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TITLE: Species Diversity and Diurnal Activity of Stomoxyine flies, *Stomoxys* spp. (Diptera: Muscidae) in Wang Nam Khiao District, Nakhon Ratchasima Province, Northeastern Region, Thailand

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THESIS

SPECIES DIVERSITY AND DIURNAL ACTIVITY OF STOMOXYINE FLIES, *STOMOXYS* SPP. (DIPTERA: MUSCIDAE) IN WANG NAM KHIAO DISTRICT, NAKHON RATCHASIMA PROVINCE, NORTHEASTERN REGION, THAILAND

SUPATTA KEAWRAYUP

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Supatta Keawrayup 2012: Species Diversity and Diurnal Activity of Stomoxyine flies, *Stomoxys* spp. (Diptera: Muscidae) in Wang Nam Khiao District, Nakhon Ratchasima Province, Northeastern Region, Thailand. Master of Science (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Professor Theeraphap Chareonviriphap, Ph.D. 61 pages.

A study of species diversity of *Stomoxys* spp. and diurnal variations of activity of the most abundant was performed during a 1-yr period at a local dairy cattle farm in Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand. Four species of stomoxyine flies were identified by morphological, including *Stomoxys indicus* Picard 1908, *S. calcitrans* (Linnaeus 1758), *S. sitiens* Rondani 1873 and *S. uruma* Shinonaga and Kanao 1966. The most common species were *S. indicus (50.2%)* and *S. calcitrans* (49.5%). *Stomoxys sitiens* and *S. uruma* were found in small proportions (<1%). Seasonal abundance of stomoxyine flies was determined during three different climatic seasons: cool summer rainy. The number of captured flies was significantly different among the three seasons with the greatest number in the rainy season (mean=66%, df = 2, $P < 0.05$). A major seasonal peak of abundance of *S. indicus* and *S. calcitrans* was found in this season. Statistically significant difference between the number of males and females of *S. indicus* and *S. calcitrans* ($P < 0.05$) was observed.

Diurnal activity of *S. indicus* and *S. calcitrans* during three seasons were observed from different periods of two days a month with 2 hr interval, beginning from 06.00 to 18.00 hr by using Vavoua trap. Both sexes of *S. indicus* and *S. calcitrans* showed unimodal activity pattern in cool season. Similarly in summer period, both sexes of *S. indicus* and males of *S. calcitrans* showed also unimodal activity pattern. Whereas, a bimodal activity pattern was recorded in rainy season for both sexes of *S. indicus* and males of *S. calcitrans*. For females *S. calcitrans*, activity was observed thoughtout the day during summer and rainy season. A better understanding of stomoxyine fly behavior, especially the daily flight activity, can assist in prioritization and design of appropriate vector prevention and control strategies.

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Supatta keawrayup

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LIST OF ABBREVIATIONS

S.	=	*Stomoxys*
hr	=	Hour
yr	=	Year
P	=	Probability Value

SPECIES DIVERSITY AND DIURNAL ACTIVITY OF STOMOXYINE FLIES, *STOMOXYS* SPP. (DIPTERA: MUSCIDAE) IN WANG NAM KHIAO DISTRICT, NAKHON RATCHASIMA PROVINCE, NORTHEASTERN REGION, THAILAND

INTRODUCTION

Stomoxyine flies are blood-sucking Diptera belonging to genus *Stomoxys* (Muscidae: Stomoxyinae), which contains eighteen different species in the world (Zumpt, 1973). One of these, *Stomoxys calcitrans* (Linnaeus 1758), normally referred to as “stable fly”, is the most cosmopolitan species and a significant economic pest of livestock and other warm-blooded animals in many parts of the world (Greenberg, 1971; Zumpt, 1973; Harwood and James, 1979; Mullens *et al.*, 1988; Muenworn *et al.*, 2010). Both male and female stable fly have been reported to have a wide vertebrate host range in which several species of domestic and wild vertebrate animals which appear to be common (Wall and Shearer, 1997). This cosmopolitan species is found in close proximity with large domestic mammals, especially cattle and horses. In the United Kingdom *S. calcitrans* preferred to feed primarily on cattle and horses (Warnes and Finlayson, 1987). In Egypt domestic donkeys and horses remain the most preferred vertebrate hosts (Hafez and Gamal-Eddin, 1959). Although livestock is a major blood source, humans can also be bitten by this species, which has a flight range of 1-2 km more or less (Jones *et al.*, 1991).

When under mass attract of stable fly, significant economic losses due to reduction of anticipated gross weight gain and 30-40% decrease in milk yields have been observed (Hall *et al.*, 1982; Mullens *et al.*, 1988). Campbell *et al.* (2001) reported weigh gains by grazing cattle were reduced an average of 0.20 kg per steer per day by an average of 2.79 flies per leg, representing a 19% reduction in weight gain or 7% per stable fly. In addition, stable flies have been known as mechanical or biological vectors for several pathogens such as *Anaplasma marginale* (anaplasmosis), *Trypanosoma* spp. (trypanosomosis) as well as different viruses, including bovine leucosis virus, bovine herpesvirus-2, lumpy skin disease virus (Mihok *et al.*, 1995; Torr *et al.*, 2005) and polio virus, equine anemia (Greenberg, 1971; Wall and Shearer, 1997).

Surveys of adult stomoxyine flies populations can be assessed by different techniques. Direct counts or collections from the animals, especially leg counts, to assess fly numbers appear to be one of the most common investigative techniques (McNeal and Campbell, 1981; Berry and Campbell, 1985). In addition to a direct count technique, various trapping devices have been developed to collect flies. In the United States, sticky traps (Broce trap and William trap) are commonly used for sampling stomoxyine flies (Williams, 1973; Broce, 1988). These trap devices have exploited the attractiveness of UV-reflecting Alsynite® panels. Recently, a friendlier field trap device, originally designed for tsetse fly collection in Africa, was used for stomoxyine flies collection. This "Vavoua trap", whose name is derived from the name of an African Village, has been proved to be a very efficient way to sample *Stomoxys* spp. in many African countries (Holloway and Phelps, 1991; Mihok *et al.,*1995; Mihok and Clausen, 1996), in La Reunion Island (Gilles *et al.*, 2007a) and in Thailand (Tainchum *et al.*, 2009; Muenworn *et al.*, 2010).

In Thailand 4 known species of stomoxyine flies were identified with the most common being *S. calcitrans* (Masmeatathip *et al*., 2006; Muenworn *et al.*, 2010). This species has been found in abundance in many parts of Thailand, particularly in the north and northeastern regions where dairy and beef cattle farms are most available (Sucharit and Tumrasvin, 1981). Recently, stomoxyine flies were sampled from different geographic regions of Thailand in which 3 species previously reported were found (Muenworn *et al.*, 2010). The same authors also described the diurnal activity and seasonal abundance of *S. calcitrans* from 2 different geographical settings, rural areas and national parks, and found that *S. calcitrans* were more active near livestock compared to the natural setting. Tainchum *et al.* (2009) observed gene flow and genetic differentiation between *S. calcitrans* populations from several localities throughout Thailand. Results demonstrated that there was no correlation between genetic and geographical distances and that all 9 Thai sampled populations fit closely in the same cluster taxa. A good knowledge of biological and ecological data is absolutely crucial to understand the epidemiology of pathogen transmission and to design vector control activities. To assist in improving this base of information, we studied the species diversity, the seasonal variation of density, and daily activity of stomoxyine flies in a local dairy cattle farm during a one year period of time.

OBJECTIVES

1. To observe species diversity of *Stomoxy* spp. in a dairy cattle farm, Wang Nam Khiao district, Nakhon Ratchasima Province

2. To document diurnal activity of *Stomoxys indicus* and *Stomoxy*s *calcitrans* in a dairy cattle farm, Wang Nam Khiao district, Nakhon Ratchasima Province

3. To study climatic factors affect to the number of stomoxyine flies in a dairy cattle farm, Wang Nam Khiao district, Nakhon Ratchasima Province

LITERATURE REVIEW

1. Taxonomic of Stomoxyine flies (*Stomoxys* spp.)

Taxonomic catalogue	Name
Phylum	Arthropoda
Subphylum	Mandibulata
Class	Insecta
Subclass	Pterygota
Order	Diptera
Suborder	Cyclorrhapha
Division	Schizophora
Section	Calypterate
Family	Muscidae
Subfamily	Stomoxyinae
Tribe	Stomoxini
Genus	*Stomoxys* Geoffroy 1762

Source: Zumpt (1973)

2. Biology of Stomoxyine flies (*Stomoxys* spp.)

The Genus *Stomoxys* is a member of subfamily Stomoxyinae, family Muscidae is known by several common names including: Stomoxyine flies (*Stomoxys* spp.) is similar to the house fly in size and be easily distinguished by the Piercing-sucking mouthparts in which are used to pierce the skin with a long, thin proboscis pointing forward from under the head. This is composed of the labium with short labella, the hypopharynx, and the labrum (Zumpt, 1973). Key characteristics to the Stomoxyine flies are the arista hairs found only on the dorsal side of the antenna (Zumpt, 1973). The wing venation of the *Stomoxys* fly is quite different from other Muscid flies having a slight bend upward on vein M1+2 unlike the slight curve to the house fly (Castro, 1967). The Stomoxyine flies have four longitudinal black stripes on the thorax and a checkered abdomen larger than that of the house fly (Foil and Hogsette, 1994). This genus contains at least eighteen species that are of meaningful veterinary importance as major pests of confined and pastured livestock (Zumpt, 1973). Stomoxyine flies is known by several common names, including stable fly, biting house fly, dog fly, barn fly, and power mower fly (Hall and Smith, 1986).

The "stable fly" is the common name that refers to *Stomoxys calcitrans*. It occurs in a small predation near the stables as compare to house fly. It is sometimes called the biting house fly because it may found indoors, especially in the extreme weather condition of season and could feed on human beings viciously. It is often observed along the sandy, vegetation-strewn shores of lakes and along the seashore. The stable fly is typically an outdoor feeder and it active during the day is usually to be found when large numbers of domestic animals occur, horses and cattle affording an abundant food supply. The Stable fly is the only species found in North America (Skidmore, 1985). Its introduction to the New World may have occurred during the mid 17th century (Hall and Smith, 1986). The Stable fly is a strong flier and has been observed to travel long distances (Hoffman, 1968).

2.1 Feeding Habits

Stomoxyine flies can feed on a variety of warm-blooded animals. Both sexes are blood-feeder that drawing blood quickly and feeding to full capacity in 3-4 minutes (Harwood and James 1979) and with recent studies showing as few as 2.5 min. (Schofield and Torr, 2002). Parr (1962) reported that the average blood meal (25.8 mg) is three times the average body weight (8.6 mg). Bailey and Meifert (1973) observed that the adult Stomoxyine flies usually approaches a host only to feed. The female is anautogenous, which mean she requires several blood meals to complete ovarian development. The males require at least one blood meal to produce seminal fluid and to stimulate sexual drive (Klowden, 1996). Ordinarily, the Stomoxyine flies cannot obtain a full blood meal on one host because of the defensive behavior elicited by the painful bite, thus the flies alight repeatedly on the same host or fly from one animal to animal until the meal is completed (Harwood and James, 1979). There are three phases in the Stomoxyine flies search for their host. The first one is the appetitive-searching phase, this is followed by the activation and orientation phases, when the flies encounter the chemical stimuli (kairomones) indicating the presence of a host. Attraction occurs when the insect (having located a host) begins to feed (Lehane, 1991).

Stable flies are diurnal. Holloway and Phelps (1991) found that *S. calcitrans* has a bimodal diurnal pattern of feeding, locating hosts by responding to carbon dioxide and octenol; temperature was found to be the most important weather factor. For stable flies' highest biting activity was observed to be at approximately 30° C, whereas at 14° C the flies were no longer attracted to host animals (Zumpt, 1973). They have been observed feeding at dawn and in the late afternoon under natural conditions, but can feed at any time during the daylight hours (Mitzmain, 1913). They feed on large mammals instance rats, guinea pigs, rabbits, monkeys, cattle, horses and man.(Castro, 1967) This fly can also feed on nectar for immediate energy needed for flight, but cannot successfully produce eggs when a blood meal is not readily available (Jones *et al*., 1985).

Stable flies tend to feed on the lower extremities, such as below the knees and hocks of animals but can move to the sides and back if the populations are too high on the lower extremities (Foil and Hogsette, 1994). On cattle, it will first land and rest on the back of the animal. As soon as the cattle are disturbed, the flies land on the outside of the forelegs; where it will bite the cow near the knees and begin feeding. On horses, flies prefer feeding on the sides of the neck, lower legs, and underbelly. After feeding, this fly rests nearby, usually on warm, sunny sites such as fences, walls and vegetation near the hosts. When disturbed it will fly and then return to the original spot of feeding (Bishop, 1913). Stomoxyine flies are not specific host insect. They prefer cattle, being less frequent on horses and pigs, under laboratory conditions, the flies consume blood of laboratory mammals and birds (Greenburg, 1971). After feeding, the stable fly is sluggish, and remains motionless near the host. After hatching, the larvae begin feeding on local microbial flora and fauna (Bishop, 1913).

2.2 Breeding Habits

Adults assemble on sunlit objects from which the males dart out after flying females and engage in aerial interactions (Buschman and Patterson, 1981). According to Buschman and Patterson (1981), males mount the females in the air or on the ground with copulation occurring on a perch. The same male can mate with more than one female, but females cannot be coupled with more than one male (Zumpt, 1973). Buschman and Patterson (1981) found that flies used basking stations not only for mating, but also for thermoregulation when ambient temperatures were too high or too low.

The female can begin mating 3-5 days after emerging and can begin laying eggs when 5-8 days old (Foil and Hogsette, 1994); that is if she has had available bloodmeal. The habitat of the stomoxyine flies, as suggested by their common name, are almost anywhere where horses, cattle, and other agricultural animals can be found especially inside barns and stables (Bishop, 1913). Excellent breeding in a number of habitats commonly found in agricultural area where afforded by the leftover soggy hay, alfalfa or grain in the bottoms of or underneath out-of-doors feed racks in connection with cattle feed lot, such as decaying straw, oats, rice, barley, wheat, silage, horse manure, lot manure and cow manure. These materials become soggy and ferment. The female must be engorged for reproduction. The female has a greatly extended pseudovipositor with which she deposits eggs into decaying straw where there is moisture. The stomoxyine flies does not breed in excrement under field conditions unless mixed with decaying vegetable material. The material must be humid, since dryness could prevents larval development (James and Harwood, 1969). After taking a blood meal, the female fly will seek out a suitable oviposition site and deposit eggs throughout the media. The first egg batch is mature approximately 2 days after copulation (Parr, 1962).

2.3 Life History

Stomoxyine flies have a holometabolous development; their life cycle consists of the egg, three larval instars, and the pupal and the adult stages (Ross *et al*., 1982). After taking a blood meal, and the proper time for blood meal digestion and ovarian development, the female will seek out a suitable oviposition site and deposit eggs throughout the media. The suitable temperature for oviposition is between 22°C and 28°C. The number of eggs laid by one female was low during the cold months, but rose to about 120 eggs at 25°C, and to about 200 eggs at 30°C. (Zumpt, 1973). The eggs are about 1 mm long and 0.2 mm wide, resembling a banana in shape, and have a median longitudinal groove (Harwood and James, 1979, Hall and Smith, 1986). The eggs hatch 12-24 hours after being oviposited (Foil and Hogsette, 1994). Female laid egg between 40 and 60 eggs in each gonotrophic cycle (Lysyk, 1998). The life fecundity ranges between 30 and 700 eggs. The duration of this period is affected by ambient temperature, relative humidity, and how long the egg was retained by the female.

The hatching larva forces open the operculum and emerge. Larvae hatch from the eggs in 24-48 hrs at 22◦C and 50-80% RH (Hoffman, 1968). The newly-emerged larva measures an average of 1.08 mm in length, larvae molt to the second instars in less than 24 h.; the second instars attains a length of 2.80 mm, larvae molt to third instars 1 day later, under optimal conditions (Parr, 1962).; and the third instars is 11.12 mm developing ranges from 8 day (26◦C and 80% RH) during summer months (Parr, 1962), to several months during winter (Harwood and James, 1979). Larvae are whitish-yellow, characterized by a pointed anterior with the mouth opening, and a blunt truncated posterior with the anal opening and caudal spiracles (Peterson, 1960, Skidmore, 1985). Larvae have 12 visible segments and a single mouth hook (Peterson, 1960, Zumpt, 1973). The nutritive value of the medium and temperature play large roles in the duration of the larval stages (Parr, 1962). Female stable flies oviposit batches of approximately 35 eggs in moist media (Parr, 1962, Hall and Smith, 1986). Females can deposit up to 600 eggs during their lifetime (Killough and McKinstry, 1965). Third instar larvae and pupae have been recorded overwintering in silage, pilled manure and piled grass (Berkebile *et al*., 1994).

The pupal development takes place inside the puparium, which is the hardened cuticle of the 3rd instar larva (Castro, 1967). At 21- 26◦C, the pupal stage lasts from 6 to 26 day (Parr, 1962, Harwood and James, 1979). The reddish-brown puparia are 6 to 7 mm long and barrel shaped. Adults emerge from the puparium by inflating the ptilinum, an eversible sac on the head (Ross *et al*., 1982). Once the flies emerge, the body elongates. The fly has a pale gray color and crumpled wings; and the mouthparts are bent posteriorally between the forelegs (Castro, 1967). Once an adult emerges the body elongates and turns is darker within 30 minutes, the wings expand, the proboscis folds forward, and the insect is ready to fly away (Castro, 1967).

Adult stable flies are generally gray body and can be identified by four characteristic longitudinal stripes across the thorax as well as several dark spots on top of the abdomen. On the vertex and fronts, there are three ocelli and two large compound eyes (Bishop, 1913). Broad frons at vertex measuring is about 1/2 of eye length in female and 1/3 in male (Zumpt, 1973). The total time for development of the fly was from egg laying to emergence to be 33-36 days at 21˚c (Mitzmain, 1913).

3. Distributions and Abundance

All species of *Stomoxys* are native to Old world (Skinmore, 1985). The stable fly is the only species found in North America (Skidmore, 1985). Its introduction to the New World may have occurred during the mid 17^{th} century (Hall and Smith, 1986. The stable fly is a strong flier and has been observed to travel long distances (Hoffman, 1968). A wind assisted flight maximum range of 225 km in Florida by using mark release recapture (Hogsette and Ruff, 1985). They lifetime migration radius was about 140 km. (Gersabeck and Merritt, 1985). This was based on movement of 7 km per day (Bailey *et al*., 1973). Movement of stable flies in time and space is probably a function of host activity patterns, duration of feeding and potential of the insect to fly. The flight of the stable fly is direct and swift and of long range, the fly sometimes traveling many miles (James and Harwood, 1969).When provided with an abundant host source, i.e., feedlots, 90% of marked flies were captured within 0.8 km of the release site (Gersabeck and Merritt, 1985). Scholl (1986) observed that 80% of dispersing flies recovered between two feedlots, of 0.8 km apart, were males. The information on the distribution and dispersal pattern of the stable fly should be an important step in developing a pest management strategy (Gersabeck and Merritt, 1985).

4. Problems and Economic Importance

4.1 Medical and veterinary importance

Stomoxyine flies had economically effect to humans in two different ways: disease transmission and livestock reduction. However, it has been confirmed that stable flies can serve as carriers can transmit numerous pathogenic organisms, including cutaneous leishmaniasis, anthrax, brucellosis, equine infectious anemia, and bovine diarrhea virus (Greenburg, 1971). A large number of pathogens have been recorded due to the flies interrupted feeding habits (Harwood and James, 1979). They are the intermediate host of nematode worms, including *Setaria cervi,* a parasite of cattle, and of several species of *Habronema*, stomach parasites of horses (Greenburg, 1973). The infective larvae of *Habronema microstoma* interfere with the ability of the fly to penetrate the skin with the proboscis and take a normal blood meal (Zumpt, 1973). They are mechanical transmission of *Trypanosoma brucei*, *T. rhodesiense T. gambiense* and *T. evansi* also occurs, the cause of surra. This disease is always fatal to horses and mules, often affects camels and dogs seriously and is asymptomatic in cattle (Harwood and James, 1979). Lameness in horses has also been reported to be due to the continuous stomping, and swelling; and stiff joints in other animals bitten by stable flies are common (Zumpt, 1973).Tabanids and stable flies are able to transmit a viral disease known as equine infectious anemia to equine species (Foil and Hogsette, 1994). They become involved in accidental traumatic myiasis or enteric pseudomyiasis in man.

4.2 Economic Importance

Genus *Stomoxys* is the one of the most important sources of annoyance to livestock by causing economic losses in the form of reduced feed intake and feed efficiency, resulting in reduced weight gain. Injury is brought in various ways, worry caused by the mass attack of flies, loss of blood, loss of flesh, lowered milk production, reduced vitality, and loss of pasturing time (Harwood and James, 1979). In the past two decades, stable fly has become the most important pest of pastured cattle. Campbell *et al*. (2001) and Greenburg (1971) reported that the stable fly affect the cattle industry by destroying the hides of cattle due to the holes created by the piercing of the skin for feeding. Further impact that stable flies have on cattle is when cattle seek protection from stable flies by standing in water, which results in water pollution with fecal matter, in addition to reduced foraging time. When Stable fly numbers are high, they may terminate eating and crowd in bunches which then causes heat stress and weight loss (Foil and Hogsette 1994). In the beef cattle and dairy cattle farm, fly feeding may result in a 10 to 15 % loss of beef cattle body weight when stable fly population is high and reduced yield of dairy cattle has been reported to be as high as 40 to 60 % in some cases, just as, Catangui *et al.* (1997) demonstrated that they can affect to weight gain reductions of 0.02 to 0.05 kg per day and feed efficiency reduction of 11 to 13 percent have been documented in feedlots. Several economic thresholds have been estimated. According to Steelman (1976) estimated at about 25, the number of flies that landed on an animal per day. In the United States, a single fly-to-cow ratio was enough to reduce milk production by 0.7% (Bruce and Decker, 1958). The economic threshold for stable flies attacking feeder heifers, when weight gain, feed efficiency, and cost of control with insecticides are considered, to be less than two stable flies per host leg. Total losses to the producer were estimated at $8.51 per animal with 5 stable flies per front leg, with worse feed efficiency accounting for 88% of the total loss (Campbell *et al*., 1987). Campbell *et al*. (2001) reported a 7% reduction in weight gain per stable fly on grazing yearling cattle. If steer prices averaged $1.98/kg, the loss would be valued at $33.26 per animal or 2.33 cents per fly. These costs do not include the cost of spraying the yearling cattle indicating the need for a superior stable fly management plan over spraying. Mc Neal and Campbell (1981) used an economic threshold of 5 stable flies per cow's front leg, while Catangui *et al.* (1997) established an economic threshold of 7 per cow per leg.

As a result of host's defensive behaviors, stable flies make numerous visits, biting repeatedly before obtaining a full meal. Cattle attacked by stable flies, attempts to find a position within the bunch to protect their front legs, which are the favored feeding site of the flies. Considerable energy is expended by foot stamping, tail twitching, and throwing the head toward the front legs in an effort to dislodge the flies or prevent feeding. Stable flies can reduce weight gain, milk production, and feed efficiency both from their feeding and because of the bunching behavior of the cattle, which may induce or increase heat stress and hence reduce feed intake (Weiman *et al.*, 1992). Bruce and Decker (1958) estimated the effect of stable flies on the hosts increases proportionally with an increase in the number of bites. This trend eventually reaches a plateau, due to the fact that the stable fly is only a daytime feeder.

5. Prevention and control

Control programs for stable flies are comprised of 2 affective ways: (1) reducing stable fly development (2) reducing adult stable flies. These include chemical compounds, traps, ecological modifications of the environmental conditions, utilization of biological control agents, and the Sterile Male Technique (SMT) (Zumpt, 1973). The cultural control practice of sanitation is the most important technique for on-site reduction of stable flies (Greene, 1993). Decaying organic mixed with manure and moisture provides good conditions for larval development that can be hauled off, spread thin to dry out, composted, or burned to kill the maggots (Harwood and James, 1979). However, insecticides in most cases became the first line of defense. Residual insecticides are often used on stable fly resting areas to provide control (Campbell and Hermanussen, 1971). It was therefore, not surprising to learn of the rapid development of insecticide resistance to the commercially available insecticides (Georghiou and Bowen, 1966; MacDonald *et al*., 1983). Biological controls using natural enemies reported include species of beetles and mites preying on fly eggs and larvae. Some species of pteromalid wasps were found parasitoids of stable and house flies pupae. Pteromalids lay eggs inside pupae through its ovipositor, immatures then feed on the pupae and cause death (Mullen and Durben, 2004). Geetha-Bai and Sankaran (1977) conducted a survey for natural enemies of house fly and stable fly developing in dairy manure heaps in India. They reared three species of pteromalid pupal parasites, *Spalangia cameroni* Perkins, *Spalangia endius* Walker and *Spalangia nigroaenea* Curtis and one chalcid parasite, *Dibrinus trichiophthalmus* Masi. *Spalangia cameroni*, *S. endius* and *S. nigroaenea* were the only species which parasitized both house fly and the stable fly, supporting the worldwide pattern established for these species of parasites. Harwood and James (1979) pointed out that reproductive manipulation may have some distinct possibilities because stable fly populations tend to be focal in nature, easily reared, and the female apparently mates only once.

Genetic control strategies offer an environmentally safe alternative to insecticides for insect control. One strategy, Sterile Insect Release Method (SIRM), is based on the introduction of sterilized insects into a population to suppress or eradicate by reducing population fertility. Using a parasite species can work in most situations but a survey of the habitat location is key in the developing population of the parasites (Greene *et al*., 1989). Harwood and James (1979) pointed out that reproductive manipulation (SMT) may have some distinct possibilities because stable fly populations tend to be focal in nature, easily reared, and the female apparently mates only once. Buschman and Patterson (1981) contemplate that if wild males are more successful than laboratory-reared sterile males in holding favorable waiting stations, they could account for many more mating than their numbers indicated. Petersen (1989) reported potential pathogens, predators and parasites that may control stable flies. Inundative release of sterile males as in screw worm control cannot be applied in stable flies because both sexes are blood feeders, thus releasing males will increase nuisance problems in livestock (Buschman and Patterson, 1981).

Trapping is also one of the components of pest management strategy to control stable flies. Efficacy of box traps has been greatly improved through the discovery of adhesive alsynite fiberglass sticky panel (Williams, 1973). Broce (1988) improved the original box trap design and use the cylindrical alsynite trap made from cheap and thin plastic with less adhesive required. Additional innovations include the use of volatile compound to enhance attractiveness of traps. Hoy (1969) found that malaise traps baited with CO_2 caught three times more than the unbaited ones. Cilek (1999) reported the use of dry ice, acetone, and octenal as powerful attractants for stable flies when using cylindrical alsynite traps. Other traps uses high voltage electrocutor grid that causes fly disintegration. This could be effective however has disadvantage of releasing bacteria and pathogen during the disintegration (Urban and Broce, 2000). Natural product attractant baited have not been proven sufficient to reduce biting flies in significant numbers (Ashworth and Wall, 1994).

Insecticides intended for fly control includes water soluble formulation of pyrethroids. Permethrin has been reported to show quick knockdown effect in adult stable flies (Mock and Greene, 1989). Pyrethrins have been shown to control house flies, adventages of which include negligible impact on the natural enemies (Geden *et al*., 1992). Treating animals with insecticides has also been used to control of flies (Foil and Hogsette, 1994). Insecticide residual spraying on walls, bunk and shelters also showed efficacious control (Campbell, 1993), though currently used insecticides showed short duration of efficacy. In a number of instances however, use of insecticides resulted to the development of resistance (Cilek and Greene, 1994) resulting to fewer available alternatives.

6. Trap

Williams (1973) found that a box trap containing an inverted plastic cone positioned inside a plywood box was effective in catching stomoxyine flies on sandy beaches. However, the box trap was not as effective along inland sites as other traps. Although cumbersome at times because of the eight surfaces, the Williams trap, a translucent fiberglass sticky panel, has been found to be highly effective for monitoring *Stomoxy* flies (Williams, 1973). Broce (1988) invented the now commercial cylinder design of the Alsynite plastic traps that uses a cheaper, thinner plastic with less adhesive than the old Williams traps. He found that the new trap was equal to the Williams traps in catching house flies and stable flies; however, because of its smaller surface area, the cylinder trap had a greater trapping efficiency than the Williams trap. Hogsette and Ruff (1990) supported Broce's findings and found that the cylinder trap captured fewer total flies, but more flies per cm^2 than any of the Williams traps used in their experiments. There are several visual, thermal, and chemical cues that stable flies use in search of hosts. There are several traps for stable flies which incorporate many of these cues. The use of carbon dioxide, ultraviolet light, and plexiglass were all tested as attractants with an electrocutor grid to find the most effective combination against stable flies. The electrocutor grid trap with carbon dioxide attractant has proved highly selective against *S. calcitrans* during the winter in north central Florida (Schreck *et al*., 1975). Hoy (1969) found that the Malaise traps baited with CO_2 caught 3 times as many stable flies than did either of the CO_2 and control Malaise traps. Cilek (1999) used Alsynite cylinder traps with various volatile substances, such as dry ice, acetone, and octenol, and found that CO_2 from the dry ice was a very powerful attractant for collecting stable flies. The only draw-back to these types of traps is the use of CO_2 as dry ice can be costly.

When using traps of any design, their placement is a key factor on their performance. Traps should be placed where stable flies are assembling, in a place that is easy to service, and out of the way of workers and animals to avoid being trampled or tripped on (Hogsette and Ruff, 1987). Insecticides should be used as a last measure in fly control, not only because sanitation is more permanently effective, but also because insecticide resistance can develop (Harwood and James, 1979). Cilek and Greene (1994) found that in Kansas feedlots resistant stable flies were found even where insecticide use was minimal. They thought the insecticide-resistant stable flies came from nearby feedlots not using proper management practices against biting flies. Permethrin-impregnated yarn wound around either Williams or cylindrical traps can also be used to reduce fly numbers (Hogsette and Ruff, 1996). Tseng and Hogsette (1986) found that a distance of 2.54 cm between strands and in a continuous coil, not crisscrossed, yielded maximum catch as compared to the strands being laid right next to each other. This yarn can be left in a field for about 3 months and can be placed on several traps (Foil and Hogsette, 1994). Proper sanitation, such as spreading manure out to dry, composting, and burning manure is key to reducing the number of stable flies that successfully mate and reproduce. Proper use of insecticides and traps in conjunction with reducing the breeding media can be effective in decreasing fly populations.

MATERIALS AND METHODS

1. Collection site

A local dairy cattle farm in Wang Nam Khiao district, Nakhon Ratchasima Province (14°25´6´´N, 101°51´0´E) was used for this study. The majority of Wang Nam Khiao district area is covered with organic farms near Tub Lan National Park, one of the biggest national parks in Northeastern region Thailand. Approximately 100 cows were housed in this local farm. Absolutely no insecticide has been treated to protect cows from insect bites.

Figure 1 locality where Stomoxyine flies were collected in Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand

2. Trapping method

Eight Vavoua traps (Laveissiere and Grebaut, 1990) were placed around the farm and left operational during the night before collection at 06.00 hr. Ambient air temperature and relative humidity were recorded every 2 hr throughout the period of collection.

Figure 2 The Vavoua trap used for collecting Stomoxyine flies during January to December 2010

3. Fly collections

Adult stomoxyine flies were captured with Vavoua traps every 2 hr at 06.00, 08.00, 10.00, 12.00, 14.00, 16.00 and 18.00 hr (local time), respectively. Collections were made during 2 consecutive days per month from January to December in the year 2010. Captured flies were preserved in vials, containing 95% ethanol and recorded by date and hour of capture. Specimens were subsequently brought back to the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand for the morphological identification following Zumpt (1973) with some modifications.

4. Climatic parameter

Ambient air temperature and relative humidity were recorded every 2 hr at a dairy cattle farm, Wang Nam Khiao district, Nakhon Ratchasima Province during period of the collections, whereas rainfall data was obtained from Nakhon Ratchasima Meteorological station. In this area, the year is usually divided in three different seasons: rainy was from July to October, summer was from March to June and cool was from November to February, in the year 2010. Each season was of 4 months long and the same effort of fly-collection (64 day-traps per season) the variation of apparent fly density all year long.

5. Data analysis

Captured flies were compared by a two-way analysis of variance (ANOVA). Differences among seasons on one side and day time periods on the other were performed using Fisher's least-significant difference. The accepted level of significance was determined at 5% (*P*-value < 0.05). All data were analyzed using SPSS program package (Ver 17, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Results

Species diversity

A survey of stomoxyine flies was carried out at a local dairy farm in Wang Nam Khiao district, Nakhon Ratchasima Province, Northeastern region Thailand during a 1 yr period from January to December 2010. Four species of stomoxyine flies were morphologically identification in this area, including *Stomoxys indicus* Picard 1908, *S. calcitrans* (Linnaeus, 1758), *S. sitiens* Rondani 1873 and *S. uruma* Shinonaga and Kanao 1966. A total of 3,449 flies were captured with the most abundant species being *S. indicus* with 1,731 specimens, representing 50.2% and one thousand seven hundred and seven (1,707) specimens of *S. calcitrans* representing 49.5% of the total collection. *S. sitiens* and *S. uruma* were found in small proportions (<1%), *S. sitiens* were found in a relatively low number with 8 specimens (0.2%) and *S. uruma* were found 3 specimens (0.1%), respectively. The number of *S. indicus* and *S. calcitrans* was not statistically significant difference in this study for this area ($P > 0.05$).

The results have been grouped for both sexes of *S. indicus* and *S. calcitrans*. Statistically significant difference was observed between males and females both species ($P < 0.05$). Female *S. indicus* was collected more than male as show in Table 1. While female *S. calcitrans* was collected in low number compare to males flies (Table 1).

Table 1 Stomoxyine flies collected by Vavoua traps from a dairy cattle farm, Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand (from January to December 2010)

Species	Number of flies		
	Male	Female	Total
Stomoxys indicus	556	1,175	1,731
Stomoxys calcitrans	1,193	514	1,707
Stomoxys sitiens	5	3	8
Stomoxys uruma	1	2	3
Total	1,755	1,694	3,449

Seasonal abundance

The variations of seasonal abundance were determined during 3 different climatic seasons: cool, summer and rainy. In general, flies were found to be more abundant in the rainy period of the year (Table 2). The total number of flies captured in the rainy season was statistically significant difference from other seasons ($P < 0.05$). Flies were found in rainy period 2,269 specimens representing 65.79% followed by cool period 786 specimens representing 22.79% and in summer period 397 specimens representing 11.51% (Table 2).

A total of 75.5 % of *S. indicus* were captured in rainy season (1,307), followed by those captured in summer (221) representing 12.8 %, and in cool season (203) representing 11.7 %. Similarly, the greatest number of *S. calcitrans* were captured in the rainy season (958) representing 56.1% of the captures of this species. Lower proportions were collected in the cool season (578) representing 33.9% and in summer (171) representing 10% (Table 2). The total number of *S. indicus* and *S. calcitrans* captured were statistically significant difference among three seasons ($P < 0.05$) (Table 3). During three seasons, female of *S. indicus* was collected more than male while female *S. calcitrans* was collected in low number compare to males for each season as show in Table 2. The total number of *S. indicus* and *S. calcitrans* the both male and female were statistically significant difference among three seasons ($P < 0.05$). *S. sitiens* was found all of 3 seasons in low number representing 8 specimens (<1%), in contrary *S. uruma* was found only in cool season representing 3 specimens (<1%) (Table 2).This analysis could not be made for *S. sitiens* and *S. uruma* because of the relatively low number of flies of these species captured for each season.

The climatic parameters including temperature and relatively humidity recorded at a dairy cattle farm, Wang Nam Khiao district, Nakhon Ratchasima Province, and rainfall obtained at Nakhon Ratchasima meteorological station is presented in figure 3. During the period of this field survey, climatic parameters were observed from January to December 2010, Average rainfall and relative humidity were very high in rainy season representing 252.3 mm and 71% respectively, following summer representing 72.28 mm and 62% respectively and cool representing 14.7 mm and 61.5% respectively. Whereas average temperature was high in summer (34.25°C) followed by rainy season (31.25°C) and cool season (31.25°C) as present in figure 3.

Table 2 Total number of Stomoxyine flies captured per season with Vavoua traps at Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand.

Season	*S .indicus*		*S. calcitrans*		*S. sitiens*		*S. uruma*		Total
	M*	F*	M	F	M	F	M	F	
Cool[1]	74	129	386	192	2	0	1	2	786 (22.79%)
Summer[2]	79	142	90	81	2	0	0	0	397 (11.51%)
Rainy[3]	403	904	717	241	1	3	0	0	2,269 (65.79%)

[1]: Cool season: November-February, [2]: Summer season: March-June, [3]: Rainy season: July-October

*: M: Male, F: Female

Table 3 Two-way ANOVA of total flies /seasons (cool, summer and rainy), as discriminating

Factors.	df	Sum of squares	Mean square	F	Significant
Season	2	69838.071	34919.036	17.98	< .0001
Season × SI	2	28554	14277	10.526	< .0001
Season × SC	2	11064.5	5532.25	15.551	< .0001

SI: *S. indicus*, SC: *S. calcitans*

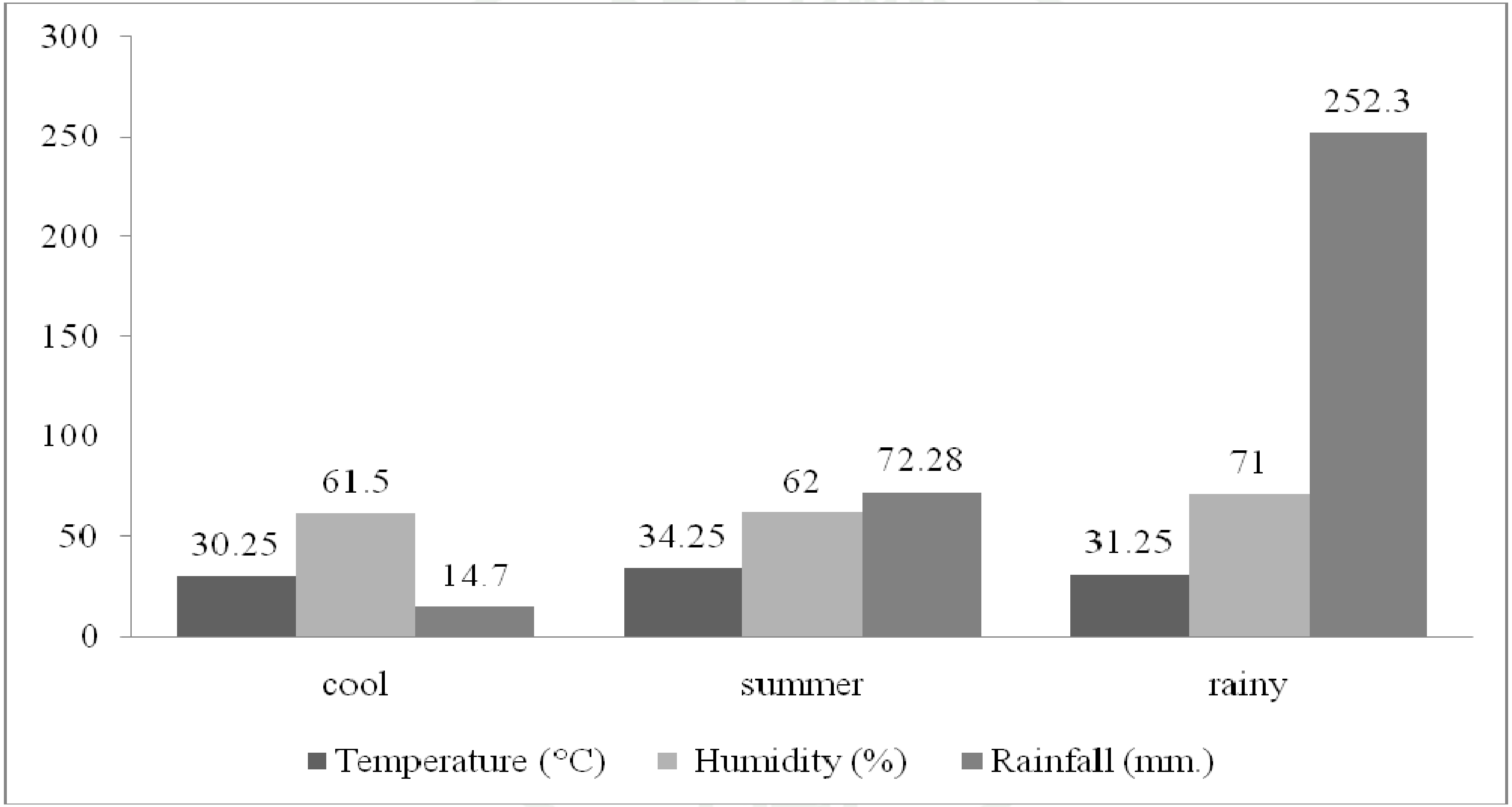

Figure 3 Monthly mean rainfall, ambient temperature and relative humidity by season in the year 2010

Monthly activity

Monthly fly collections were shown in table 5 during a 1 year period, and the total number of flies captured per species and per month was analyzed. Statistically significant differences in the total number of flies collected during the 12 months were observed ($P<0.05$) as showed in table 4. 828 flies were captured the most in August from a total of 3,449, representing 24 %. After these months the number of flies declined and showed the lowest number of flies in March in which 41 specimens of 3 species. After that, the number of stomoxyine flies increase as presented in Table 5.

S. indicus was statistically significant differences ($P<0.05$) (Table 4), during the 12 months showing peak activity in September representing 526 specimens. The number of *S. indicus* declined and showing the lowest number of flies in January and April representing 14 specimens. After April the numbers of *S. indicus* flies increase until September. The total numbers of *S. calcitrans* was statistically significant differences ($P<0.05$) (Table 4) during this study which showed a peak activity observing in August representing 396 specimens (Table 5). We collected the lowest number of *S. calcitrans* flies in March representing 9 specimens. Whereas *S.sitiens* and *S. uruma* could not be made for statistically analysis because of the relatively low number of flies of both species captured for each season. Three specimens of *S. uruma* were captured only in November (Table 5).

Monthly relative humidity ranged from 50 to 80 % with the highest in September. Monthly mean temperatures ranged from 29 to 35°C, with the highest in March. And monthly mean rainfall ranged from 4.4 to 389 mm with the highest in October. Average rainfall was 114.06 mm during periods of collection. Average temperatures were about 31.9°C and relative humidity was 64.83% throughout period of our study (Figure 4).

Table 4 Two-way ANOVA of total flies/month (January to December 2010) as discriminating

Factors.	df	Sum of squares	Mean square	*F*	Significant
Month	11	106897	9717.909	5.819	< .0001
Month × SI	11	43492.714	3953.8473	17.98	< .0001
Month × SC	11	20049.75	1822.705	6.618	< .0001

SI: *S. indicus*, SC: *S. calcitans*

Table 5 Monthly captures of Stomoxyine flies (*Stomoxys* spp.) with Vavoua traps at Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand (from January to December 2010).

Collection Site	Months	*S. indicus*	*S. calcitrans*	*S.sitiens*	*S.uruma*	Total
Wang Nam Khiao district	January	14	44	2	0	60
	February	39	189	0	0	228
	March	31	9	1	0	41
	April	14	43	1	0	58
	May	97	50	0	0	147
	June	79	69	0	0	148
	July	159	114	0	0	273
	August	430	396	2	0	828
	September	526	228	1	0	755
	October	192	220	1	0	413
	November	57	115	0	3	175
	December	93	230	0	0	323
Total		1,731	1,707	8	3	3,449

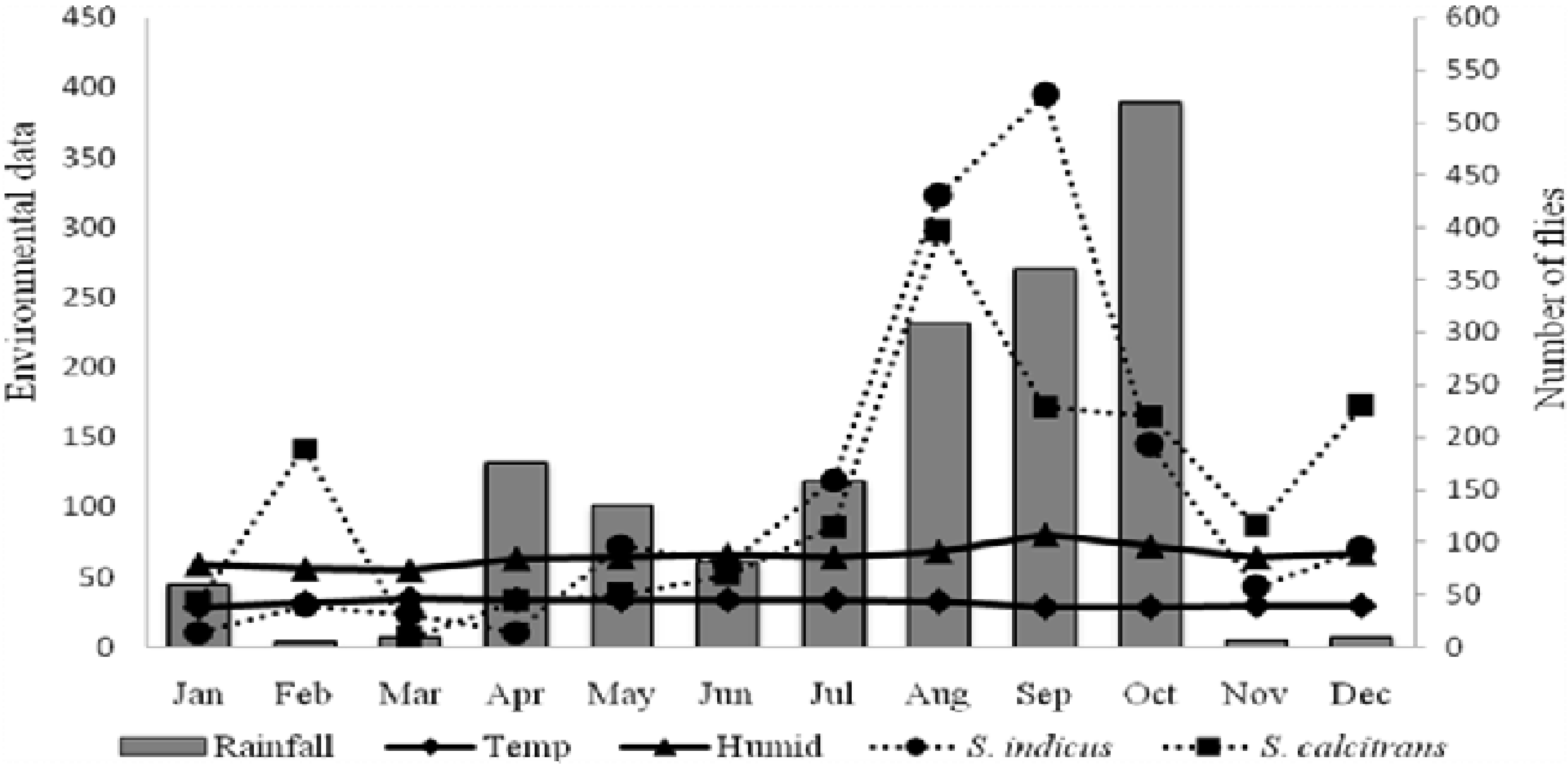

Figure 4 Monthly means ambient temperature, relative humidity and rainfall data obtained from Nakhon Ratchasima meteorological station.

Diurnal activity

Diurnal activity of Stomoxyine flies is given in figure 10 where all captures of the year have been totalized. Despite the low number of captured flies, their activity could not be easily observed among the 2 species including *S.sitiens* and *S. uruma*. Diurnal activity of *S. indicus* and *S. calcitrans* during three seasons, collected from different periods of the day with 2 hr intervals, beginning from 06.00 to18.00 hr. In cool season, peaks of activity were observed for *S. indicus* the both male and female, with the most activity in evening (16.00 to18.00 hr) (Figure 5, A-1). In contrast, a main peak of activity of male and female of *S. calcitrans* occurred in the afternoon (12.00 to14.00 hr) (Figure 5, A-2). For summer period, the both sexes of *S. indicus* were pronounced peak of activity in the morning before 06.00 hr (Figure 6, B-1). Male of *S. calcitrans* was observed the peak activity in the morning (06.00 to 08.00 hr) but female of this species showed activity all along the day (Figure 6, B-2). During rainy season, two peak of activity of *S. indicus*, the both male and female were observed with the most prominent activity right before sunset (16.00 to18.00 hr), followed by a less pronounced peak after sunrise (06.00 to 08.00 hr) (Figure 7, C-1). Male of *S. calcitrans* was also observed two peak activities with the most activity in afternoon (12.00 to14.00 hr.) and a less one in the morning (06.00 to 08.00 hr), while female showed activity throughout the day(Figure 7, C-2). Statistically significant difference ($P<0.05$) in the numbers of *S. indicus* and *S. calcitrans* was found during different periods of the day and the numbers of male and female of both species during different periods of the day were also statistically significant difference ($P< 0.05$) among three season.

Average temperature and relative humidity were recorded every 2 hr. In cool season, average temperature during the day ranged from 24 to 36 °C, with very low in the morning and very high in the afternoon. However, relative humidity ranged from 48 to 79 %, with very high in morning and evening (Figure 5). While in summer period, temperature was very high during the day, maximum temperature was recorded on the afternoon and ranged from 26 to 39 °C. Relative humidity ranged from 48 to 86 %, with maximum relative humidity in the morning (Figure 6). During rainy season, average temperature ranged from 26 to 34 °C, with the lowest average temperature in the morning and the highest in the afternoon. Whereas, relative humidity ranged from 64 to 86 % during the day and the highest was recorded in the morning and the evening (Figure 7).

Table 6 Two-way ANOVA of species/season (cool, summer, rainy) and time as discriminating

Factors.	df	Sum of squares	Mean square	*F*	Significat
Time× Season	2	69838.071	34919.036	17.98	< .0001
(Time× Season) × mSI	12	3918.929	326.577	3.054	0.002
(Time× Season) × fmSI	12	9631.833	802.653	1.28	0.253
(Time× Season) × mSC	12	1841.286	153.44	0.545	0.876
(Time× Season) × fmSC	12	531.81	44.317	2.081	0.031

mSI: Male *S. indicus*, fmSI: female *S.indicus*
mSC: male *S. calcitrans*, fmSC: female *S.calcitrans*

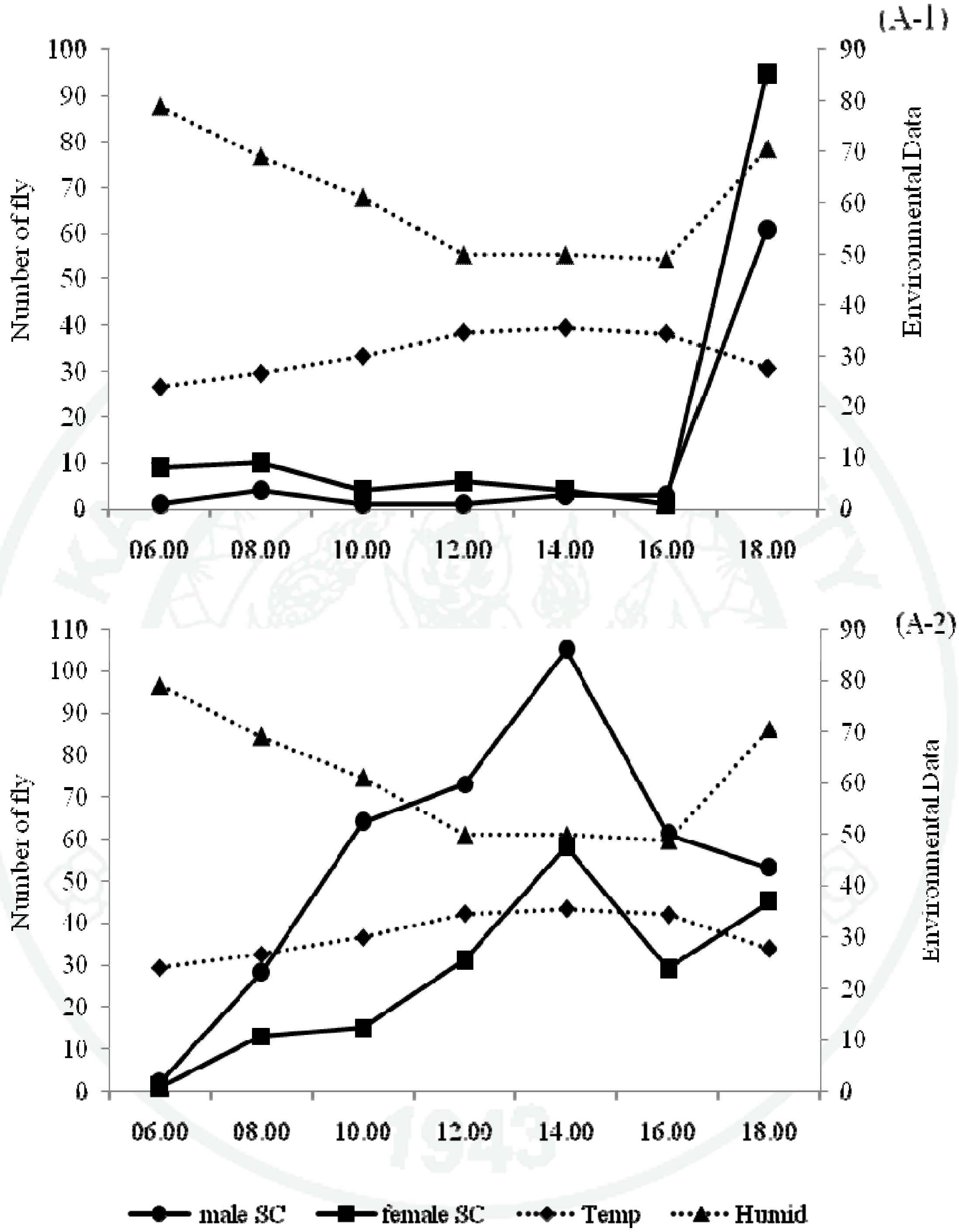

Figure 5 Diurnal variations of activity of *Stomoxys indicus* (A-1) and *S. calcitrans* (A-2), collected during summer with Vavoua traps at a Dairy Cattle Farm in Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand.

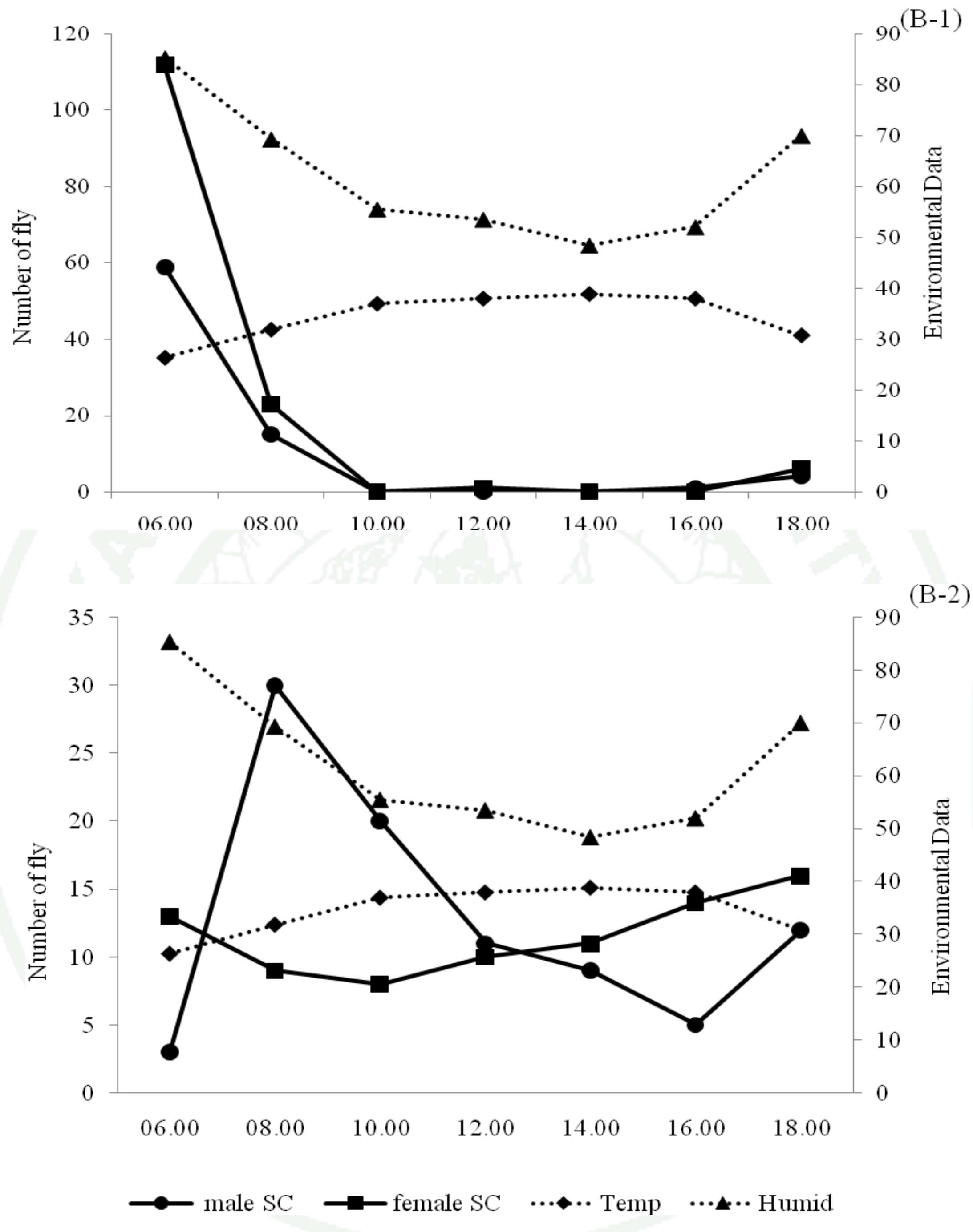

Figure 6 Diurnal variations of activity of *Stomoxys indicus* (B-1) and *S. calcitrans* (B-2), collected during summer with Vavoua traps at a Dairy Cattle Farm in Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand.

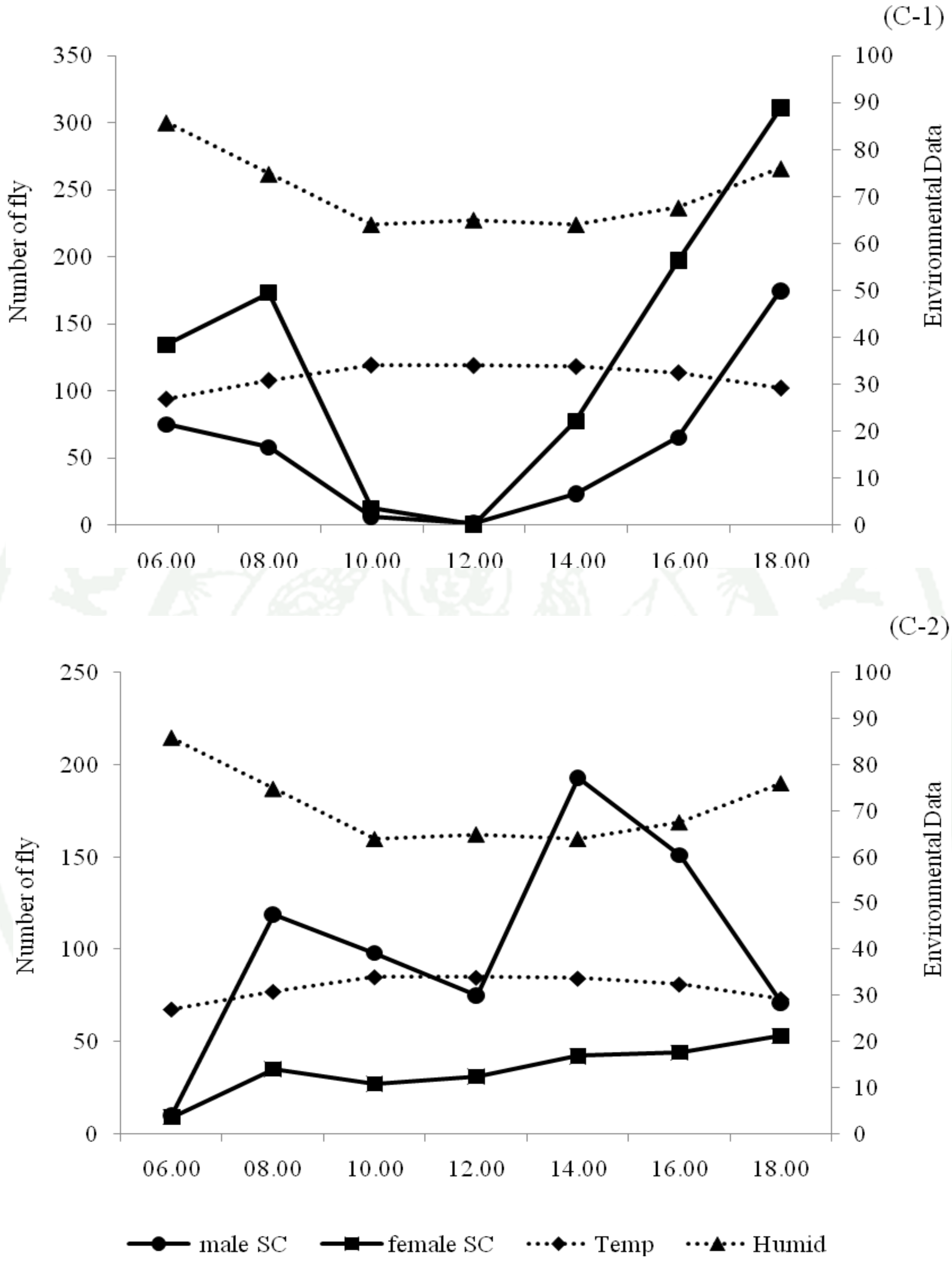

Figure 7 Diurnal variations of activity of *Stomoxys indicus* (C-1) and *S. calcitrans* (C-2), collected during rainy season with Vavoua traps at a Dairy Cattle Farm in Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand.

Discussion

Species diversity

Few studies of stomoxyine flies have been conducted in Southeast Asian countries, especially Thailand (Sucharit and Tumrasvin, 1981; Masmeatathip *et al.,* 2006; Muenworn *et al*., 2010; Tainchum *et al*., 2010). Most studies of stomoxyine flies have been well documented from Africa, the United States and France (Zumpt, 1973; Jones *et al*., 1991; Gilles *et al*., 2007a, 2007b, 2008; Mavoungou *et al*., 2008; Dsouli *et al*., 2011a, 2011b). Recently, 4 species of stomoxyine flies were identified from Thailand in which the prevailing species in the rural setting area is *S. calcitrans* (Masmeatathip *et al*., 2006; Muenworn *et al*., 2010).

During our survey, four species of stomoxyine flies were identified, including *S. indicus, S. calcitrans, S. sitiens* and *S. uruma*. Two species of *S. indicus* and *S. calcitrans* were found to be the most abundant in this local farm. There was no significant difference in the total numbers of *S. indicus* and *S. calcitrans* ($P > 0.05$).

S. indicus is considered as the most common *Stomoxys* species in the Oriental region (Zumpt, 1973). It has been recorded from many countries, from India and Sri Lanka in the West to Samoa Island in the East, and this species is commonly found in cattle barns (Zumpt, 1973). In Thailand, *S. indicus* was first reported by Masmeatathip *et al.* (2006) from Nakhonpathom Province. In their work on the phylogeny of *Stomoxys* flies, Dsouli *et al*. (2011a) have shown that *S. indicus* could be the oldest species of *Stomoxys*, indicating that this genus could have originated in the Oriental region.

In contrast, *S. calcitrans* is native to the Old world and known as a cosmopolitan species, commonly found in many areas in tropical and temperate zones. This species is regarded as a synanthropic fly which followed human beings during their peregrinations everywhere in the world (Zumpt, 1973).

S. sitiens has been recorded from many places in the Ethiopian region ranging from the Gambia to Egypt all the way to South Africa, but this species is very rare in collections. It occurs also in the Oriental region from India to the Philippines, but the material is as rare as that from Africa (Zumpt, 1973). And *S. uruma* has been reported from the Iriomote and Ishigaki Islands, Ryukyus, Hong Kong and some specimens from India, Vietnam, Taiwan, and Thailand (Zumpt, 1973).

For the sexes, statistically significant difference was observed for *S. indicus* and *S. calcitrans* this could be explained by a different behavior of both sexes.

Monthly activity and seasonal abundance

For the study of seasonal abundance, the results showed statistically different numbers ($P<0.05$) between seasons. The greatest number of flies was captured during the rainy season while their number during the summer and cool seasons were not different. The high number of stomoxyine flies collected in this local dairy cattle farm is the consequence of appropriate environmental conditions, i.e. moisture, light intensity, rainfall, and temperature to maintain suitable breeding habitats. In the USA a single seasonal peak of density for *S. calcitrans* was observed during the summer season, whereas marked bimodal and trimodal peaks have been documented in other locations (Mullens & Meyer, 1987; Lysyk, 1998).

In Thailand, former observations showed that a peak of density of *S. calcitrans* was during the rainy season (Masmeatathip *et al.*, 2006; Muenvorn *et al.*, 2010). In our study, the greatest number of adult stomoxyine flies was captured during the rainy season as well. A major seasonal peak of abundance of *S. indicus* and *S. calcitrans* was found in this season. A minor peak has been observed in February, probably due to unusual important rainfalls in January 2010. The summer and cool seasons showed lower numbers of flies; that could be explained by the very low rainfalls and high temperature, which are unsuitable conditions for larval development (Zumpt, 1973). It should have been useful to extend such a survey on a 14 month period for a better explanation of the variations of fly density throughout the year. The differences observed in sex-ratios of our captures of *S. calcitrans* and *S. indicus* all along the year require further studies.

Diurnal activity

The diurnal variations of activity were observed among different period times (06.00 to 18.00) during three seasons. The patterns of activity between the most abundant species were quite different. For *S. indicus* the peak activity was effect by season, unimodal feeding activity was showed in the evening in cool season and summer in early morning. On the order hand, bimodal activity in rainy season was recorded in the evening and another one in the morning. The greatest number of *S. indicus* was found in the morning and the evening that our results confirm the crepuscular activity of *S. indicus* already indicated by Zumpt (1973), who wrote that those flies are more active in the evening and they are readily collected by using light-traps set in cow-sheds.

For *S. calcitrans*, the patterns during all seasons indicate diurnal variations of activity. For males, this pattern is unimodal in cool season (peak in the afternoon) and summer season (peak in the morning), but bimodal in rainy season. For females *S. calcitrans*, a more or less constant activity was observed all along the day during all seasons. Many authors who have worked on the activity of stomoxyine flies focused only on S. *calcitrans*. Bimodal feeding activity patterns for *S. calcitrans* were reported by Mitzmain (1913), Simmonds (1944), Labrecque *et al.* (1975), Kunz & Monty (1976), and Charlwood & Lopes (1980). In contrast, Coaker & Passmore (1958) and Harley (1965) observed unimodal feeding activity patterns on daily feeding in Uganda. In Thailand Masmethathip *et al*. (2006) reported that *S. indicus* showed the highest activity at sunset and dawn; in the same experiment, *S. calcitrans* showed an activity all through the day with a peak between 08.00 am to 10.00 am. Muenworn *et al.* (2010) observed a peak of flight activity of males *S. calcitrans* at 10.00 and 16.00 hr, whereas females showed an increase of activity all along the day until 16.00 h. Berry & Campbell (1985) found that the pattern of daily activity of *S. calcitrans* was affected by temperature, humidity, and the level of solar radiation. In our study *S. calcitrans* had the highest activity when temperatures range from 30 to

35°C. This finding is the same as Hafez & Gamal-Eddin (1959), who worked on diurnal rhythm and seasonal variation, and reported that highest biting activity occurred about 30°c.

CONCLUSION

Our study confirmed that *S. indicus* and *S. calcitrans* are the most abundant species of *Stomoxys* in a local dairy local farm in Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand. *S. indicus* appears in Asia as a vicariant species of *S. niger*, which is abundant in farms in Africa, along with the cosmopolitan *S. calcitrans*. It showed also that both species had their seasonal peak of abundance during the rainy season (August - September). And that their daily variations of activity were different during the seasons. Those results let us propose that, in this environment, control methods should be implemented in summer season to limit the development of their populations at the beginning of next rainy season. A better knowledge of larval breeding sites should help to control at the same time adult and larval stages.

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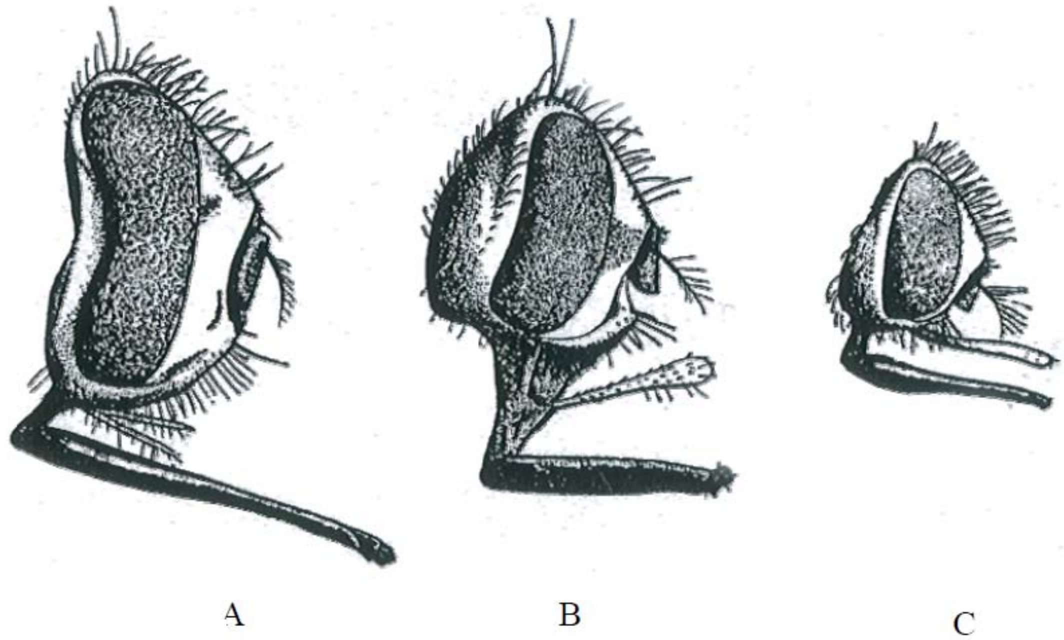
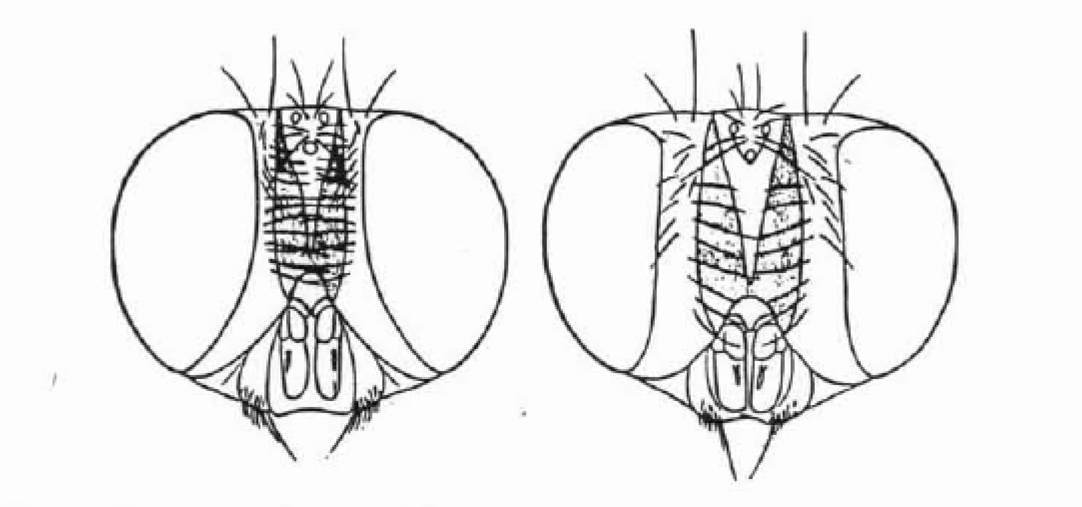
APPENDIX

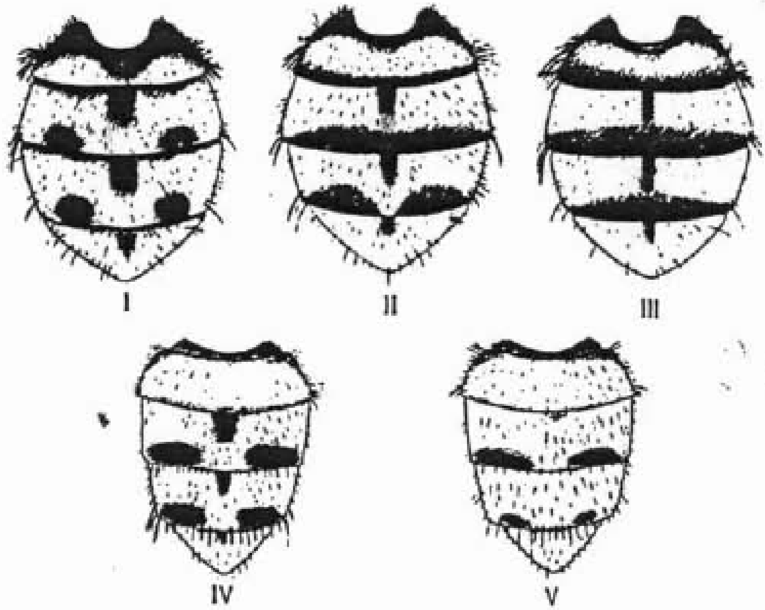


Appendix Figure 1 Head of A) *Stomoxys calcitrans*, B) *Haematobosca stimulans*, (C) *Haematobia irritans*



A B

Appendix Figure 2 Head of *Stomoxys* spp. (A) Male and (B) Female



Appendix Figure 3 Abdominal pattern of (I) *Stomoxys calcitrans* (L.), (II) *S. niger niger* (Mcq.), (III) *S. indicus* Pic., (IV) *S. sitiens* Rond., and (V) *S. nigra bilimeata* Grunberg

Appendix Table 1 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on January 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	23	78		1				2			3
06.00-08.00	24	66	1				1	1			3
08.00-10.00	26	64		2							2
10.00-12.00	35	38	5	2							7
12.00-14.00	32	47	24	4							28
14.00-16.00	34	44	1	2							3
16.00-18.00	26.4	74	1	1	2		6	4			14
Total			32	12	2	0	7	7	0	0	60

*M = male, F = female.

Appendix Table 2 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on February 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	26	72									0
06.00-08.00	30	68	17	8			3	9			37
08.00-10.00	32	56	24	3			1	1			29
10.00-12.00	36	45	45	17			1	4			67
12.00-14.00	40	42	17	12			3	4			36
14.00-16.00	37	44	17	9			3	1			30
16.00-18.00	32	62	9	11			3	6			29
Total			129	60			14	25			228

*M = male, F = female

Appendix Table 3 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on March 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	25.5	85		2			6	15			23
06.00-08.00	29	71		1	1		2	7			11
08.00-10.00	38.2	56									
10.00-12.00	40.5	43	1								1
12.00-14.00	39.5	38									
14.00-16.00	41	36									
16.00-18.00	33	59	2	3				1			6
Total			3	6	1		8	23		0	41

*M = male, F = female.

Appendix Table 4 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on April 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	25.5	84	1	2	1		5	5			14
06.00-08.00	33.5	63	4	2			1				7
08.00-10.00	36	52	3	2							5
10.00-12.00	37	56	3	2							5
12.00-14.00	37	54.5	4	5							9
14.00-16.00	38	54.5	4	9							13
16.00-18.00	29.5	73.5	1	1			1	2			5
Total			20	23	1	0	7	7	0	0	58

*M = male, F = female.

Appendix Table 5 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on May 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	27	88	2	5			25	56			88
06.00-08.00	32	74	12	2			6	9			29
08.00-10.00	36	62	10	4							14
10.00-12.00	38	56	0	4				1			5
12.00-14.00	39	50	1	1							2
14.00-16.00	37	55	0	1							1
16.00-18.00	32	65	1	7							8
Total			26	24	0	0	31	66	0	0	147

*M = male, F = female.

Appendix Table 6 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on June 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	27.5	84	0	4			23	36			63
06.00-08.00	33	69	14	4			6	7			31
08.00-10.00	38	52	7	2			0	0			9
10.00-12.00	37	59	7	4			0	0			11
12.00-14.00	40	51	4	5			0	0			9
14.00-16.00	36	63	1	4			1	0			6
16.00-18.00	28.5	83	8	5			3	3			19
Total			41	28	0	0	33	46	0	0	148

*M = male, F = female.

Appendix Table 7 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on July 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	27	88		4			45	74			123
06.00-08.00	32	74	6	3			1	18			28
08.00-10.00	36	62	12	2				1			15
10.00-12.00	38	56	16	10							26
12.00-14.00	39	50	15	8			1				24
14.00-16.00	37	55	6	5				1			12
16.00-18.00	32	65	20	7			4	14			45
Total			75	39	0	0	51	108	0	0	273

*M = male, F = female

Appendix Table 8 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on August 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	27	88	2				11	22			35
06.00-08.00	29	74	58	14		1	32	67			172
08.00-10.00	37	62	47	9		1	4	5			66
10.00-12.00	39	56	38	15			1				54
12.00-14.00	37	60	74	6			1	1			82
14.00-16.00	34	68	85	9			36	79			209
16.00-18.00	31	70	25	14			52	119			210
Total			329	67	0	2	137	293	0	0	828

*M = male, F = femal

Appendix Table 9 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on September 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	27	87	4	5			9	24			42
06.00-08.00	34.5	74	38	12		1	16	72			139
08.00-10.00	33.5	62	26	13			1	4			44
10.00-12.00	28	80	4	3							7
12.00-14.00	26.75	83	47	12			21	76			156
14.00-16.00	26.5	84	21	9			29	115			174
16.00-18.00	26.25	89	14	20			51	108			193
Total			154	74	0	1	127	399	0	0	755

*M = male, F = female

Appendix Table 10 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on October 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	26.5	80	4				10	14			28
06.00-08.00	28	77	17	6			9	16			48
08.00-10.00	29.5	70	13	3			1	2			19
10.00-12.00	30.5	68.5	17	3							20
12.00-14.00	32	63.5	57	16							73
14.00-16.00	32	63.5	39	21				2			62
16.00-18.00	27.5	81	12	12	1		68	70			163
Total			159	61	0	0	88	104	0	0	413

*M = male, F = female

Appendix Table 11 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on November 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	24	84									0
06.00-08.00	26	70	4	1							5
08.00-10.00	30.5	63	14	3						2	19
10.00-12.00	34	54	9	6							15
12.00-14.00	35	53	16	11							27
14.00-16.00	33.5	55	10	7							17
16.00-18.00	25.5	67	16	18			17	40	1		92
Total			69	46	0	0	17	40	1	2	175

*M = male, F = female

Appendix Table 12 Number of stable flies collected at Wang Nam Khiao district, Nakhon Ratchasima Province on December 2010.

Period time	Temp.	RH	*Stomoxys* spp.								Total
			S.calcitrans		*S. sitiens*		*S. indicus*		*S. uruma*		
			M*	F*	M	F	M	F	M	F	
18.00-06.00	23.5	82	2				1	7			10
06.00-08.00	26.5	72	6	4							10
08.00-10.00	31	61	26	7				3			36
10.00-12.00	33	62	14	6				2			22
12.00-14.00	34.5	57	48	31							79
14.00-16.00	32.5	52	33	11							42
16.00-18.00	26.5	79	27	15			35	45			124
Total			156	74	0	0	36	57	0	0	323

*M = male, F = female

CURRICULUM VITAE

NAME	: Ms. Supatta Keawrayup		
BIRTH DATE	: December 10, 1984		
BIRTH PLACE	: Phranakhon Si Ayutthaya, Thailand		
EDUCATION	: **YEAR**	**INSTITUTE**	**DEGREE**
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