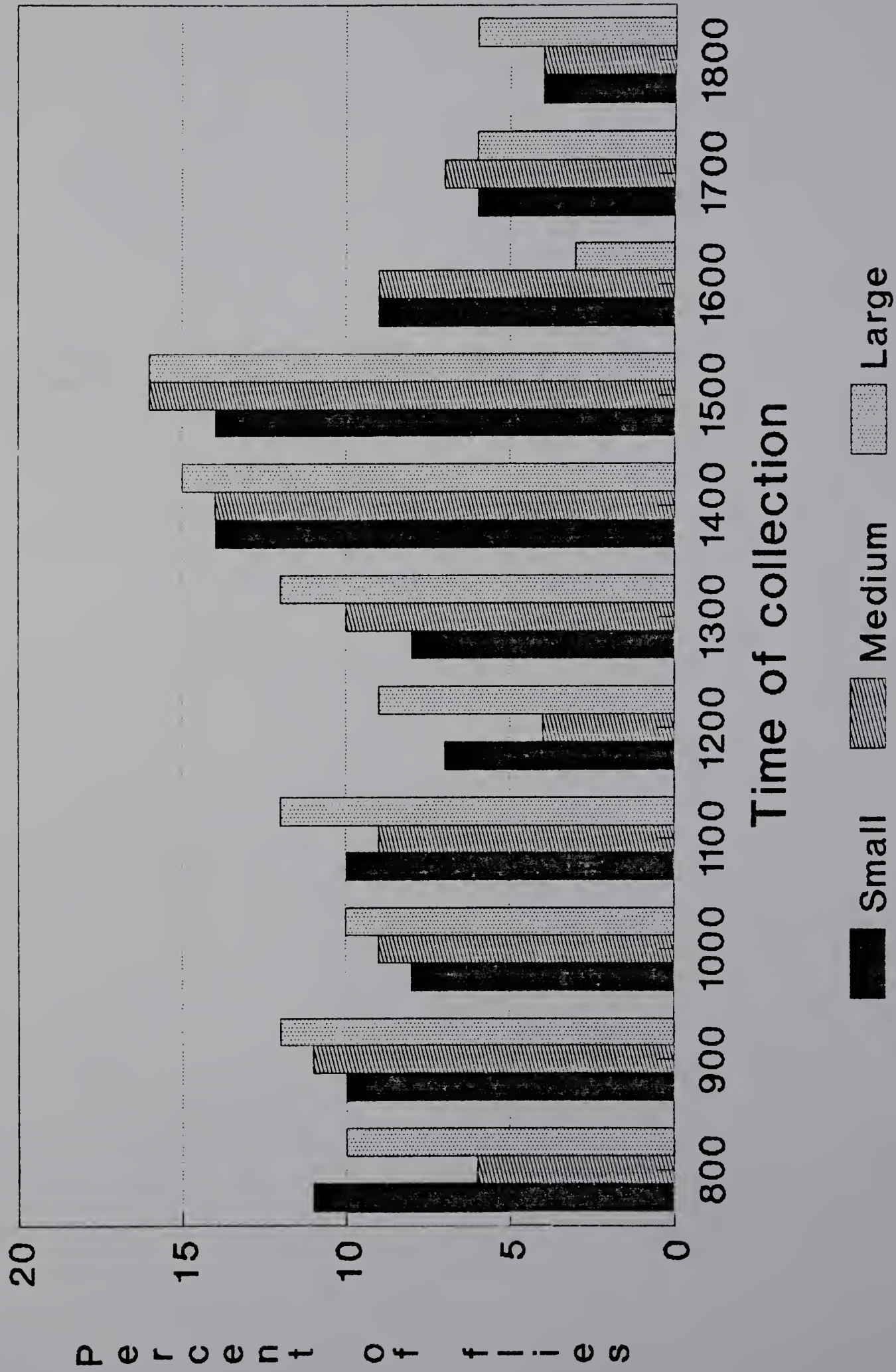


Figure 2.5 Number of flies attracted to box traps daily, during a. 1991.

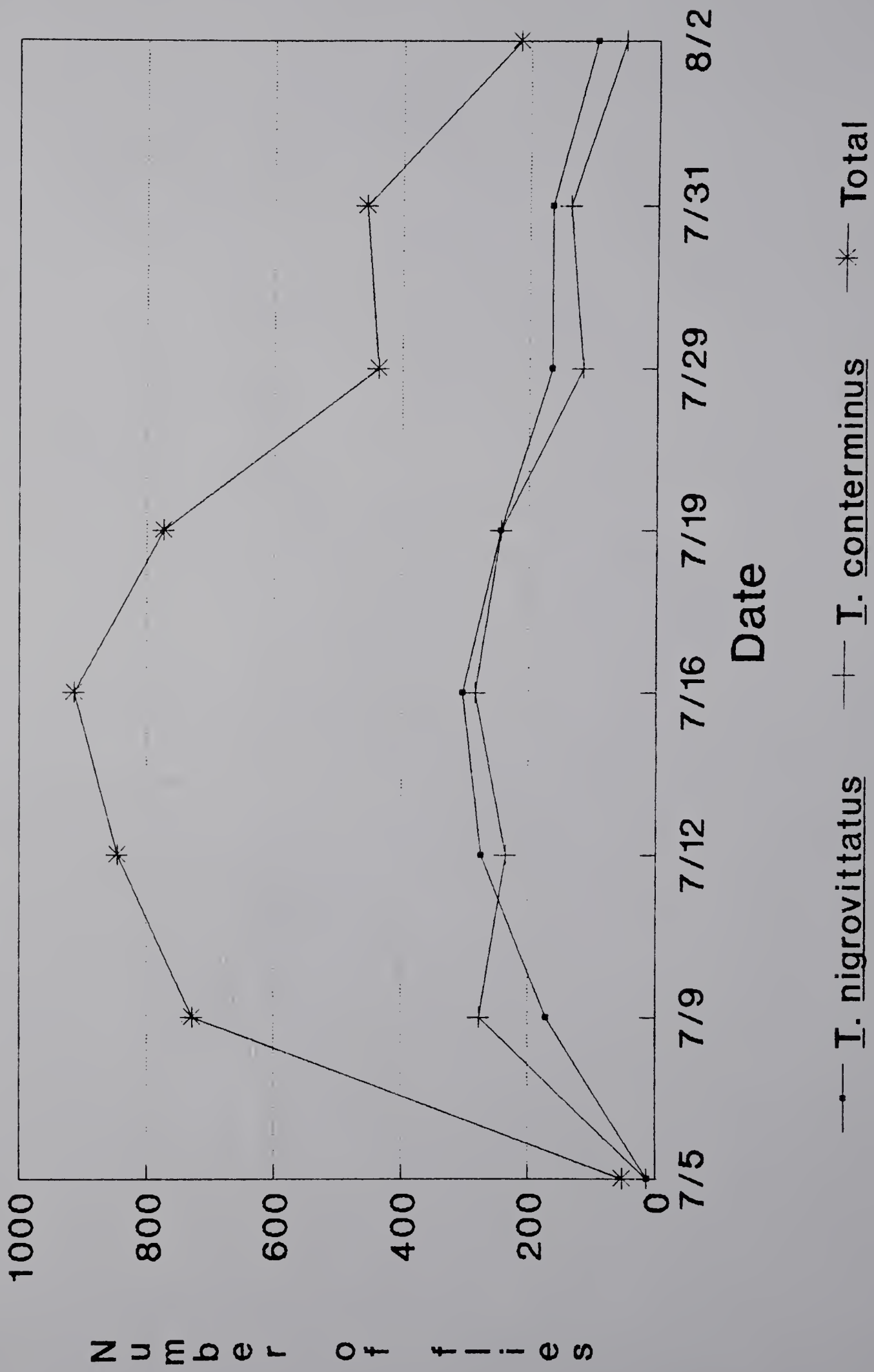
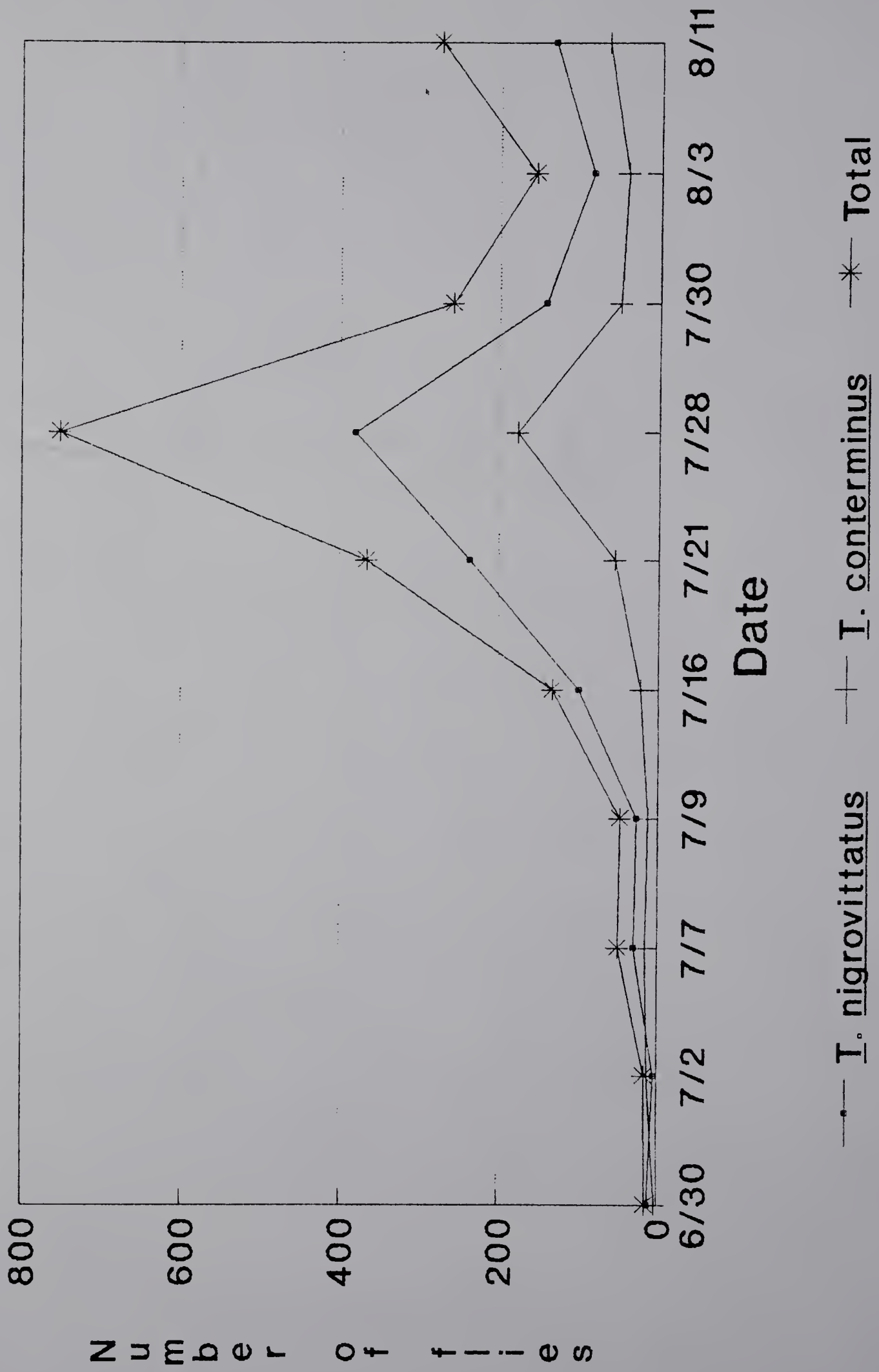


Figure 2.5 continued, b. 1992.



CHAPTER III
QUALITY AND QUANTITY OF CARBOHYDRATES IN THE CROP
OF FIELD CAUGHT GREENHEAD FLIES

Introduction

The greenhead flies, Tabanus nigrovittatus and T. conterminus, are found breeding on salt marshes along the Atlantic coast from Nova Scotia to southeastern Georgia (Sofield et al. 1984a). These flies are autogenous for their first gonotrophic cycle, but require a blood meal for maturation of any subsequent batches of eggs (Bosler and Hansens 1974, Magnarelli and Stoffolano 1980). These flies have become a major nuisance, as well as a possible vector of pathogens (Foil 1989, Krinsky 1976).

Previous studies have shown that carbohydrates in the form of nectar and honeydew are important food sources of tabanids (Hoppe 1989, Kniepert 1980, Leprince and Bigras-Poulin 1990, Leprince and Lewis 1986, Magnarelli and Anderson 1981, Magnarelli et al. 1979, Schutz and Gaugler 1989). This food source, as in mosquitoes, may be required for flying to find another carbohydrate food source, increasing longevity, increasing fecundity, finding a mate, or locating a host. Further, current efforts to control these flies rely upon traps which attract females only after they have already laid their first batch of eggs (Bosler and Hansens 1974, Wall and Doane 1980). Carbohydrate feeding occurs before the first batch of eggs is laid and carbohydrates are probably essential for maturation of the first batch of eggs (Friend and Stoffolano 1991). Thus, understanding carbohydrate feeding at that particular time could help develop a method of eliminating females before the first batch of eggs has been laid. One study has already examined the relationship between carbohydrate acquisition and the male hovering behavior in salt marsh horse flies (Schutz and Gaugler 1989). Many studies have tested for fructose in wild tabanids as an indicator of nectar feeding (Hoppe 1989, Kniepert 1980, Leprince et al. 1983, Leprince and Bigras-Poulin 1990, Leprince and Lewis 1986, Magnarelli and

1983, Leprince and Brigras-Poulin 1990, Leprince and Lewis 1986, Magnarelli and Anderson 1977, Magnarelli and Anderson 1981, Magnarelli et al. 1979). There have been no studies that have assessed either the quality (i.e., concentration of sugar) or the quantity of the sugar meal (i.e., crop size in mg) in tabanids. This study centers on analyzing both the quality and quantity of carbohydrates stored in the crop by the female greenhead flies that have already laid one batch of eggs and were host-seeking. A previous study determined that most host-seeking *T. nigrovittatus* are carrying a store of carbohydrates in their crops (Bosler and Hansens 1974), thus my hypothesis was that female tabanids caught in box traps would be carrying a significant amount of carbohydrates (> 1 microliter) in their crops.

Materials and Methods

This study was conducted in Provincetown, Massachusetts, at Hatches Harbor salt marsh in the Cape Cod National Seashore. A dike runs along side of the marsh and adjacent to the dike is a brackish marsh that provides fresh water flowers for additional carbohydrate sources. The female flies were collected from box traps hourly twice a week from the last week in June to mid-August, during two summer seasons (1991-1992).

Collection Methods

Blue colored box traps, provided by the Cape Cod Mosquito Control District, were used to trap flies. The trap tops were modified into a pyramid shape each with a collection jar on top (Figure 1.2) (Dale and Axtell 1976). Each collection jar contained a piece of pesticide strip (active agent 2,2-dichlorovinyl-dimethyl-phosphate) to kill flies as they enter the trap. Rapid killing stopped the flies from moving and flying thus preventing utilization of carbohydrate stores. Flies were immediately placed on ice after removal from traps. The flies were then returned to the laboratory on ice at the end of the day and immediately placed in a freezer at -20^o C.

Sugar Concentration in Crop

Three flies were randomly chosen from each trap each hour to be dissected for crop analysis. The crops of these flies were dissected out by opening the fly at the point where the thorax and abdomen meet. Often the midgut was removed and the crop was pulled out with it. This was done not only to prevent the crop from breaking but also helped to locate crops that were empty, as they were difficult to see. The crop duct closes when the fly is not feeding or when the fly is killed so the contents remain in the crop. After dissection, the crop was externally blotted dry on filter paper. The crop was then teased open on to a piece of parafilm.

Crop contents were then pulled into a microdispenser which measured the volume to a minimum of 0.1 microliters. The crop contents were immediately placed on a refractometer (Bellingham and Stanley, England) to determine the sugar concentration. The refractometer was specially designed to measure sugar concentration in one microliter of liquid or more. The readings from this refractometer did not need temperature compensation because of the small amount of liquid being analyzed. Any crop containing less than one microliter of fluid could not be analyzed for sugar concentration of crop contents. Crop content analysis was completed in less than 15 seconds thus reducing the possibility of liquid evaporation and sugar content concentration.

Twice during the 1991 season, 25 live flies were removed from box traps and placed in a metal cage. These flies were starved (no water, etc.) for 36 hours to allow their crops to empty. At the end of that period, the flies were offered 19% sucrose solution. This percent of sugar in the sucrose solution was chosen because it was the highest concentration of sugar that would dissolve in to the solution without applying heat, while still being concentrated enough for crop diversion (Friend and Stoffolano 1991). The flies were allowed to feed for 3 minutes then the sucrose solution was removed. The cage with

the flies was immediately placed into a freezer (-20 °C). Crops were removed from these flies and the quantity of crop contents was measured following the methods previously described. The results of this procedure were used to determine the maximum crop capacity for greenhead flies.

Results

Flies were categorized as being small sized, medium sized, or large sized. Data was divided into small, medium, and large sized flies' crop contents. Medium sized flies had head widths between 3.50 and 3.84 mm. Small flies are flies with a head width smaller than 3.50 mm and large flies were flies with a head width greater than 3.84 mm. An attempt was made to identify flies to species using the method described by Sofield et al. (1984b), but results obtained by using this method were inconclusive and therefore flies could not be positively identified to species (see Chapter 2).

Quantity of Crop Contents

The volume of crop contents of female flies trapped in 1991 and 1992 is summarized in Tables 3.1a & b. Both years showed that a smaller percentage of small flies had at least one microliter of crop contents compared to the percentage of medium and large flies. On the other hand, almost the same percentage of medium and large flies had at least one microliter of crop contents. Only 17% of the small flies examined in the first season had one or more microliters of crop contents, whereas 27% of the medium and large flies had at least one microliter of crop contents. In the second season, 29% of the small flies had one or more microliters of crop contents and 43 and 44% of the medium and large flies, respectively, had at least one microliter of crop contents. The number of small sized flies storing over one microliter of fluid in their crop was significantly different than the number of medium and large flies storing over one microliter of fluid in their crop in both the 1991 season ($X^2=7.51$, $p < 0.01$) and the 1992 season ($X^2=12.47$, $p < 0.005$).

Average crop volume for flies with at least one microliter of crop contents was also examined. Data collected during both seasons showed the trend that increasing body size related to increasing average crop volume (Tables 3.1a & b). There was about a 20% increase in average crop volume from small flies to medium flies and from medium flies to large flies. The second season's data showed higher average crop volumes for each fly size compared to the first season. On the other hand, ANOVA analysis of the data collected during both years determined that the crop volume was not significantly different between different size groups ($F=1.552$, $p=0.200$).

The average amount of sucrose solution found in the crops of flies starved and then offered 19% sucrose solution ($n=50$, $\bar{x}=9.756$, $S.E.=0.35$) was higher than the average quantity of crop contents found in the wild flies that were not offered any sucrose in the lab. The maximum amount of sucrose solution found in the crops of greenhead flies offered 19% sucrose solution was 25.2 ml.

Amount of Sugars in the Crop

Only flies with over one microliter of crop contents were analyzed to determine crop sugar content. A summary of the amount of sugar found in flies caught during the 1991 & 1992 season is given in Tables 3.2a & b. Sugar content in the crop followed the same trends as crop volume. The large flies had more sugar in their crops than medium flies, which had more sugar in their crops than the small flies. The flies caught in the second season had a greater amount of sugar compared to flies of the same size in their first season. The minimum amount of sugar found in flies whose crop contents were analyzed was 0.21 mg and a maximum of 3.60 mg. The percent of sugar in the crop contents of the flies with over one microliter of crop contents was also examined (Table 3.3). The greatest number of flies had over 50% sugar in their crop contents. No fly had under 20% sugar in their crop contents.

Discussion

Laboratory studies conducted by Magnarelli and Stoffolano (1980) conclude that *T. nigrovittatus* require carbohydrates in the form of sugar for energy to survive long enough to mature oocytes. Past studies report observations of this species obtaining carbohydrates from plant sources (Roberts 1967, Pratt and Pratt 1972). Many studies have used Van Handel's (1972) fructose test on other wild tabanids as an indicator of nectar feeding (Magnarelli and Anderson 1977, Magnarelli et al. 1979, Kniepert 1980, Magnarelli and Anderson 1981, Leprince et al. 1983, Leprince and Lewis 1986, Hoppe 1989, Leprince and Bigras-Poulin 1990). Wilson and Lieux (1972) have reported finding a few pollen grains in the guts of individual tabanids, further proving the role of plants in carbohydrate feeding of tabanids.

Schutz and Gaugler (1989) found that male *T. nigrovittatus* feed on aphid honeydew as a source of carbohydrates immediately following hovering periods. A conscious effort was made during this two season study period to identify the carbohydrate source for female *T. nigrovittatus*. Flowers were observed at different hours of the day throughout the season. No female *T. nigrovittatus* were ever observed to land and attempt to feed on any flowering plant on or near (< 1/2 mile) of the marsh.

In this study, it was shown that less than half (32.2%) of the flies examined had at least one microliter of carbohydrates in the crop. This differs from the results obtained by Bosler and Hansens (1974) who found that 59.3% of the flies caught in box traps had full crops. It is not clear what amount of crop distension was being described by the term full crop, but some differences in these observations could have been caused by availability of different carbohydrate sources. The concentration of sugar in the carbohydrate sources could affect the amount of carbohydrates being stored by the fly. While the percent of sugar found in the crop contents of the flies in this study may appear high at first glance, the level of concentration is not unusual for nectar. There are many plants that produce nectar with over 40% sugar concentration and some plants that produce nectar with over 50% sugar concentration (Shaw 1956). It is now important to positively identify the

carbohydrate source(s) these flies are utilizing.

The small percentage of tabanids with any carbohydrates found in their crops raises the question of where these flies are getting their energy to fly, mate, etc. One possible explanation is that these flies are obtaining energy through the blood meals. This has been suggested in mosquitoes that do not often feed on nectar in the wild (Edman et al. 1992). Magnarelli and Stoffolano (1980) showed that laboratory populations of *T. nigrovittatus* could not survive long enough to develop oocytes even when fed strictly a blood diet. This field study further supports these laboratory data which suggest that these flies are not using blood as a source of energy. Only two individuals, out of over 7000 examined during both seasons of this study, were found that had any evidence of blood feeding (fresh or dried blood in their midgut). No blood was observed in the crop of any flies examined in this study. Another possibility is that flies are taking many small carbohydrate meals during the day. These small meals could be used immediately with little carbohydrate being stored in the crop. Further, if flies are feeding on diluted sources of carbohydrates, the carbohydrates would not be diverted to the crop in the first place but would go directly to the midgut (Friend and Stoffolano 1991). Therefore, the flies would not have a large amount of carbohydrates being stored in the crop because it is immediately being used by the fly for energy.

Although the small flies had a smaller average crop volume, statistically the crop volumes for different size individuals were not different. This was due to the amount of variation of crop volume within a body size category. This could be due to the small sample size. On the other hand, the percentage of small flies with at least a crop volume of one microliter was significantly smaller than the percentage of medium and large flies with at least one microliter of crop fluid. The body size of a fly appears to affect the amount of carbohydrates being stored by that fly.

This is the first study to examine the quality and quantity of carbohydrates being stored in the crop of a field-caught tabanid. Future studies on the role of body size and energy expense are needed to explain the connection between body size of the fly and the amount of carbohydrates being stored in the crop of the fly.

Table 3.1 Percent of flies with over one microliter of crop contents and the average crop volume of greenhead flies, during a. 1991.

Fly size	Total number of flies examined	Number and % of flies with > 1ul crop contents	Average crop volume (ul) \pm S.E.*
Small	189	32 (16.9%)	1.44 \pm 0.21
Medium	365	97 (26.6%)	1.63 \pm 0.14
Large	270	73 (27.0%)	2.07 \pm 0.18

* S.E. = Standard error

Table 3.1 continued, b. 1992.

Fly size	Total number of flies examined	Number and % of flies with > 1ul crop contents	Average crop volume (ul) \pm S.E.*
Small	200	59 (29.5%)	1.96 \pm 0.19
Medium	425	185 (43.5%)	2.36 \pm 0.13
Large	180	79 (43.9%)	3.14 \pm 0.64

* S.E. = Standard error

Table 3.2 Average amount (mg) of sugar found in the crops of flies with over one microliter of crop contents, during a. 1991.

Fly size	n	Avg. amount of sugar \pm S.E.*	Average amount of sugar	
			Minimum	Maximum
Small	32	0.67 \pm 0.088	0.35	3.10
Medium	101	0.85 \pm 0.067	0.21	3.00
Large	51	0.97 \pm 0.103	0.21	3.60

* S.E. = Standard error

Table 3.2 continued, b. 1992

Fly size	n	Avg. amount of sugar \pm S.E.*	Average amount of sugar	
			Minimum	Maximum
Medium	27	0.62 \pm 0.084	0.32	2.60
Large	23	0.85 \pm 0.011	0.34	1.80

* S.E. = Standard error

Table 3.3 Distribution of the percent of sugar found in the crop contents of flies with over one microliter of crop contents.

Percent sugar (%)	1991 Season (n=186)		1992 Season (n=320)	
	Number of flies	Percent of total (%)	Number of flies	Percent of total (%)
20-29	5	2.6	8	2.5
30-39	21	11.3	37	11.6
40-49	78	41.9	100	31.2
50 +	82	44.1	175	54.7

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