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Studies on the oviposition behavior of the salt marsh greenhead, Tabanus nigrovittatus Macquart (Diptera: Tabanidae).

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STUDIES ON THE OVIPOSITION BEHAVIOR OF THE SALT MARSH GREENHEAD, TABANUS NIGROVITTATUS MACQUART (DIPTERA: TABANIDAE).

A Thesis Presented

By

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SALT MARSH GREENHEAD, _TABANUS NIGROVITTATUS_

MACQUART (DIPTERA: TABANIDAE)

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DEDICATION

To my parents, for their many years of love, understanding and encouragement.

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ABSTRACT

Two forms of Tabanus nigrovittatus Macquart, recently shown in New Jersey to be sibling species (Jacobson et al. 1981), were discovered in the salt marshes of Wellfleet, Massachusetts. The oviposition behavior and the characteristics of the egg masses differed between the two forms. The smaller, typical flies deposited gray, double-tiered egg masses, while the larger, grayer females deposited tan, single-layered shingled egg masses. In the laboratory the smaller, typical females faced head-down on the adaxial surface of Spartina alterniflora Loisel blades during oviposition. In contrast the larger, grayer females faced head-up on the blades while ovipositing in both field and in laboratory situations. Both types of egg mass were most frequently deposited on the upper third of the short form of S. alterniflora.

In the salt marsh, 100% of the 63 egg masses discovered were deposited on S. alterniflora. Eighty-one percent of these were deposited on the short form in the low marsh, while 19% were deposited on the tall form, in the transition zone between the low marsh and the high marsh. During 14 days of observation, no females oviposited in the high salt marsh, on Spartina patens (Ait.) Muhl. or on any other high marsh vegetation.

When given a choice of S. alterniflora, S. patens or Distichlis spicata (L.) as potential oviposition sites, 98% of the ovipositing females selected S. alterniflora, while only 2% of the females oviposited on the other two species of marsh plant in the cage. When

presented with only one of the three species of marsh plant in a single cage, 98% of the females oviposited on S. alterniflora in the cage containing only this species; the other 2% of the females oviposited on the wooden beams of the cage. In the two cages containing either S. patens or D. spicata, 2% of the egg masses were found on each of the two plant species, while 98% of the egg masses were found on the various cage surfaces in each cage.

Oviposition behavior was observed from June 6 through August 2 during 1979-1980. During this period, oviposition activity occurred between the hours of 0900 and 1700, with a peak in activity from 1200 to 1300 hours. Seventy percent of all observed oviposition took place between the hours of 1100 to 1400.

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CHAPTER I

LITERATURE REVIEW

The Salt Marsh Greenhead, Tabanus nigrovittatus Macquart

Taxonomic position. Distribution of the family Tabanidae is world wide. There are approximately 3,500 species, 100 subspecies, 121 genera and 77 subgenera presently recognized (Fairchild 1969). The family Tabanidae is currently divided into three subfamilies: Pangoniinae, Chrysopinae and Tabaninae, based on the characteristics of the genitalia (Mackerras 1954). The family was established in 1850 by Agassiz in his *Nomenclator Zoologicus*. Linnaeus in 1758 established the genus *Tabanus* in the 10th edition of *Systema Naturae*.

In this study, the concentration has been primarily on salt marsh greenheads, *Tabanus nigrovittatus*, inhabiting the marshes of southern Massachusetts. Two forms of *T. nigrovittatus* have been recognized in a number of areas. In one form, the adult flies are larger and have grayer mesonotums; males of this form have a greater extension of the large eye facets. Flies of the larger form are often found within the same habitat as the smaller, typical form (Pechuman 1981).

Morphological and ecological observations on the immature stages of *T. nigrovittatus* led Freeman (1962) to first discuss the possibility that *T. nigrovittatus* consisted of a pair of sibling species. Structural differences in both the larval and in the pupal stage occurred between the two forms. Freeman (1962) also reported differences in the larval habitats of the two forms. Larvae which emerged as the larger, grayer adults (which Freeman designated *T. sp. 3*) were found along drainage ditches where *Spartina alterniflora* Loisel grew taller and was more sparsely distributed than the shorter form. Larvae which emerged as the smaller, typical adults inhabited areas of the marsh dominated by the shorter, more highly vegetated form of *S. alterniflora*. Teskey (1969) believed Freeman's (1962) *T. sp. 3* was identical with *T. sp. A* reported by Jamnback and Wall (1959), who discovered unusual larvae in the marsh sod where *S. alterniflora* grew tall and sparse. These larvae did not fit the discriptions for either of the most prevalent salt marsh tabanids, *Tabanus lineola* F. or *T. nigrovittatus*, thus they designated these larvae *T. sp. A*. The larger grayer adult was once recognized as the valid species *Tabanus conterminus* Walker (Hine 1906), however, this classification was held in abeyance by Stone (1938) as a potential variety due to apparent intergrades which he found between the two forms. Stone (1938) stated that if varietal rank were given to the larger form, then the name of the species would be *Tabanus simulans* (Walker) due to the rule of priority. Recently, the taxonomic status of *T. nigrovittatus* seems to have been clarified. Examination of adult flies by starch gel electrophoresis of enzymes has shown that *T. nigrovittatus* may actually consist of a pair of sibling species along the marsh lands of New Jersey (Jacobson *et al*. 1981).

Jacobson and his co-workers also reported a significant difference in the total lengths of the two forms which they designate as separate species.

Economic importance. The salt marsh greenhead, *T. nigrovittatus*, is seasonally abundant on coastal salt marshes and adjacent areas along the eastern seaboard of North America (Jamnback and Wall 1959). Following autogenic egg maturation, females aggressively seek blood meals from vertebrate hosts, thus becoming a severe nuisance to man and other vertebrates (Rockel 1969, Anderson 1971, Bosler and Hansens 1974). The large numbers of flies which emerge during the summer months, when recreational activity is at a peak, present a major economic concern to the resort industry.

Oviposition behavior. The specificity and selectivity of oviposition sites varies among the members of the family Tabanidae. Some species are quite selective, and deposit their egg masses only on a particular plant species, while other species show no apparent preference for specific oviposition sites within their habitats. Most frequently, the eggs of Tabanidae are laid in separate masses in aquatic or semi-aquatic environments (Hays, 1956); however, a number of species are restricted to terrestrial environments (Marchand, 1920). Hine (1903) reported that certain tabanid species showed a preference for specific oviposition sites. He discovered *Tabanus vivax* Osten Sacken deposited egg masses on stones, *Tabanus atratus* F.

oviposited on *Scirpus* sp., and *Tabanus stygius* Say generally oviposited on *Sagittaria* spp. *T. atratus* was found to be less selective by other investigators; Stone (1930) discovered an egg mass on *Typha* sp., Pechuman (1957) discovered a female ovipositing on a cement bridge abutment and Jones and Anthony (1964) reported that this species had been found to oviposit on various surfaces of buildings. Gjullin and Mote (1945) discovered egg masses of *Chrysops discalis* Williston only on the stems of *Scirpus americanus* Pers., while Roth and Lindquist (1948) found egg masses of this species on *S. americanus* and on objects placed in the shallow waters of a lake. Tidwell and Hays (1971) conducted an oviposition site preference study among various species of *Chrysops* in Alabama. Artificially placed stations containing several species of aquatic plants were placed at various intervals from 2 to 100 ft. from the shore of a pond. From distances up to 100 ft. from shore, *Chrysops* species randomly deposited egg masses on *Typha latifolia* (L.). At stations near the shore, deerflies showed a definite preference for *Polygonum hydropiperoides* Michx. over the other experimental marsh plants. Shin and Hyun (1975) reported that *Tabanus amaneus* Walker preferred to oviposit on 3 species of gramineae found in rice fields. Most of these egg masses were discovered at heights of 2-35 cm on the grasses.

A description of the oviposition behavior of *Chrysops callidus* Osten Sacken and *Chrysops moerens* Walker was given by Hine (1903, 1906). The oviposition behavior of *T. nigrovittatus* has not previously been described, although descriptions of the egg masses have been given by several authors (Jamnback and Wall 1959, Thompson et al. 1979, Magnarelli and Stoffolano 1980).

The oviposition sites of several salt marsh Tabanidae have been documented. T. lineola was found to oviposit primarily on the tips of S. alterniflora (Orminati and Hansens 1974). Magnarelli and Anderson (1979a, b) found egg masses of Chrysops atlanticus Pechuman and of Chrysops fuliginosus Wiedemann on the adaxial surface of S. alterniflora. Jamnback and Wall (1959) found an egg mass of T. nigrovittatus on S. alterniflora; however, the female which deposited this egg mass was never identified nor were the larvae which emerged from the egg mass, therefore the species which deposited the egg mass remains questionable.

Oviposition activity patterns of tabanids have not been previously reported; however, flight and biting activity patterns of many species of Tabanidae have been documented. North American Tabanidae, including T. nigrovittatus, were found to be most active during the day light hours (Miller 1951, Roberts 1966, Burnett and Hays 1977). Schulze et al. (1975) reported T. nigrovittatus to be most active from 0800 to 1600 hours.

CHAPTER II

THE RELATIONSHIP BETWEEN FEMALE SIZE AND THE TYPE OF EGG MASS DEPOSITED, WITH A DESCRIPTION OF THE OVIPOSITION BEHAVIOR OF *TABANUS NIGROVITTATUS* MACQUART.

Introduction

The salt marsh greenhead, *Tabanus nigrovittatus* Macquart, is seasonally abundant in coastal salt marshes and adjacent areas along the eastern seaboard of North America (Jamnback and Wall 1959). Following autogenic egg maturation, females readily seek blood meals from vertebrate hosts, thus becoming serious pests of man and other vertebrates (Rockel 1969, Anderson 1971, Bosler and Hansens 1974).

Two forms of *T. nigrovittatus* are recognized in a number of areas. In one form, the adult flies are larger and have grayer mesonotums; males of this form have a greater extension of the large eye facets. Flies of this larger form are often found within the same habitat as the smaller typical form (Pechuman 1981). Identification of specimens sent to Dr. Pechuman revealed that both the larger, grayer form and the smaller typical form of *T. nigrovittatus* exist in the salt marshes of Wellfleet, Massachusetts. The larger, grayer flies were once classified as the valid species *Tabanus conterminus* Walker (Hine 1906), however this classification was held in abeyance by Stone (1938) as a potential variety because of apparent intergrades which he found between the two forms. Stone

(1938) also stated that if varietal rank were given to the larger form, then the name of the species would be *Tabanus simulans* (Walker) due to the rule of priority. Recently, the taxonomic status of *T. nigrovittatus* seems to have been clarified. Examination of adult flies by starch gel electrophoresis of enzymes has shown that *T. nigrovittatus* actually consists of a pair of sibling species along the marsh lands of New Jersey (Jacobson et al. 1981). Jacobson et al. (1981) also reported significant differences in the total lengths between the two forms which they designated as separate species.

Before the most effective control measures can be undertaken, all stages of the life history of a species should be well understood. At present, nothing is known about the oviposition behavior and the characteristics of the egg masses deposited by the two forms of *T. nigrovittatus* in the salt marsh. Subsequently, the objectives of the present study were two-fold: (1) to determine whether the egg masses deposited by the two forms of *T. nigrovittatus* differed in any manner, and (2) to investigate the oviposition behavior of the two forms of greenhead fly.

Materials and Methods

Study site. Research on the oviposition behavior of *T. nigrovittatus* was conducted on a salt marsh at Great Island, Massachusetts. Great Island is a barrier spit, located within the Cape Cod National Seashore, on the northwest coast of the town of Wellfleet.

The vegetation at Great Island is typical of Northeastern salt marshes. Two distinct zones are discernible: a low marsh dominated by Spartina alterniflora Loisel, and a high marsh, dominated by Spartina patens (Ait.) Muhl. The low marsh (the portion of the marsh which is inundated by daily high tides) has two subzones colonized by the two forms of S. alterniflora. The shorter and more densely vegetated form of S. alterniflora occurs in flat areas removed from tidal creeks, and covers the greatest area of the low marsh. The much less extensive and more sparsely vegetated subzone of tall S. alterniflora is restricted to the edges of the tidal creeks and along the margins of the bay where tidal exchange is at a maximum. The high marsh is inundated at least once a month by the high spring tides. Although the dominant species is S. patens, two other grasses, Distichlis spicata (L.) and Puccinellia maritima (Huds.) Parl. also exist in the high marsh. The upper limit of the high marsh is bordered by the dune strand, dominated in New England by Ammophilla breviligulata Fernald.

Fly size (form) and egg mass type: laboratory studies. During the summer of 1980, parous T. nigrovittatus females were collected from box traps and transported to the laboratory in 1 mm mesh metal screened cages (24 x 24 x 25 cm). In the laboratory females readily fed on warmed (34-38°C), citrated human blood applied through Kimwipes placed on top of the cages (Stoffolano 1979) and were allowed to feed to repletion. Engorged flies were captured upon leaving the feeding site, whereupon the total length of each fly was measured with an ocular micrometer to the nearest millimeter. Based on total length, females were then placed into one of eight groups of increasing 1 mm intervals (the eight groups consisted of flies ranging in length from 9-10 mm to 16-17 mm). A 1 mm mesh wooden screened cage (61 x 61 x 61 cm) was set up for each of the eight different size groups of flies. Eight sections of S. alterniflora (each section was approximately 30 x 50 cm wide) were dug out of the salt marsh, transported to the laboratory and potted into plastic bags. One section of grass was placed into each of the eight cages. Females were then placed into the cages and provided with granulated sucrose cubes and distilled water throughout the duration of the experiment. Due to a high mortality of captive T. nigrovittatus and a scarcity of the smaller flies in the Wellfleet marshes, females were continually collected from the marsh, blood fed, measured and placed into the appropriate cages. This process was continued until approximately 50 egg masses could be collected from each of the 8 cages. S. alterniflora sections were replaced when the grass thinned out due to the daily removal of blades containing egg masses, or when the grass showed signs of dessication. The structure, dimensions, coloration and number of eggs per mass were recorded for the masses collected from each of the 8 cages. The dimensions of the egg masses and of the individual eggs in each mass were measured using an ocular micrometer. Flies were maintained in the laboratory under ambient conditions of temperature, daylight and relative humidity.

Fly size (form) and egg mass type: field studies. To determine whether a relationship existed between fly size (form) and the type of egg mass deposited, ovipositing *T. nigrovittatus* females and their respective egg masses were collected from the salt marsh and transported to the laboratory where the length of each fly was measured using an ocular micrometer. Females were keyed to species according to Pechuman (1981); egg mass structure, coloration, size and individual egg size was recorded for each egg mass deposited in the salt marsh by the respective female. As with the field studies, egg masses were measured using an ocular micrometer.

Oviposition behavior in the salt marsh. The oviposition behavior of *T. nigrovittatus* was observed during the summers of 1979 and 1980. In order to locate ovipositing females, the observers crouched to the level of the *S. alterniflora* blades; from this angle, flies were silhouetted against the sky and could be seen ovipositing from a distance with the aid of field binoculars. Upon encountering an ovipositing female, the observers were able to move to within a few inches of the fly, easily observing its behavior. Oviposition behavior of five females was video taped for future viewing.

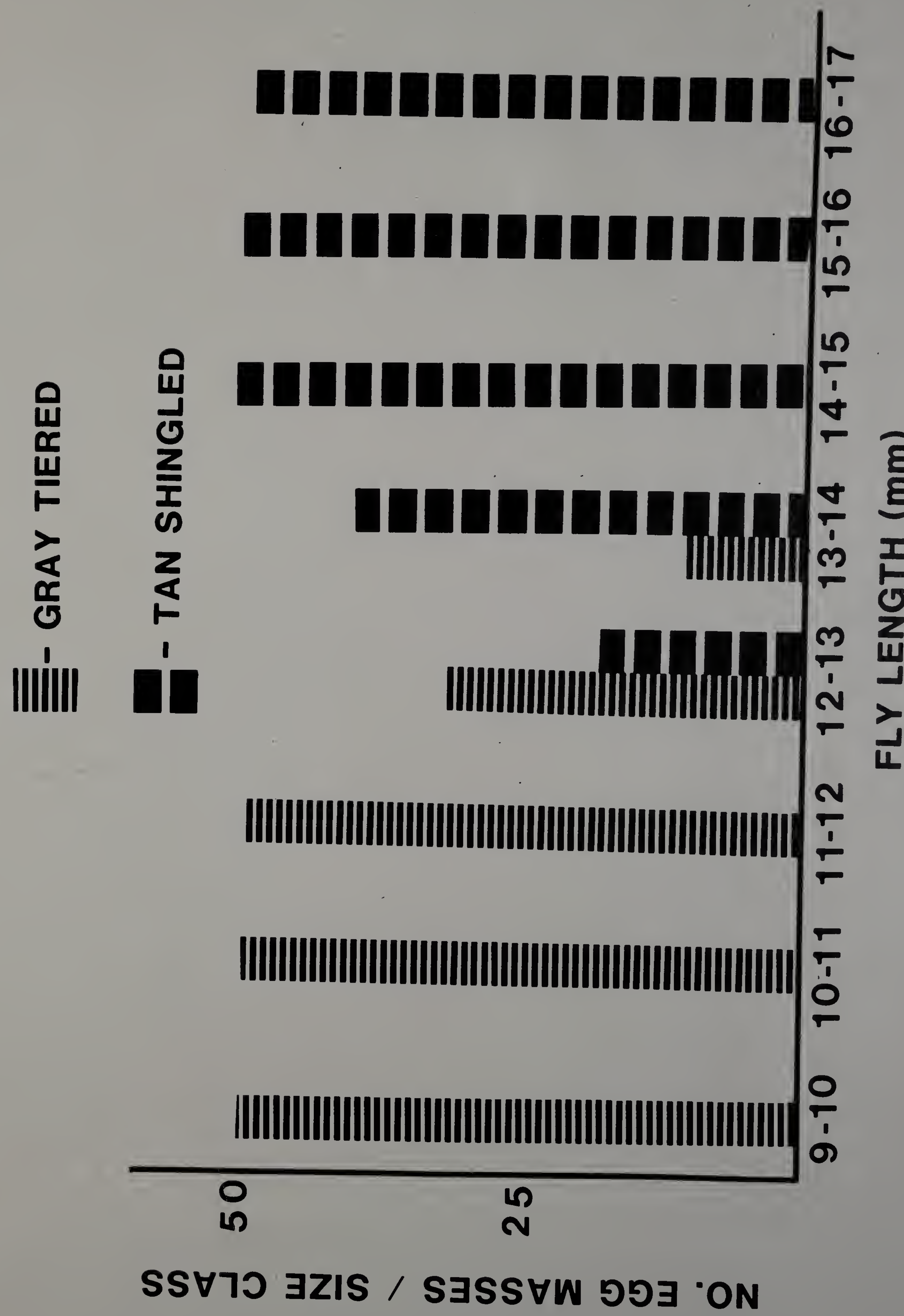
Results

Fly length (form) and egg mass characteristics. Egg masses deposited by the two forms of *T. nigrovittatus* differed in structure, coloration, dimension and in the size of the individual eggs from each type of mass. The relationship between female length (form) and the type of egg mass deposited on *S. alterniflora* in the laboratory can be seen in Fig. 1. Smaller flies in the 9-12 mm range deposited double tiered, medium, gray masses, similar to those described by Jamnback and Wall (1959), Thompson et al. (1979) and Magnarelli and Stoffolano (1980). Larger flies in the 14-17 mm range deposited tan, single layered, shingled egg masses. Females in the middle size range (12-14 mm) deposited either gray, tiered egg masses or tan, shingled egg masses; egg masses having intermediate characteristics of the two types of mass were never found.

In the salt marsh, ovipositing females were found ranging in length from 13.9-16.3 mm. According to Pechuman (1981), flies of this length were classified as the larger, grayer form of *T. nigrovittatus*. These females deposited tan, shingled egg masses, similar to those deposited by the larger flies in the laboratory. When first deposited in the salt marsh the egg masses were shiny white, however, within a few hours they darkened to a light tan, and remained so, if at this time they were removed from the salt marsh and brought back to the laboratory. When the egg masses were allowed to remain in the salt marsh for 48 hours, the outer portions of the eggs (those not covered by overlapping portions of other eggs, but exposed to sunlight and air) darkened to a chocolate brown. The egg masses which were originally deposited in the laboratory and which remained there, darkened only to a light tan.

Descriptions of the two types of egg masses. Egg masses deposited by the smaller, typical form of *T. nigrovittatus* were similar in appearance to those described by Jamnback and Wall (1959); one end of

Fig. 1. Relationship between fly length and the type of egg mass deposited.


- GRAY TIERED
- TAN SHINGLED
NO. EGG MASSES / SIZE CLASS
50
25
9-10
10-11
11-12
12-13
13-14
14-15
15-16
16-17
FLY LENGTH (mm)

the mass tapered to a point apically and was bluntly rounded basally. The upper portion of the individual eggs of the lower tier overlapped the lower portion of the eggs of the upper tier (Fig. 2). Twenty-six gray, tiered egg masses deposited on S. alterniflora in the laboratory had a mean length of 6.7 $\pm$ 0.5 mm, width of 2.7 $\pm$ 0.2 mm, height of 2.1 $\pm$ 0.1 mm and contained a mean of 156.8 $\pm$ 13.9 eggs (Table 1).

Egg masses deposited by the larger, grayer form of T. nigrovittatus were long and lanceolate shaped, tapered both apically and basally, and were deposited along the midline of the adaxial surface of S. alterniflora blades (Fig. 3 and 4). These masses were composed of single layered rows of shingled eggs, which is contrary to the generalization that Tabanus species deposit double tiered egg masses and Chrysops species deposit single layered, shingled egg masses (Roberts 1966). As greater numbers of egg masses are described for species of Chrysops and Tabanus, it becomes apparent that no general rule exists which specifies egg mass characteristics. The egg masses found in the salt marsh had a mean length of 24.7 $\pm$ 4.4 mm, width of 2.9 $\pm$ 0.8 mm, height of 0.9 $\pm$ 0.1 mm, and mean egg number of 204.0 $\pm$ 12.1. A significant difference was found in the number of eggs per mass between the gray tiered masses deposited in the laboratory and the tan shingled masses deposited in the field. No significant difference in number of eggs was found between the tan shingled masses deposited in the laboratory and either the gray tiered massed deposited in the laboratory or the tan shingled masses deposited in the field.

Fig. 2. Gray, tiered egg mass deposited on the side of a wooden screened cage.

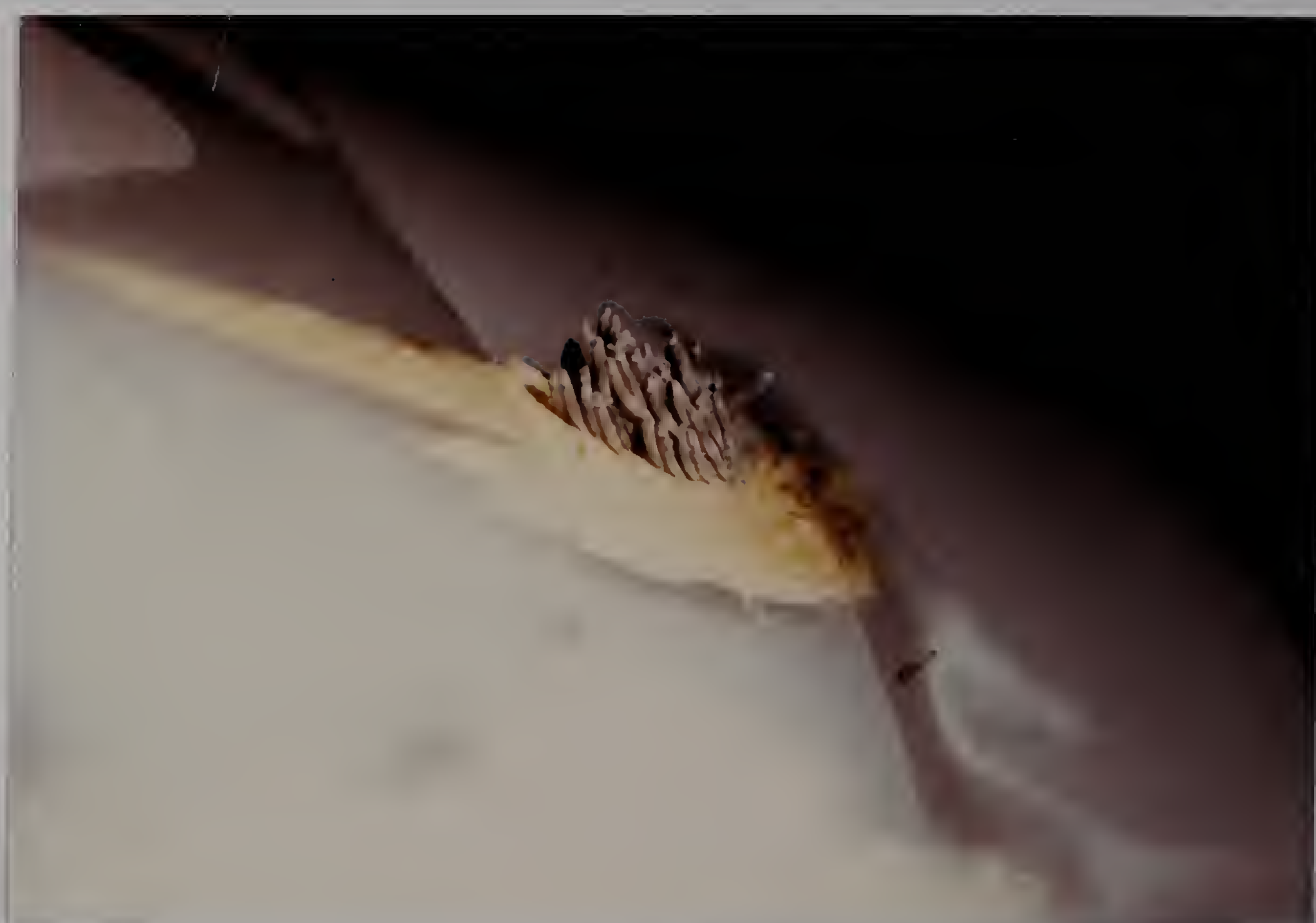


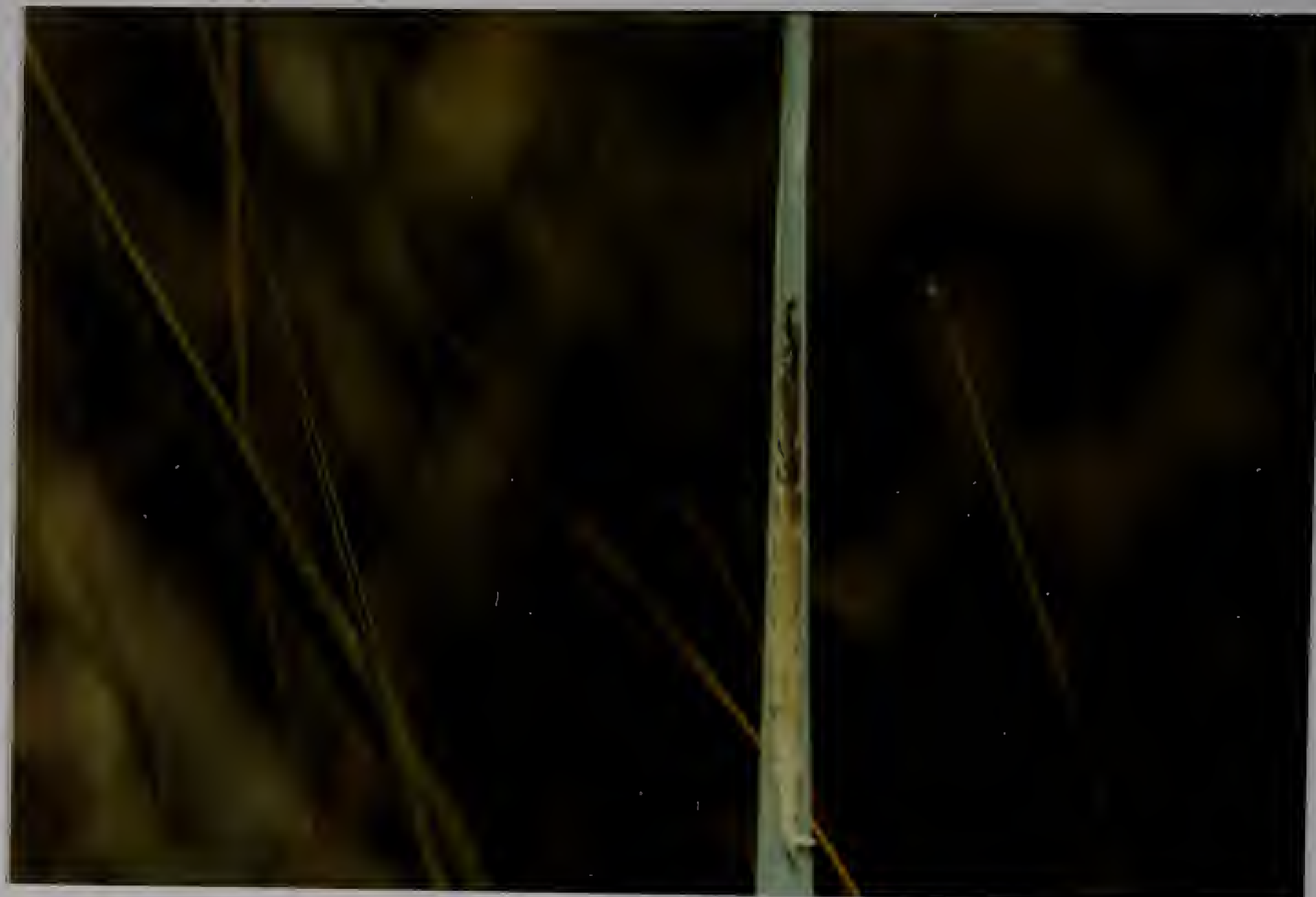
Table 1. Characteristics of the egg masses deposited on *Spartina alterniflora* by the two forms of *Tabanus nigrovittatus*.

Form of *T. nigrovittatus*	Sample size	Coloration and mass structure	No. eggs per mass (x ± SE)	Dimensions of egg masses (x ± SE in mm)		
				Length	Width	Height
Smaller, typical form						
A. Lab deposited egg masses	26	Gray/tiered	156.8±14.0 a	6.7±0.5 a	2.7±0.2 a	2.1±0.1 a
Larger, grayer form						
A. Field deposited egg masses	13	Tan/shingled	204.0±12.1 b	24.7±4.4 c	2.9±0.8 a	0.9±0.1 b
B. Lab deposited egg masses	26	Tan/shingled	178.0±16.5 ab	13.6±0.7 b	2.8±0.1 a	1.1±0.1 b

Within a column, means which are not followed by the same letter are significantly different at $P < 0.01$.

Fig. 3. Egg mass deposited by the larger form of Tabanus nigrovittatus, 24 hours after deposition in the salt marsh.

Fig. 4. SEM photograph of a tan, shingled mass (40x).



A highly significant (P<0.01) difference was found in both the length and in the height, between the gray, tiered egg masses and the tan, shingled egg masses deposited in the laboratory. A highly significant difference (P<0.01) of length and height was also noted between gray, tiered egg masses deposited in the laboratory and tan, shingled egg masses deposited in the salt marsh (Table 1). For the tan, shingled egg masses, a significant difference in length was noted between the masses deposited in the laboratory and the masses deposited in the field; however, the egg masses from the two locations did not differ significantly in width, in height, or in the number of eggs per mass.

Measurements of individual eggs from the two types of egg mass. The individual eggs from the gray, tiered egg masses differed significantly ($P \leq 0.01$) in both length and width from the individual eggs of the tan, shingled egg masses (Table 2). Four eggs from each of 25 gray, tiered masses, had a mean length and width of 1.56 ± 0.01 mm by 0.28 ± 0.01 mm, respectively. Four individual eggs from each of 25 tan, shingled egg masses had a mean length of 1.98 ± 0.04 mm and width of 0.34 ± 0.01 mm.

Oviposition behavior in the salt marsh. The oviposition behavior of *T. nigrovittatus* can be categorized into three parts: (1) Preoviposition behavior, (2) Oviposition behavior, and (3) Postoviposition behavior. Oviposition behavior of the smaller, typical form was never observed in the field nor were any egg masses

Table 2. Size and color differences between individual eggs deposited by the two forms of *Tabanus nigrovittatus* in the laboratory.

Species/form	Egg mass coloration	Sample size	Length ($x \pm$ SE in mm)	Width ($x \pm$ SE in mm)
Smaller, typical form	Gray	25	1.56 ± 0.01 a	0.28 ± 0.01 a
Larger, grayer form	Tan	25	1.98 ± 0.04 b	0.34 ± 0.01 b

Within a column, means which are not followed by the same letter are significantly different at $P < 0.01$.

found in the field. Consequently, the emphasis here is on describing the oviposition behavior of the larger, grayer form. The larger form of T. nigrovittatus exhibited a stereotyped preoviposition behavior which began with an apparently random flight from blade to blade of numerous S. alterniflora plants. Females landed head up on the adaxial surface of S. alterniflora blades. Generally, females landed near the tip of the blade, then turned 180° and walked down the blade to the point at which the blade met the stem, whereupon they again turned 180° and walked back up the plant to the original landing site at the tip of the blade. If this behavioral sequence was completed without encountering a suitable oviposition site, females would fly to other plants and repeat the cycle. Occasionally females landed lower on the blades away from the tip; in these cases, they walked to the tip of the blade and exhibited the behavioral sequence which was described for the females which landed near the tips of the blades. This down-up walking sequence was usually performed once on a single blade, and on only one of the approximately six blades on any S. alterniflora plant. Occasionally, flies were observed walking down and up on the same blade a number of times, and were seen eventually returning to these blades and ovipositing.

In the transition zone between S. alterniflora and S. patens, one female exhibited preoviposition behavior alternately between the two species of grass. The female eventually selected S. alterniflora for an oviposition site.

While walking down and up the blades of S. alterniflora, females slightly arched the centers of their abdomens, thus dragging their last few abdominal sclerites along the midribs of the blades. While dragging their abdomens, females scanned the width of the blades by continuously bringing both foretarsi together in a scanning movement in to the midline of the blades, then moving the tarsi out to the opposite edges of the blades. The adaxial surface of a blade of S. alterniflora is shown in Fig. 5.; note the parallel orientation of the grooves and ridges on this surface of the blade.

To determine whether the flight from blade to blade and subsequent scanning behavior of the S. alterniflora blades was actually preoviposition behavior, 35 females exhibiting this behavior were captured, taken back to the laboratory, and dissected to determine whether they had mated and to determine their state of ovarian development according to Mer's (1936) modifications of Christopher's 1911 classification. All 35 females had mated and contained fully matured stage 5 oocytes. Females exhibiting preoviposition behavior in the salt marsh were observed and followed; all females which we could keep in eye contact eventually oviposited. Thus, we concluded that this behavior was indeed preoviposition behavior.

Most frequently, oviposition sites were located on the upper third of the adaxial surface of the short form of S. alterniflora, midway between the two edges of the blade, and where the blade was from 4-6 mm in width (Table 3). Out of 19 females ovipositing, only four females (marked with asterisk) failed to oviposit on the upper third of the plants, and, of these four, three oviposited on the upper half of the tall form of S. alterniflora (Table 3). During

Fig. 5. SEM photograph of the adaxial surface of a blade of Spartina alterniflora (39x).

Table 3. The location on *Spartina alterniflora* where the egg masses of the larger form of *Tabanus nigrovittatus* were deposited in the salt marsh.

Measurement on plant where the first egg (lowest egg on the the blade) was deposited.			
Plant height (cm)	Egg mass distance from salt marsh surface (cm)	Plant width (cm)	Egg mass width (cm)
63.5	43.2	5.0	2.7
61.0	42.0	5.2	3.0
56.0	43.0	6.0	3.0
74.0	42.0	5.0	3.0
38.1	25.4*	5.1	2.5
46.0	30.5	6.0	3.0
61.0	40.1	5.5	3.1
61.0	43.1	6.1	3.2
38.1	25.4*	4.0	2.0
76.2	43.0	5.0	3.0
74.0	48.0	6.0	3.7
91.4	60.0	10.0	3.0
99.0	60.0	10.0	3.1
53.3	43.1	4.2	3.2
35.0	26.0*	4.0	2.0
53.0	27.0*	5.0	2.5
43.0	31.0	6.0	3.0
64.0	44.2	4.0	2.0
89.4	50.0	13.0	4.0
$\bar{x}$= 61.9 ± 9.6	$\bar{x}$= 40.4 ± 2.4	$\bar{x}$= 6.0 ± 0.5	$\bar{x}$= 2.9 ± 0.6

*Female did not oviposit on the upper third of the plant.

oviposition the larger, grayer form of T. nigrovittatus faced head up on the plants (Fig. 6). The flies gradually moved upward toward the tips of the blades as deposition of the egg mass took place. Each egg was deposited individually by curling the tip of the abdomen under, toward the abdominal sternites, while extruding an egg. When the abdomen was extended back to its original position an egg was drawn out. After each egg was deposited, females scanned the newly deposited egg and a few of the surrounding eggs with the tips of their abdomens in a forward, backward movement. After scanning the fresh egg, females continued filling in gaps in the egg mass, or moved upwards toward the tip of the plant, depositing new sections of the egg mass as they progressed. Once oviposition had begun, females clung to the edges of the blades with their foretarsi, keeping the axis of their bodies straight without moving from side to side. The upper tips of the eggs were attached to the adaxial surface of the S. alterniflora blades with a sticky exudate from the accessory reproductive glands (Fig. 7). Egg masses were so firmly attached to the blades of S. alterniflora that a layer of epidermal plant tissue remained on the eggs after masses were dislodged from the plants (Fig. 8). For comparison, Fig. 9 shows an SEM photograph of an intact epidermal ridge on the adaxial surface of S. alterniflora.

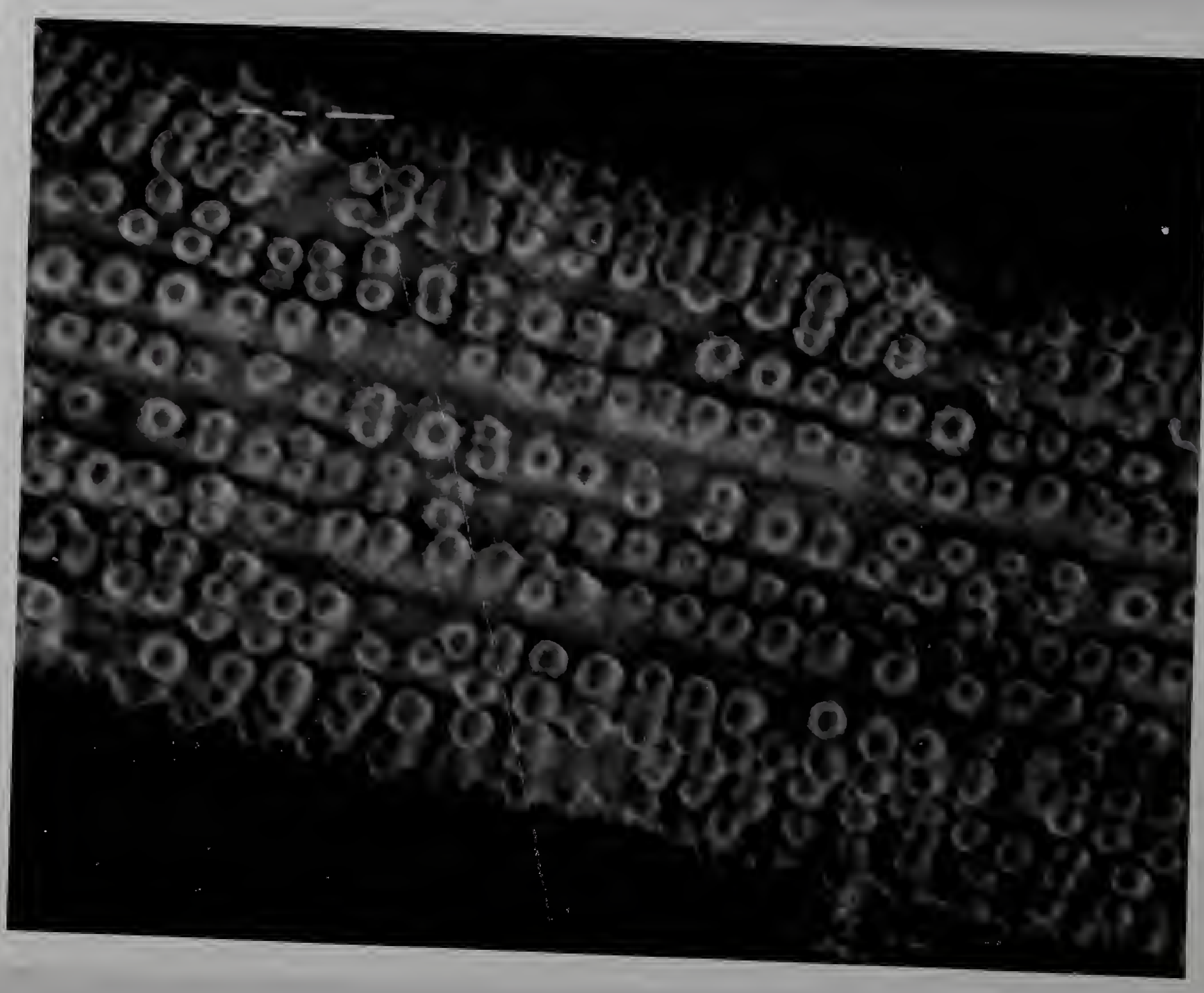
If females were disturbed during oviposition, they did not return to complete the original egg mass, but flew to other plants where they engaged in the preoviposition behavioral sequence until they encountered another suitable oviposition site. In the salt marsh, no more than one egg mass was found on any single blade of S.

Fig. 6. Tabanus nigrovittatus ovipositing on the adaxial surface of a blade of Spartina alterniflora in the salt marsh.

Fig. 7. Accessory reproductive gland exudate (arrows) on eggs deposited by the smaller, typical form of *Tabanus nigrovittatus* on a clear plastic lid.

Fig. 8. SEM photograph of a tan egg, with a layer of epidermal plant tissue remaining attached, after dislodgement from a blade of *Spartina alterniflora* (210x and 2100x).

Fig. 9. SEM photograph of an intact epidermal ridge of the adaxial surface of *S. alterniflora* (630x).



alterniflora.

Deposition of a complete egg mass required a considerable amount of time. One female was timed while depositing an average sized mass of 200 eggs; the process took 40 minutes, with an average of 12 seconds per egg. Once oviposition was completed, females flew to other blades of S. alterniflora, where they rested head down on the lower half of the plant.

D Approximately one week after oviposition, the larvae hatched from the egg mass and dropped into the mud where they remain from one to two years completing their developmental cycle (Freeman 1962), then presumably emerging as adults during the following summer.

The smaller, typical form of T. nigrovittatus were rather scarce in the Wellfleet area, thus they were not seen ovipositing in the salt marsh. Several of the smaller flies were, however, seen ovipositing in the laboratory. This smaller form of T. nigrovittatus faced head down on the blades of S. alterniflora while ovipositing, while the larger, grayer form faced head up on the blades during oviposition, both in the laboratory and in the salt marsh.

Discussion and Conclusions

In this study, adult T. nigrovittatus varied in total length from 9-17 mm. This "species" deposited two distinct types of egg mass. Larger flies (12-17 mm, x = 14.8) deposited tan, shingled masses, while smaller flies (9-13 mm, x = 11.0) deposited gray, tiered masses. In New Jersey, Jacobson et al. (1981) also discovered

2 groups (forms) of *T. nigrovittatus* based on electrophoretic examination of enzymes and on total body length. Their larger group of flies had a mean total body length of 12.8 mm, while their smaller flies had a mean length of 10.5 mm; these measurements are somewhat smaller than those shown here. Typically, morphological characteristics of egg masses are rather constant among members of a given species (Bailey 1948, Jamnback and Wall 1959, Thompson et al. 1979), thus it seems likely that *T. nigrovittatus* may consist of a pair of sibling species on the marshes of the outer Cape Cod area. Accordingly, variation in larval morphology and habitat led Jamnback and Wall (1959) in New York and Freeman (1962) in New Jersey to suspect that *T. nigrovittatus* actually included two or more species. In New Jersey, the electrophoretic examination of enzymes by Jacobson et al. (1981) provided strong evidence that the two forms of this fly are actually a pair of sibling species.

While the egg masses of the two forms are morphologically distinct, there can also be some variation of certain measurements and in the number of eggs per mass within each form. In our study, the gray, tiered egg masses averaged 6.7 mm in length, 2.7 mm in width, 2.1 mm in height, and contained a mean of 156.8 eggs. These measurements were somewhat larger than those given by Magnarelli and Stoffolano (1980) who reported a mean length of 4.4 mm, a width of 2.3 mm, a height of 1.5 mm and a mean of 154.8 eggs per mass. In contrast, our measurements were smaller than those given by Jamnback and Wall (1959), who reported lengths of 7-28 mm and widths of 3-4 mm. These variations in egg mass size and in number of eggs per mass may have been due to a number of factors, including quantity of stored nutrients carried over from larval stages, the amount of vertebrate blood consumed, number of gonotrophic cycles completed (Magnarelli and Stoffolano 1980), and the substrate on which the egg masses were deposited.

Individual eggs from the gray, tiered egg masses in the present study had a mean length of 1.56 $\pm$ 0.01 mm and mean width of 0.28 $\pm$ 0.01 mm. These measurements were smaller than those given by Thompson et al. (1979) who reported a mean length of 1.67 mm and mean width of 0.29 mm. In contrast, our measurements were larger than those obtained by Magnarelli and Stoffolano (1980), who reported that fully matured oocytes had an average mean length of 1.2 $\pm$ 0.07 mm and a mean width of 0.2 $\pm$ 0.01 mm. Variation in individual egg size may be related to female size; i.e., larger females may tend to produce larger eggs.

Egg masses deposited in the laboratory by the larger form of *T. nigrovittatus* were significantly shorter than those deposited in the salt marsh, however, egg masses which were deposited in the two habitats did not differ significantly in the number of eggs per egg mass, in individual egg size, or in the width of the mass at the widest point. Masses deposited in the laboratory were shorter (with a more rectangular shape) than the masses deposited in the salt marsh, which were longer and more spindle-shaped.

In the salt marsh, females scan numerous blades of *S. alterniflora* and appear to fly great distances in search of a suitable oviposition site. In the laboratory, females have a restricted flight range and a limited selection of *S. alterniflora* blades; this could result in the choice of oviposition sites which would normally be rejected in the salt marsh. These factors may account for the observed differences in the shapes of egg masses deposited in the two habitats. In the field, no egg masses were discovered on diseased, dying or damaged blades, or on blades which already contained an egg mass. However, in the laboratory, several egg masses were often found on individual blades of *S. alterniflora* while other normal, healthy blades remained unoccupied. Again, this is likely due to the limited selection of *S. alterniflora* blades in the laboratory vs. an almost unlimited choice in the salt marsh. In the laboratory, several females may choose the same blade as an oviposition site due to any number of physical and/or chemical factors which make that blade an attractive oviposition substrate. Studies with other dipterous species showed that oviposition site selection was made through information recieved from chemoreceptors on the tarsi, the antennae, or the ovipositor (Hudson 1956, Wallis 1962, Bar-Zeev 1967, Crnjar et al. 1978).

Deposition of an egg mass occurred most frequently on the upper third of the adaxial surface of *S. alterniflora*, where the blade was from 4-6 mm in width. Jamnback and Wall (1959) also found this to be so. At this width, females were able to cling to the edges of the blades with their foretarsi, and may have obtained a more secure grip on the plants than they would have at other positions on the blades. The salt marsh is often windy enough to cause the blades of *S. alterniflora* to sway, which might possibly dislodge females ovipositing at other positions on the blades. Also, had oviposition occurred where the blade width was wider or narrower than the normal distance spanned by the legs, the females may not have been able to maintain a normal oviposition posture throughout egg laying. Egg masses deposited on the upper third of the blades are usually high enough to avoid flooding from all but the high spring tides. Consequently, deposition of egg masses at this height may prevent overexposure to salt water, although no information is available on the ability of eggs to survive flooding or excessive salt spray.

Oviposition on the adaxial rather than on the abaxial surface of S. alterniflora may prevent the egg mass from dislodgement and dessication. This surface of the blade curls in on itself (enveloping any existing egg masses) during periods of water stress (i.e., extreme high tides), preventing dessication of the blade (Godfrey, unpubl.). Therefore, egg masses deposited on the adaxial surface may benefit from the protection which this surface may offer.

The adaxial surface is composed of vascular bundles which form vertical rows of deep furrows and ridges. Females may orient themselves and their egg masses along these furrows during oviposition. These undulations are much less pronounced on the abaxial surface. Also, small papillae are present on the adaxial surface but are lacking on the abaxial surface (Anderson, 1974). The furrows and papillae-covered ridges on the adaxial surface may enhance the adhesion of the egg mass to the blade.

The smaller, typical form of T. nigrovittatus were scarce on the Wellfleet marshes, and therefore only the larger flies were observed ovipositing in the marsh. However, three of the smaller flies were seen ovipositing in the laboratory. Unlike the larger form, the smaller flies faced head-down during oviposition on *S. alterniflora* blades.

In summary, the two forms of *T. nigrovittatus* produced morphologically distinct types of egg masses. Females of the larger, grayer form produced tan, shingled egg masses, while females of the smaller, typical form produced gray, tiered egg masses. Further, females of the two forms appeared to exhibit dramatic differences in oviposition behavior. Other dipterous "species" have been subdivided into 2 or more species or subspecies on the basis of intraspecific differences in behavioral (Wagner 1944) and electrophoretic (Bedo 1977) patterns. Similarly, my results support other evidence which suggests that *T. nigrovittatus* is actually a pair of sibling species.

CHAPTER III

OVIPOSITION SITE AND OVIPOSITION ACTIVITY PATTERN OF THE SALT MARSH GREENHEAD, *TABANUS NIGROVITTATUS* MACQUART.

Introduction

The salt marsh greenhead, *Tabanus nigrovittatus* Macquart is autogenous in the first gonotrophic cycle (Anderson 1971, Magnarelli and Anderson 1977). In subsequent ovarian cycles, vertebrate blood is required for maturation of oocytes. During anautogenous ovarian cycles, females readily attack vertebrate hosts, thus becoming a severe nuisance to man and other vertebrates inhabiting coastal areas of New England (Rockel 1969, Anderson 1971, Bosler and Hansens 1974).

The specificity and selectivity for an oviposition site exhibited among species of the family Tabanidae varies widely. Some tabanid species are very general in their selection of an oviposition site, while other species show specific site preferences (Khan 1952, Tidwell and Hays 1971). Egg masses of Tabanidae have been found on a wide variety of materials. Eggs are usually deposited on plants; however, egg masses have been found on a number of inanimate objects, such as rocks, sticks, buildings, bridges and debris (Hine 1906, Marchand 1920, Roth and Lindquist 1948, Khan 1952, Tidwell and Hays 1971). Many tabanid species oviposit in aquatic/semiaquatic environments (Hays 1956), while other species are restricted to terrestrial habitats (Marchand 1920). To date, no studies have examined the problem of oviposition site preference of the salt marsh tabanid, despite its possible implications for control strategies. The first objective of this research was to determine whether *T. nigrovittatus* showed a preference for a particular oviposition site.

In general most North American Tabanidae, including *T. nigrovittatus*, are diurnally active. Schulze et al. (1975) reported *T. nigrovittatus* to be most active from 0800 to 1600 hours. It seemed probable from these observations that *T. nigrovittatus* oviposited at some time during the daylight hours. Consequently, the second objective of this research was to determine the time of day during which oviposition occurred.

Materials and Methods

Study site. Research on the oviposition behavior of *T. nigrovittatus* was conducted at a salt marsh on Great Island, a barrier spit on Cape Cod. Great Island is located in Wellfleet, Massachusetts, and is part of the Cape Cod National Seashore. The vegetation at Great Island is typical of Northeastern salt marshes. Two distinct zones are discernible; a low marsh, dominated by *Spartina alterniflora* Loisel, and a high marsh, dominated by *Spartina patens* (Ait.) Muhl. The study was conducted from June to September during 1979 and 1980.

Salt marsh oviposition site. In order to determine the oviposition site of *T. nigrovittatus*, a belt transect, 169 m long and 60 m wide, was laid out across the salt marsh from the lower limit of *S. alterniflora* to the upper limit of the *S. patens* zone. The transect was divided into three distinct zones: (1) The low marsh zone, composed mainly of the short form of *S. alterniflora*, with the tall form of this species occurring only along the drainage ditches and along the edge of the bay; this zone encompassed the first 62 m of the transect. (2) The transition zone (21 m of the transect) was made up of both the tall and the short form of *S. alterniflora*, although the tall form was the more prevalent; in this zone, *S. patens* was scattered among the *S. alterniflora*. (3) The high marsh zone (86 m of the transect) was dominated by *S. patens*, with *Distichlis spicata* (L.) occurring along the upper margins of the zone. The three major zones of this transect were scanned with field binoculars to locate ovipositing females. The number of females and their respective oviposition sites were recorded for 14 days from 1000 to 1400 hours. Observations were limited to clear, warm days since oviposition activity was infrequently observed under other weather conditions.

Selection of an oviposition site in the laboratory. Studies were conducted during the summer of 1980 to determine whether *T. nigrovittatus* females exhibit an oviposition preference for a certain species of marsh plant. Females were collected from box traps (painted flat black or royal blue) located on Wellfleet salt marshes, and were transported to the laboratory in 1 mm mesh metal screened cages (24 x 24 x 25 cm).

In the laboratory, females readily fed on warmed (34-38° C), citrated, human blood applied throughFKimwipesBplaced on top of the metal screened cages (Stoffolano 1979). After feeding to repletion, females were collected from the feeding site; 200 females were placed into each of four 1 mm mesh, wooden, screened cages (61 x 61 x 61 cm). Large sections of the various marsh plants to be tested (approximately 30 x 30 cm with 15 cm of sod) were dug out of the salt marsh, potted into plastic bags and placed into the appropriate cages. Three of the cages contained single species of grass, with one cage each for S. alterniflora, S. patens and D. spicata. The fourth cage contained a combination of all three plant species. Flies were maintained in the laboratory under ambient conditions of temperature, daylight and humidity. Throughout the course of the experiment, flies had access to granulated sucrose cubes and distilled water. After one week, the numbers of egg masses deposited on each species of plant and/or on the cage surfaces, were tabulated for each cage. The present experiment was replicated four times in order to obtain a sufficient amount of data from each of the treatments. The replications were necessary due to the extremely high mortality of caged T. nigrovittatus (approximately 3/4 of the tested flies died within one day of being transported from the field to the laboratory).

Oviposition activity pattern. The low marsh was observed for ten days with field binoculars in order to discover the oviposition activity pattern exhibited by T. nigrovittatus. From 0430 to 1930 hours, the number of females observed ovipositing during the first 45 minutes of each hourly period were tabulated. Observations were made only on clear, warm days, due to the low occurrence of oviposition activity under other weather conditions. Observations were also made throughout six nights, during each of the lunar phases.

The dates of the first and last oviposition of the season were recorded during 1979 and 1980 to obtain a rough estimate of the seasonal oviposition period.

Results

Salt marsh oviposition site. Of the 63 female *T. nigrovittatus* observed ovipositing in the salt marsh, the majority (81%) oviposited throughout the low salt marsh on the short form of S. *alterniflora* (Table 4). The remaining 19% deposited egg masses on the less extensive tall form of *S. alterniflora* which occurred in the transition zone between the the low marsh and the high marsh. During the 14 days of observation (6 hours/day), females were never observed ovipositing in the *S. patens*-dominated high marsh.

Preferred oviposition site in the laboratory. When placed in the cage containing a pure section of *S. alterniflora*, *T. nigrovittatus* females deposited 53 egg masses (98.1%) on the adaxial surface of the grass, while only 1 egg mass (1.9%) was deposited on the surface of the cage (Table 5). In the cage containing a pure section of *S. patens*, females deposited only 1 egg mass (2%) on the plants themselves, while 46 egg masses (98%) were deposited on the cage surfaces. Similarly, in the cage containing a pure section of *D.*

Table 4. Location and number of egg masses deposited by *Tabanus nigrovittatus* on three dominant species of grass in the salt marsh.*

Date (1980)	Low marsh *Spartina alterniflora* (Short form)	Transition zone Low/high marsh *Spartina alterniflora* (Tall form)	High marsh *Spartina patens* (Decumbent)
7/13	4	0	0
7/14	4	2	0
7/15	3	0	0
7/16	5	3	0
7/17	5	2	0
7/18	7	0	0
7/19	5	0	0
7/20	4	0	0
7/21	0	0	0
7/22	4	2	0
7/24	3	0	0
7/25	1	0	0
7/28	2	1	0
7/30	4	2	0
Totals	51	12	0
Percent of totals	81%	19%	0%

*Field observations were conducted between 1000 and 1600 hours on each of the recorded dates.

Table 5. Location and number of egg masses deposited by *Tabanus nigrovittatus* in three laboratory cages containing pure specimens of marsh grass.

	Number of egg masses deposited			
	On plant		On cage	
Plant species	#	%	#	%
Spartina alterniflora	53	98.1	1	1.9
Spartina patens	1	2.0	46	98.0
Distichlis spicata	1	1.8	54	98.2

Four replicates of this experiment were conducted with 200 flies per replicate; most of the egg masses were from only one of the four trials.

spicata, 1 egg mass (1.8%) was deposited on the grass, and 54 egg masses (98.2%) were deposited on the surfaces of the cage.

In the cage which contained all three species of marsh grass, females preferred to oviposit on the adaxial surface of *S. alterniflora* (Table 6). In this treatment, 49 egg masses (96%) were found on *S. alterniflora*, while 1 egg mass (2%) was found on each of the other two plant species. No egg masses were found on the cage surfaces in this treatment.

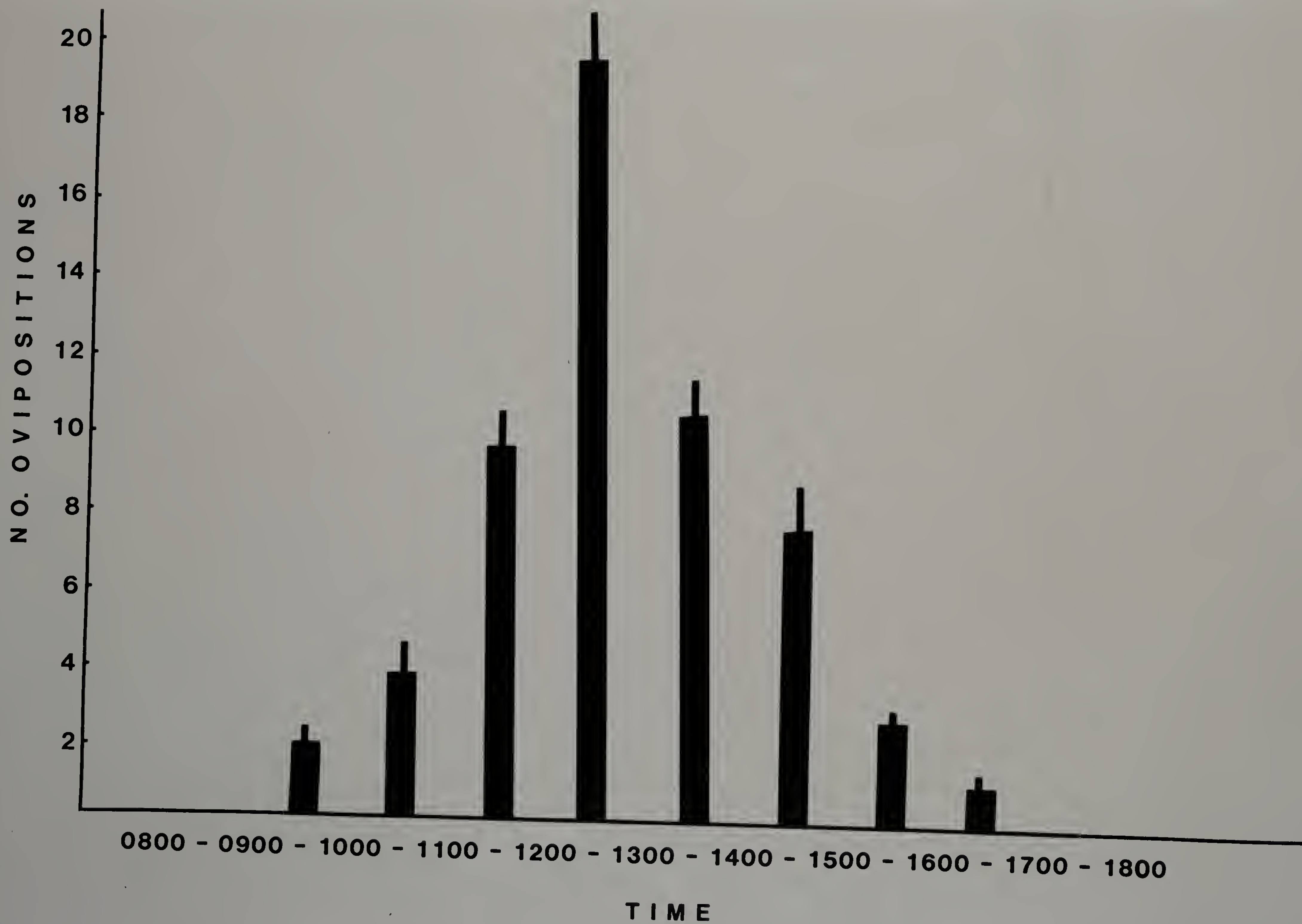
Oviposition activity pattern. In the salt marsh, *T. nigrovittatus* females oviposit between 0900 and 1700 hours with a peak of oviposition activity occurring from 1200 to 1300 hours (Fig. 9). Of the 59 females observed ovipositing, 2 females (3% of the total number of ovipositing females) initiated egg laying between 0900 and 1000 hours. Four flies (7%) were discovered ovipositing from 1000 to 1100 hours, and between 1100 and 1200 hours, 10 females (17%) were seen ovipositing. The peak period of oviposition activity came between 1200 and 1300 hours; 20 females (34%) oviposited at this time. From 1300 to 1400 hours, 11 females (19%) deposited egg masses. During the time interval of 1400 to 1500 hours, 8 flies (13%) were observed ovipositing. From 1500 to 1600 hours, 3 flies (5%) oviposited. Oviposition activity was last recorded from 1600 to 1700 hours, when one fly (2%) deposited an egg mass. No flies were seen ovipositing in the salt marsh between 1700 hours and 0900 on the following mornings. In summation, 70% of the flies which were observed ovipositing did so in the three hour period between 1100 and

Table 6. Location and number of egg masses deposited by Tabanus nigrovittatus in a single cage containing three species of marsh grass.

Plant species	Number of egg masses deposited: Number	Percent
Spartina alternaflora	49	96.0
Spartina patens	1	2.0
Distichlis spicata	1	2.0
Cage surface	0	0.0

This experiment was replicated four times with 200 flies per replicate; most of the egg masses were found in only one of the four trials.

Fig. 10. Oviposition activity pattern of <u>Tabanus nigrovittatus</u> in the salt marsh.


20
18
16
14
12
10
8
6
4
2
NO. OVIPOSITIONS
0800 - 0900 - 1000 - 1100 - 1200 - 1300 - 1400 - 1500 - 1600 - 1700 - 1800
TIME

1400 hours.

T. nigrovittatus was first sighted on the salt marsh on June 16 in 1979 and on June 22 in 1980. Oviposition activity was first noted on July 6 in 1979 and on July 9 in 1980. The last oviposition observed was on July 19 in 1979 and on July 30 in 1980. The number of adult greenhead flies dwindled to an insignificant few by August 1 in 1979 and by August 10 in 1980.

Discussion

Spartina alterniflora has been reported to be the favored oviposition site of many salt marsh tabanids. Tabanus lineola lineola F. oviposits primarily on the adaxial surface of the tips of S. alterniflora, with occasional egg masses found on Distichlis spicata (L.) (Orminati and Hansens 1974). Egg masses of Chrysops fuliginosus Wiedemann (Magnarelli and Anderson 1979a) and Chrysops atlanticus Pechuman (Magnarelli and Anderson 1979b) were also found on the adaxial surface of S. alterniflora.

The results in Chapter 2 indicate female T. nigrovittatus may choose the adaxial surface of S. alterniflora as an oviposition site due to various structural components of the blade (e.g. blade width, curvature of the adaxial surface, undulations and papillae of the adaxial surface, and blade orientation). Females most frequently oviposited on the upper third of the shorter form of S. alterniflora where the blade was 4-6 mm in width (Table 3). S. patens and D. spicata, the other dominant marsh plants tested for oviposition preference, had much narrower blades (ca. 2 mm). *T. nigrovittatus* females, being wider than 2 mm, would not fit into the adaxial curvature of these plants, which is the area where they actively search for an oviposition site and oviposit on *S. alterniflora* (cf. Chapt. 2). Since previous results (Table 1) showed that 13 field-deposited egg masses had a width measurement of 2.9 ± 0.8 mm, it is impossible for females to deposit a normal egg mass on a plant having a width of 2 mm. *S. patens* may also be rejected as an oviposition site because it exists in the decumbent form in the salt marsh; since the larger form of *T. nigrovittatus* faces head up into the sun while ovipositing on upright plants, it is possible that a plant whose blades lie parallel to the marsh surface would be rejected for an oviposition site. Similarly, oviposition preferences in the southwestern corn borer, *Diatraea grandiosella* Dyar, were attributed to leaf surface orientation; more egg masses were deposited on surfaces with vertical or diagonal orientations than on surfaces with horizontal orientations (Poston et al. 1979).

T. nigrovittatus oviposited on the shorter form of *S. alterniflora* much more frequently than on the taller form. This may have been due to the fact that the shorter form of *S. alterniflora* covers most of the area of the low salt marsh, while the taller form only occurs along the drainage ditches and along the edge of the bay.

Environmental factors have been shown to influence the physiological and behavioral processes of insects, including oviposition activities. The rhythmic periodicity of oviposition may be shortened, delayed or eliminated by environmental factors (Harries

1939). My experiments showed the oviposition activity of T. nigrovittatus to be the greatest between 1200 and 1300 hours; during this time, the average daily air temperature (25° C) and light intensity were at the highest point of the day, while daily relative humidity in the salt marsh was at the lowest point of the day (60%) (Benedict 1980). Females were not seen ovipositing on excessively cool, cloudy, windy, or rainy days. In studies on other insects, oviposition activity has been shown to be influenced by a number of factors, including temperature, humidity, wind conditions, and light intensity (Parker 1959, Henneberry and Leal 1979, Riedl and Loher 1980). In the Tabanidae, Magnarelli (unpubl.) reported that the oviposition behavior of Chrysops cincticornis Walker may be influenced by cloud cover and/or solar intensity. Under laboratory conditions of natural lighting, Thompson et al. (1979) reported oviposition activity of T. nigrovittatus on five occasions during mid-day. Deposition of an egg mass at mid-day, under conditions of high temperature and low humidity, may facilitate the drying of the accessory reproductive gland fluid which acts as an adhesive attaching the egg masses to the substrate.

Conclusions

Female T. nigrovittatus oviposit preferentially on the adaxial surface of S. alterniflora. This claim is substantiated by both field observations and laboratory choice tests. The exact information (physical and/or chemical) received by T. nigrovittatus from the blades of *S. alterniflora*, during preoviposition and oviposition, is yet to be elucidated. Receptors on the tarsae, antennae and/or ovipositor may be receiving information about the physical and/or chemical make-up of *S. alterniflora*. Failure to oviposit on other common marsh grasses (*S. patens* and *D. spicata*) appears to be due to the narrow blade width which is smaller than the width of a normal egg mass.

In this study, it was not shown whether oviposition activity is under the control of a circadian rhythm; however, environmental factors (e.g. temperature, light intensity) appear to influence the expression of oviposition behavior. More experimental data is needed to fully understand how the timing of oviposition is regulated in *T. nigrovittatus*.

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