An Investigative Study on the Effects of Black fly (Diptera: Simuliidae) Sugar Meals on Reproductive Success and Parasite Transmission

By

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ABSTRACT

Black flies are opportunistic sugar-feeders. They take sugar meals from Homopteran honeydew secretions or plant nectars, depending on availability. Homopteran honeydew secretions contain both simple and complex carbohydrates while plant nectars contain primarily simple carbohydrates. In order to determine whether honeydew secretions offer more energy than plant nectars to their insect visitors a study of wild-caught black flies was undertaken in Algonquin Provincial Park, Canada during the spring of 1998 and 1999. It was hypothesized that female black flies maintained on honeydew sugars will survive longer, produce more eggs and have a greater parasite vectoring potential than those maintained on artificial nectar or distilled water. Results demonstrated that: (1) host-seeking female *Prosimulim fuscum/mixtum* and *Simulium venustum* maintained on artificial honeydew did not survive longer than those maintained on artificial nectar when fed *ad libitum*; (2) fully engorged *S. venustum* and *Simulium rugglesi* maintained on artificial honeydew did not produce more eggs than those maintained on artificial nectar when fed *ad libitum*; and (3) *S. rugglesi* did not have a greater vectoring potential of *Leucocytozoon simondi* when maintained on artificial honeydew as opposed to artificial nectar when fed *ad libitum*. However, all flies maintained on the two sugars (artificial honeydew and artificial nectar) survived longer, produce more eggs and had greater vectoring potential than those maintained on distilled water alone.

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INTRODUCTION

Black flies are found in the order Diptera and form the family Simuliidae. They are particularly abundant in the north temperate and sub-arctic zones, but many species occur in the subtropics and tropics as well.

The world fauna is comprised of fewer than 2000 described black fly species with North America, north of Mexico, containing 188 species that have been described and 72 species that are known but not described (Adler & McCreadie 1997). However, it is suspected that the total number of species in North America exceeds 300. Approximately 10% of the North American species do not require a blood-meal for egg production (i.e., autogenous species) and are incapable of cutting skin. The other 90% that do require a blood-meal to produce eggs (i.e., anautogenous species) are made up of 33% that are ornithophilic and 67% that are mammalophilic (Adler & McCreadie 1997). Of the mammalophilic species only 10% take blood from humans.

This need for blood as a nutrient source has caused enormous economic losses for many facets of human activity, such as forestry, agriculture and tourism (Adler & McCreadie 1997; Fredeen 1977; Fallis 1964). Historically, black flies have been known as nuisance pests for humans as well as livestock and sometimes severe allergic reactions to bites occur (Fredeen 1977). In addition to being nuisance pests, black flies transmit serious infections and diseases, such as human onchocerciasis (river blindness), bovine onchocerciasis, several arboviruses and avian leucocytozoonosis, all of which can potentially be fatal.

BLACK FLY BIOLOGY

Life History

The life cycle of the black fly is comprised of both aquatic and aerial terrestrial stages. The eggs, larvae and pupae (immature stages) are aquatic and the adults are aerial or terrestrial. The immature stages are found in moving water (rivers, streams, mountain trickles) of various velocities. Eggs are deposited by the female on the water surface or along edges of various substrates such as tall grasses, rocks, and rotting debris. The shape and the size of the mature eggs vary slightly among species (Crosskey 1990; Chutter 1970; Davies & Peterson 1956). The eggs can hatch within 3 to 7 days depending on the species. However, in temperate regions eggs that are laid late in the summer usually do not hatch until the following spring.

Larvae

Larvae (5-7 mm in length) anchor themselves to the substrate by pressing their posterior abdominal hooks into a pad of salivary gland silk (Adler & McCreadie 1997; Barr 1984; Crosskey 1990; Davies & Peterson 1956). The larvae are thus able to remain in an area of optimal flow for oxygen acquisition and filter-feeding. Once anchored, the larvae filter feed using labral head fan rays to remove plankton and tiny particles of organic matter from the water column (Currie & Craig 1987). Depending on the species and the environmental conditions black fly larvae can sometimes be extremely abundant. At lake outlet locations, where there are high levels of nutrients available, there are much

higher densities of larvae than at other locations throughout the stream (Carlsson *et al.* 1977; Colbo 1982). The larval stage is very important for storing accumulated nutrients because both the pupae and the newly emerged adults rely on these reserves for their energy requirements.

Black fly larvae pass through a series of six or seven instars, depending on the species, before they pupate (Crosskey 1990). Under favourable conditions the larval period may require only 7 to 12 days but, this period may be extended to weeks or even months. Larvae of some species actually over-winter developing into pupae early in the following spring.

Pupae

The final larval instar constructs an immobile slipper or cone-shaped pupal cocoon, using salivary gland silk. The cocoon is firmly attached to natural substrates (such as trailing grasses, rocks or sticks) or artificial substrates (concrete slabs, cement walls or even plastic surfaces) (Davies & Peterson 1956). The pupae have respiratory filaments, which are quite consistent in form and number from one species to the next (Adler & McCreadie1997; Currie & Craig 1987) and are useful taxonomically. The pupal stage can take from 2 days to 4 weeks depending on water temperature.

Adult

The adults emerge from the pupal exuvium, by splitting the pupal cuticle along the eclosion line and then floating to the water surface in a bubble of trapped air (Davies

et al. 1962). The newly emerged fly will then find a resting spot, usually along the waterbed, where it will tan and harden (Adler & McCreadie 1997). Once the adults have hardened they must mate and fulfill nutritional requirements. The males generally emerge first and form aerial mating swarms or ground aggregations at or near the emergence site, depending on the species (Adler & McCreadie 1997; Crosskey 1990; Davies & Peterson 1956). Mating occurs when females are caught by males. Both male and female adult black flies must acquire carbohydrate sources for flight energy and to increase longevity (Davies 1952; Magnarelli & Burger 1984). However, it is only the adult females that require both a carbohydrate source and a blood meal.

Nutrition

Carbohydrate Requirements

Adult black flies use carbohydrates for flight energy. Male black flies must fly in order to create mating swarms, whereas female black flies must fly in order to find: (1) male mating swarms (Anderson 1987); (2) blood hosts for protein (Brenner & Cupp 1980); and (3) oviposition sites (Brenner & Cupp 1980; Crosskey 1990). Since these activities are energetically costly, adults require sources of carbohydrates. Furthermore, if female black flies are unable to sugar-feed, their potential as vectors of parasites to wildlife, domestic animals and humans will be affected.

Until recently it was thought that simuliids sugar-fed only on floral nectar (Davies & Peterson 1956; Hocking 1953). In 1997 studies by Burgin & Hunter (1997a, 1997b, 1997c) were published demonstrating that black flies consume honeydew, a non-floral

carbohydrate source. Honeydew feeding has also been reported in other dipterans including sandflies (MacVicker *et al.* 1990; Wallbanks *et al.* 1991), deer flies (Janzen & Hunter 1998) and horse flies (Hunter & Ossowski 1999), indicating that there are two important sources of carbohydrate energy used by biting flies: floral nectar and homopteran honeydew.

Nectar

Flowering plants produce nectar and store it in their nectaries. After secretion evaporation may occur, resulting in concentrated nectar sources. Alternatively, nectar may absorb large quantities of water and become dilute. Therefore, nectar concentration is variable depending on weather conditions.

There are many substances in nectars such as sugars, amino acids, lipids, proteins, antioxidants, alkaloids, vitamins, organic acids, minerals, dextrins, allantoin and allantoic acids (Bernardello 1999; Magnarelli 1978; Magnarelli 1977). The most common sugars found in nectars are sucrose, fructose and glucose (Appendix I). However, occasionally galactose, mannose, rhamose, maltose, melibiose, raffinose, and other oligosaccharides are found in extrafloral nectar (Wackers 2001) (Appendix III). These sugar compositions and concentrations vary greatly in nectars from one plant species to the next (Bernardello 1999; Barnes *et al.* 1995; Van Handel *et al.* 1972; Baskin & Bliss 1969), but the relative proportions of the different sugars appear to remain relatively constant for any one species.

Honeydew

Honeydew is a syrupy liquid that is excreted by insects of the order Homoptera. This order includes aphids (Aphididae and Adelgidae), gall coccids (Kermidae), white flies (Aleyrodidae) and leaf hoppers (Cicadellidae). The Homoptera have a worldwide distribution and may be present at all levels of a forest canopy (Downes & Dahlem 1987). Honeydew has been found in localized patches directly below groups of actively feeding Homopterans and on the surface of leaves in the form of concentrated balls (Tarczynski *et al.* 1992; Byrne and Miller 1990). However, honeydew does not occur evenly over vegetation. It is dispersed from areas of concentration by both wind and precipitation.

Homopteran honeydew contains many substances such as sugars, 9 - 23 amino acids, citric acid, malic acid, ducitol, plant growth hormones and trace amounts of proteins (Auclair 1963). Composition of honeydew varies depending on the species of Homoptera, as well as the species of host plant involved (Ewart and Metcalf, 1956; Auclair, 1963; Wackers 2001). Carbohydrates are thought to make up 80% or more of the total weight of freshly excreted honeydew. This percentage is a reflection of as many as 13 different sugars in freshly excreted honeydew (Ewart & Metcalf 1956; MacVicker *et al.* 1990). The percentage composition of these carbohydrates does not change over time (Byrne & Miller 1990). The most common carbohydrates are fructose, glucose, sucrose and an oligosaccharide melezitose (Auclair 1963; Wackers 2001). Trehalulose (a disaccharide) and stachyose (a tetrasaccharide) have also been found in some honeydews (Byrne & Miller 1990; Tarczynski *et al.* 1992; Wackers 2001) (Appendix II; Appendix III). Melezitose and trehalulose are apparently unique to honeydew. Stachyose, on the

other hand, has been found in the storage organs of some plants (e.g., root of beets) and has also been implicated as a transport sugar. Normally, it is not found in nectar, with the possible exception of some orchids (Byrne & Miller 1990).

Melezitose and stachyose are indicator sugars in chromatography studies to show ingestion of honeydew. Trehalulose, on the other hand, cannot be used as a honeydew indicator sugar as it is not commercially available.

Carbohydrate Use by Black Flies

The black fly crop serves as the storage organ for ingested sugars and has no known digestive functions. Carbohydrates are ingested in the same way as blood, but instead of going directly to the midgut, carbohydrates are directed along the crop duct to the crop for storage prior to digestion (Crosskey 1990). The midgut is involved in the breakdown and the digestion of these sugars (Downes & Dahlem 1987). The carbohydrate meal is transferred to the midgut, from the crop, where it is digested with the aid of invertase, a sugar-converting enzyme (Crosskey 1990). Magnarelli and Anderson (1981) suggested that there were two strategies used by biting flies to access carbohydrates for energy metabolism. The first was to ingest a large quantity of sugars and slowly release these from the crop to the midgut when energy was required. The second was to ingest frequent small amounts of sugars for immediate metabolism. It is probable that both of these strategies are used to some degree by black flies.

Few studies have examined the carbohydrate digestion rates in black flies. Cupp and Collins (1979) estimated the carbohydrate digestion rates of *S. ochraceum*. Using the

cold anthrone reagent, fructose was detected 2 days after sugar feeding to repletion in 40% of laboratory maintained flies. Nothing is known regarding the digestion of other sugars, specifically the complex carbohydrates found in honeydew.

Flight

Flight Activity

Black flies are relatively strong fliers that can travel substantial distances, as evidenced by the fact that they can be found more than 20 miles from the closest waterway (Baldwin *et al.* 1975). In warmer temperate and tropical environments black flies are usually blood sucking and as a result are more active fliers than those in harsher environments (Crosskey 1990). Experiments, done under laboratory conditions, have shown that flies fed sugar meals have both longer flight duration and flight distance compared to those not fed sugar (Hocking 1953). Flight duration and distance affect a fly's ability to locate blood hosts as well as suitable oviposition sites.

Flight Range & Parasite Transmission

The epizootiology and extent of spread of diseases transmitted by black flies and the geographical distribution of the flies will be affected by their flight range. Bennett (1963) found *S. rugglesi*, a vector of avian leucocytozoosis, moved 2 - 6 miles. This suggests that infections can be transmitted quite substantial distances.

Feeding

Blood Feeding Behaviour

Autogenous black flies, those that do not feed on blood, are able to develop their eggs without the proteins from a blood meal. These flies use the nutrients which they acquired as larvae in their aquatic environments and from carbohydrate meals consumed as adults. On the other hand, females of anautogenous (haematophagous) black flies, in addition to sugar feeding, need to take a blood meal to fill reproductive requirements. These flies need the proteins from a vertebrate host to develop at least one of their egg batches. Haematophagous black flies differ in their choice of vertebrate hosts. The two main types of blood feeding, based on vertebrate hosts, are: (1) ornithophily, feeding on the blood of birds (Bennett 1960); and (2) mammalophily, feeding on the blood of mammals, which includes anthropophilic flies that feed on humans (Anderson 1987; Crosskey 1990; Davies & Peterson 1956). In some cases, haematophagous flies will feed only on a specific vertebrate host (i.e., they are specialists). However, there are many cases where flies are indiscriminate (i.e., generalists) and will feed on any host (Davies & Peterson 1956). Biting activity differs from species to species and is influenced by light intensity, temperature, vegetation effects (shade, light and temperature), and by the colour and odour of the vertebrate host (Crosskey 1990; Davies & Gyorkos 1990; Davies & Peterson 1956). Anderson & DeFoliart (1961) reported little effect of humidity on the blood feeding behaviour of ornithophilic species, although they speculated that climatic influences on blood feeding may depend on factors associated with individual species.

Ornithophilic feeding behaviour is prevalent in some black fly groups, and in particular the genus *Simulium*. Markedly ornithophilic species in North America include *S. meridionale*, *S. rugglesi*, *S. euryadminiculum* and *S. jenningsi* (Anderson & DeFoliart 1961; Bennett 1960; Crosskey 1990; Tarshis 1972). Females of these species have a tarsal claw that appears adapted for moving through or grasping the feathers of their avian hosts. The majority of these species are not specific to one bird species although *S. euryadminiculum* is documented as being host-specific on the common loon, *Gavia immer*. *Simulium rugglesi* which feeds primarily on waterfowl is not as specific in its host choice. Similarly, *S. jenningsi* and *S. meridionale* although known mainly from turkeys are not host-specific (Anderson & DeFoliart 1961).

Mammalophilic feeding is well understood from examination of domesticated mammals (primarily livestock) and wild trapped animals (Anderson & DeFoliart 1961; Fallis 1964; Hunter *et al.* 1993). It is problematic, however, to obtain information on wild mammals as blood engorged black flies in nature are difficult to find. Approximately 10% of simuliids found feeding on mammals will feed on humans as well (Crosskey 1990; Fallis 1964). Humans are an attractive host to some species of black flies in Ontario (e.g., *S. venustum* and *S. truncatum, and Prosimulim fuscum/mixim*). Mammalophilic species have simple claws that do not have a basal tooth.

Blood Ingestion & Digestion

Once a black fly female has found its host, it probes the host's skin until it has found a suitable place to begin gorging on blood. Black flies are considered pool feeders,

since their mandibles create a small wound in the host's skin allowing the fly to ingest blood that has welled into the wound. Once blood sucking begins, it is usually continuous until the fly has become satiated with blood. The length of time taken by flies to fully engorge and the volume of blood ingested during engorgement is variable (Anderson & DeFoliart 1961; Bennett 1960; Fallis 1964; Tarshis 1972). In mammalophilic and anthropophilic flies, it takes approximately 3 - 6 minutes to reach engorgement (Anderson & DeFoliart 1961). It is not clear whether engorgement times differ between ornithophilic and mammalophilic species. However, some ornithophilic flies are known to remain on a bird for up to 4 hours (Bennett 1960), with the proportion of time spent feeding unknown. Engorged flies more or less double their original weight (Bennett 1963).

Black fly saliva contains anticoagulant and agglutinating substances (Yang & Davies 1974). So while the flies are ingesting the blood there is no clotting until the blood reaches the midgut. Once the ingested blood reaches the midgut it quickly forms a semi-solid mass. This blood mass is then surrounded by a viscous secretion referred to as the peritrophic membrane (PM) (Yang & Davies 1977). Trypsin, which is stimulated by blood-feeding, is secreted by the cells of the midgut wall and is the primary enzyme involved in digestion of the blood (Yang & Davies 1968). Digestion starts 6 - 12 hours post engorgement. The PM is broken down during the digestion process and shrinks as it becomes digested along with the blood (Yang & Davies 1977). The time taken to completely digest the blood meal appears to vary with temperature. Yang & Davies (1977) demonstrated that several temperate North American species (e.g., *S. venustum*, *S. rugglesi*) require 120-180 hours for complete blood digestion. This digestion is closely

linked to the ovarian cycle and maturation of eggs.

Reproduction

Nutrient Source for Egg Development

Davies & Gyorkos (1990) outlined the basic sources of nutrients used by female black flies to develop eggs. Autogenous flies, that do not need to blood feed, may display obligate autogeny where only nutrients accumulated in the larval stage and sugar meals are used in development of eggs. Anautogenous flies may display either facultative autogeny or obligate autogeny depending on the species. Facultative autogeny is where the nutrients accumulated in the immature stages are used in the first ovarian cycle only, with the subsequent egg batches requiring both blood and sugar meals. Obligate anautogeny is where the egg development nutrients are derived from blood meals.

Egg Maturation

Five physiological steps in egg maturation are outlined in Crosskey (1990): (1) differentiation of oocyte and nurse cells; (2) follicle formation; (3) oocyte enlargement; (4) yolk deposition; and (5) formation of the egg wall. These stages are consecutive and the early and late periods in each stage are distinguishable. For example, when eggs are in the early stages of maturation they will occupy only a small part of the follicle. When the final stage is complete, the eggs fill the entire follicle, the egg wall is formed, and the shape of the egg becomes distinct.

Anautogenous black flies sometimes emerge with the earliest stages of oogenesis already accomplished. However, oogenesis can not proceed further until nutrients have been ingested. Normally the source is only a blood meal, although laboratory experiments show that feeding on sugar often promotes further egg maturation. Once a nutrient source has been obtained the rate of egg maturation is dependent on the amount of available nutrients as well as the ambient temperatures (Davies & Gyorkos 1990). However, all of the eggs in one batch usually mature at the same time. In most species, after an uninterrupted blood meal, egg maturation is completed in 5 to 8 days (Davies & Gyorkos 1990; Davies & Peterson 1956).

The number of eggs that each female matures varies among both species and individuals within species. However, there has been little attempt to examine factors contributing to this variability (Chutter 1970). Most species produce an average of 200-500 eggs per female. The size and shape of these eggs also vary among species, but they are similar among individuals within a species (Davies & Peterson 1956). The eggs of *Prosimulium* tend to be larger and narrower than those of *Simulium*, which have a definite triangular shape.

Number of Ovarian Cycles

Once the eggs have reached maturity, in both autogenous and anautogenous flies, the female locates an oviposition site and deposits her eggs. The period of time for a female to mature and lay a batch of eggs is referred to as the gonotrophic cycle. This cycle can vary from species to species. Anautogenous black flies can successfully

oviposit at least two egg batches. However, the number of gonotrophic cycles that a black fly can complete will obviously depend on longevity. The number of gonotrophic cycles is very important for species which vector parasites (e.g., *S. rugglesi*, *S. damnosum*) since at minimum two blood meals are required to acquire and then transmit the parasite. If these species complete enough gonotrophic cycles to accommodate parasite lifecycles then they are a potential disease threat to both humans and animals (particularly livestock).

Longevity

Life-span is a significant aspect of black fly biology since it affects the number of blood meals and gonotrophic cycles a black fly can complete. Natural longevity looks at the time a black fly can survive in the face of lethal natural factors such as accidents, predation, and parasitism (Crosskey 1990). Unfortunately, a fly's natural longevity is nearly impossible to measure. For this reason, longevity is usually inferred through laboratory experiments.

Longevity experiments have demonstrated that the life span of blood sucking black fly species is within 10-35 days. Davies (1952) was able to maintain individual *S. venustum* for 20 – 63 days in the laboratory, and the ornithophilic species *S. rugglesi* up to 28 days (Davies 1952). Bennett (1963) found a 4 – 6 day lapse between two successive blood-meals or, by inference, between one oviposition and the next. Such experiments suggest that female black flies have a natural longevity of approximately 2 or 3 weeks in both temperate and tropical faunas (Crosskey 1990; Davies 1952). This period

allows the flies to complete 2 gonotrophic cycles, each ending with oviposition.

Longevity & Sugar Feeding

There is a positive correlation between sugar feeding and survival of adults of bloodsucking black flies (Hunter 1977). The energy obtained from carbohydrates extends the flies' natural longevity. Without a carbohydrate meal flies may perish from before they can lay eggs even once. This, in turn, would decrease the proportion of the female population likely to survive beyond each oviposition. Many studies have shown importance of carbohydrates to the survival of other dipterans such as mosquitoes (Bowen 1992; Magnarelli 1977; Magnarelli 1978); sandflies (MacVicker *et al.* 1990 66; Wallbanks *et al.* 1991); stable flies (Jones *et al.* 1992); deer flies (Magnarelli & Burger 1984; Janzen and Hunter 1998) and horse flies (Hunter and Ossowski 1999).

Longevity & Parasite Transmission

Little has been published on the life expectancy of parasite-infected black flies. Basanez *et al.* (1996) reported that black fly mortality rates are affected by age and parasite induced effects. Under laboratory conditions the mean survival times of *S. ochraceum, S. guianense* and *S. damnosum* decreased as parasite uptake increased although differences were not statistically significant.

Some research has been done with other haemaphagous Diptera, such as sandflies and mosquitoes. El Sawaf *et al.* (1994) examined the effect of *Leishmania* sp. on the longevity of sandflies. Female sandflies that were infected with their natural parasite

showed the highest mortality rates on the seventh day post infection. The female sandflies with mixed infections all died by the eighth day post infection with 90% of them dying by the fifth day post infection. In both cases, infected sandflies died earlier than non infected ones. These data demonstrated that longevity is substantially decreased in *Leishmania* infected sandflies.

Research on mosquito longevity demonstrates that mortality is dependent on the species of parasite and apparently species of mosquito. *Anopheles stephensi* infected with *Plasmodium yoelii nigeriensis* showed substantially higher mortality (33%) than uninfected mosquitoes (Hogg & Hurd 1995). However, Hogg & Hurd (1997) were unable to detect mortality resulting from natural *Plasmodium falciparum* infections in *Anopheles gambiae* mosquitoes. The majority of the other field studies of mortality also found no evidence of increased mortality in infected *Anopheles* (Chege and Beier 1990; Gamage-Mendis *et al.* 1993; Robert *et al.* 1990). However, two field studies suggested that there was increased mortality in larger mosquitoes due to greater *P. falciparum* oocyst burdens (Lyimo & Koella 1992).

Fecundity

The reproductive system of a female black fly consists of a pair of ovaries, each with 20 to 600 ovarioles depending on the species (Davies & Peterson 1956). Each ovariole can produces only one ripe egg at a time. The fecundity of a black fly female is defined as the number of eggs successfully matured from each ovariole and oviposited. The usual method of assessing fecundity is to count the number of mature eggs in the

ovaries of gravid flies and is referred to as actual fecundity. A black fly's potential fecundity is fixed by the number of ovarioles. If all ovarioles are productive, a full batch of eggs can be matured and laid. Actual fecundity will then be almost identical to potential fecundity. However, this is not always the case as there are many physiological and environmental factors involved (Mokry 1980). There is usually a reduction in actual fecundity from one gonotrophic cycle to the next. Some ovarioles may become degenerate and relict eggs may also remain in the reproductive tract. There is also a difference in actual fecundity between autogenous and anautogenous cycles, as some ovarioles may remain dormant until a blood meal is taken (Chutter 1970).

Other factors thought to influence fecundity in black flies are: (1) fly size (Anderson 1987; Baba 1992; Mokry 1980) (2) access to sugar meals (Hunter 1977; Mokry 1980); (3) blood-meal diet (Crosskey 1990; Davies & Peterson 1956); (4) parasite infections (Ham & Banya 1984; Yang & Davies 1977); and (5) environmental conditions (Crosskey 1990). However, only a few authors have attempted to quantify the effects of these factors on the numbers of eggs produced.

Fecundity & Body Size

In many insects, the fecundity of individual females is positively correlated with body size, where large specimens normally produce more eggs than small ones in a given species. However, there has been little research done on the relationship between fecundity and body size in black flies. In a few studies which have examined size-related fecundity in black flies, the wing lengths of gravid flies have been the best criterion to

measure adult fly size (Chutter 1970; Colbo 1982). Chutter (1970) found that oocyte counts were high in large flies and low in small flies. There was a significant correlation between size and oocyte count in both 12h old and more than 36h old *S. vittatum* females. There was also a difference in the number of eggs that were matured at emergence. Smaller *S. vittatum* matured smaller proportions of the oocytes at emergence than did large *S. vittatum* (Chutter 1970). Colbo (1982) also found that larger flies (wing length > 2 mm) lay about 200 - 300 eggs and flies below this size almost always fewer than 200 eggs (often about 150). Baba (1992) showed for *Simulium kawamurae* that fecundity increased linearly as a function of body size.

Fecundity & Blood-Feeding

The quantity and source of blood perhaps have an important bearing on fecundity but this is one of the least studied aspects of Simuliidae reproduction. Mokry (1980) found that *P. mixtum* females of a given size produced more eggs when fed on bird blood than when fed on human blood. Large blood meals probably result in higher fecundity than small blood meals, but the relationship between the amount of blood taken in and the number of eggs produced has still to be properly studied.

Fecundity & Sugar-Feeding

It has been commonly thought that blood and nectar meals serve different functions, where carbohydrates provide flight energy and prolong life and blood meals provide nutrients needed for egg maturation (Davies & Peterson 1956). This is generally true for black flies but for certain autogenous species carbohydrates are also known to

enhance fecundity (Anderson 1987; Hunter 1977). Hunter (1977) found that autogenous *S. ornatipes* in Australia required carbohydrate nutrient to initiate oogenesis. Researchers in Canada also found that sucrose-fed females of other autogenous species produced greater numbers of eggs than those fed only water. Flies that were fed 10% sucrose solution produced more eggs than those fed on distilled water alone. Mokry's (1980) results are in agreement with previous studies that showed lack of carbohydrates resulted in a significantly decreased number of mature oocytes and greatly reduced female survival over 5 days. Carbohydrate feeding is also thought to enhance fecundity in certain species of mosquitoes, and is necessary for facultative autogenous egg development in some arctic species of mosquitoes (Anderson 1987; Lavoipierre 1961).

Fecundity & Parasite Infections

Black flies that fed on Leucocytozoon infected vertebrate hosts produced fewer eggs than females that had fed on non-infected hosts (Anderson 1987). It is thought that the parasites use the nutrients derived from the process of oocyte maturation for their own development (Ham & Banya 1984; Hogg *et al.*1997; Hogg & Hurd 1997; Jahan & Hurd 1997; Renshaw & Hurd 1994). Fecundity appeared to be lowest when higher parasite infection rates were present. Yang and Davies (1977) observed that microsporidian infections in both *P. decemarticulatum* and *S. rugglesi* increased the time required for blood digestion, and affected both the gonotrophic cycle and overall egg production.

Other Diptera that fed on infected hosts have also been found to have reduced fecundity. Work on *Leishmania*-infected sandflies showed that infections significantly

depressed the mean number of eggs that were laid per female (El Sawaf *et al.* 1994). Malaria parasites have also been shown to reduce fecundity of anopheline and aedine mosquitoes (Briegel 1990; Carwardine & Hurd 1997; Hogg *et al.* 1997; Hogg & Hurd 1995, 1997). *Plasmodium yoelii nigeriensis* infections decrease fecundity in *Anopheles stephensi* regardless of infection intensity (Carwardin & Hurd 1997; Hogg & Hurd 1995) and *Plasmodium falciparum* infections substantially decrease fecundity in *Anopheles gambiae* (Hogg & Hurd 1997).

Black Flies: Importance to Man

Black flies are of high importance to humans for economic and medical reasons (Fredeen 1977). Economically, black flies are of importance with respect to their marked effect on domesticated animals. Certain Simuliidae species occur in enormous numbers at times, leaving bites that cause allergic reactions. These reactions may be very severe, causing death to cattle, horses and other domestic animals (Crosskey 1990; Fredeen 1977). Simuliidae are also known vectors of blood parasites and protozoan parasites in birds (Anderson 1956; Bennett & Fallis 1960; Clarke 1935; Desser *et al.* 1975; Desser & Rychman 1976; Desser *et al.* 1978; Fallis *et al.* 1956; Fallis & Bennett 1958; Fallis *et al.* 1951; Khan & Fallis 1970; Khan & Fallis 1971; Noblet *et al.* 1972; Wong & Desser 1981). The most important of these diseases is the blood parasite, *Leucocytozoon*. This parasite causes great economic loss in domestic birds (chickens, turkeys and ducks).

Of medical importance Simuliidae are known vectors of human pathogens in the tropics. The most serious of these diseases is the filarial disease onchocerciasis

(*Onchocerca volvulus*), which results in blindness (Nelson 1991). In addition, some people have severe allergic reactions to relatively few bites. Aside from both economic and medical reasons, black flies are generally important to man due to their nuisance factor. Even when black flies are not biting they can be very annoying by getting into the eyes, ears and nostrils of both humans and their animals.

Parasite Transmission

Longevity and fecundity of bloodsucking Diptera play an important role in their vectoring ability. Flies need to live long enough and complete enough gonotrophic cycles in order for the parasites to complete their cycle. The majority of parasites transmitted by black flies complete their life cycle inside the fly during the maturation of an egg batch (Crosskey 1990). Once the eggs are matured and oviposited the parasites are ready to be injected into their next host. The main parasites which black flies transfer are protozoan parasites particularly *Leucocytozoon*, trypanosomes and filarial nematodes specifically those in the genus *Onchocerca*.

Family Leucocytozoidae

Both wild and domesticated birds are commonly infected with the blood sporozoan from the family Leucocytozoidae. Leucocytozoidae is closely related to the genus *Plasmodium* that contains the malarial parasites of humans. *Leucocytozoon* is widespread in distribution, wherever populations of bird hosts and simuliid vectors overlap. *Leucocytozoon* infections are not uniformly distributed in nature and can cause high mortality to the young of birds. Over a 13 year period at the Seney National Refuge

in northern Michigan mortality caused by *Leucocytozoon* in goslings 2 to 7 weeks old ranged from 16 to 87% (Fredeen 1977). Some of the most common species of *Leucocytozoon* (and the birds that they infect) are: *L. bonasae* (grouse) (Fallis & Bennett 1958), *L. danilewskyi* (owls) (Fallis and Desser, 1977), *L. dubreuili* (robins) (Wong & Desser 1981), *L. fringillarum* (sparrows) (Khan & Fallis 1970), *L. simondi* (ducks and geese) (Desser & Rychman 1976; Fallis *et al.* 1956) and *L. smithi* (turkeys) (Noblet *et al.* 1972), and *L. tawaki* (penguins) (Allison *et al.* 1978). Of particular interest are *L. simondi* infections which are most prevalent in waterfowl.

Leucocytozoon simondi Mathis and Leger, 1910

Leucocytozoon simondi is characterized by its sporogony which occurs in ornithophilic simuliid flies. The most important of these simuliids is *Simulium rugglesi* Nicholson & Mickel (Shewell 1955). The majority of *S. rugglesi* adults are found concentrated over the shores of lakes and marshes in late spring and early summer and they feed predominantly on ducks and geese. *Simulium rugglesi* are the primary vector of *L. simondi*. Some of the birds that *L. simondi* has been found in are mallards (*Anas platyrhynchos*), black ducks (*Anas rubripes*), and Canadian geese (*Branta canadensis maxima*) as well as domestic geese (*Anser domesticus*) and ducks particularly white Pekin ducks (*Anas bochas*) (Desser & Rychman 1976; Desser *et al.* 1978; Khan & Fallis 1968).

Leucocytozoon simondi Lifecycle

The life cycle of *L. simondi* is quite complicated with two main phases: (1) schizogony which is an asexual multiplication of the parasite in the avian host; and (2)

sporogony which is a sexual phase in the simuliid host (Figure 1).

(1) Schizogony in the Avian Host (Desser 1967)

Once the sporozoites of *L. simondi* are introduced into the blood of the avian host they penetrate the hepatic parenchymal cells (soft spongy tissue of the liver and sometimes the kidneys). In these cells the sporozoites transform into intracellular hepatic schizonts on day 2 post infection. The schizonts produce small merozoites, between day 5 and 6 post infection, and they either invade red blood cells and develop into round gametocytes, become engulfed by macrophage cells forming megaloschizonts, or repeat schizogony in the liver to produce more merozoites. The round gametocytes are the reproductive gametocytes that the flies ingest. Round gametocytes are present in the bird's blood in highest numbers between day 6 and day 12 post infection. Around day 7 or 8 the megaloschizonts rupture releasing merozoites that develop into elongate gametocytes. By day 13 there are more elongate gametocytes than round gametocytes circulating in the blood of the avian host. The rupturing of the megaloschizonts and the development of elongate gametocytes is the cause of high mortality rates in young infected ducklings. The peak parasitemias are observed on day 6 to day 12 post infection. Therefore, *S. rugglesi* will pick up the highest infections on or between days 10 and 12 post infection when there are greater numbers of round gametocytes than elongate gametocytes in the bird blood.

(2) Sporogony in the simuliid Vector (Desser 1967)

The simuliid vector, in this case *S. rugglesi*, ingests the round microgametocytes

and macrogametocytes while biting the avian host. Once ingested these gametocytes continue further development in the fly's mid-gut. The microgametocytes exflagellate and fertilize the macrogametocytes, which then develop into rounded zygotes. These zygotes then elongate to form motile ookinetes that penetrate the mid-gut wall and embed themselves onto its outer surface. The ookinetes grow into large spherical oocysts that are bathed by haemolymph. The parasite then multiplies inside the capsule of each oocyst by fission, producing slender sporozoites. When the sporozoites have reached maturity the oocyst wall breaks open releasing them into the haemocoel. The sporozoites migrate to the salivary glands. Release of the sporozoites into the haemocoel marks the end of sporogony and the beginning of the potential infectivity to the next bird host. When the infective black fly feeds on its next host, sporozoites pass out of the fly with the saliva into the pool-feeding wound. From here the sporozoites enter the host's peripheral blood capillaries. It takes approximately 4- 7 days for sporogony in the fly, from gametocyte intake to the appearance of sporozoites in the salivary glands, hence longevity of the black fly is of paramount importance to the life cycle of *Leucocytozoon*.

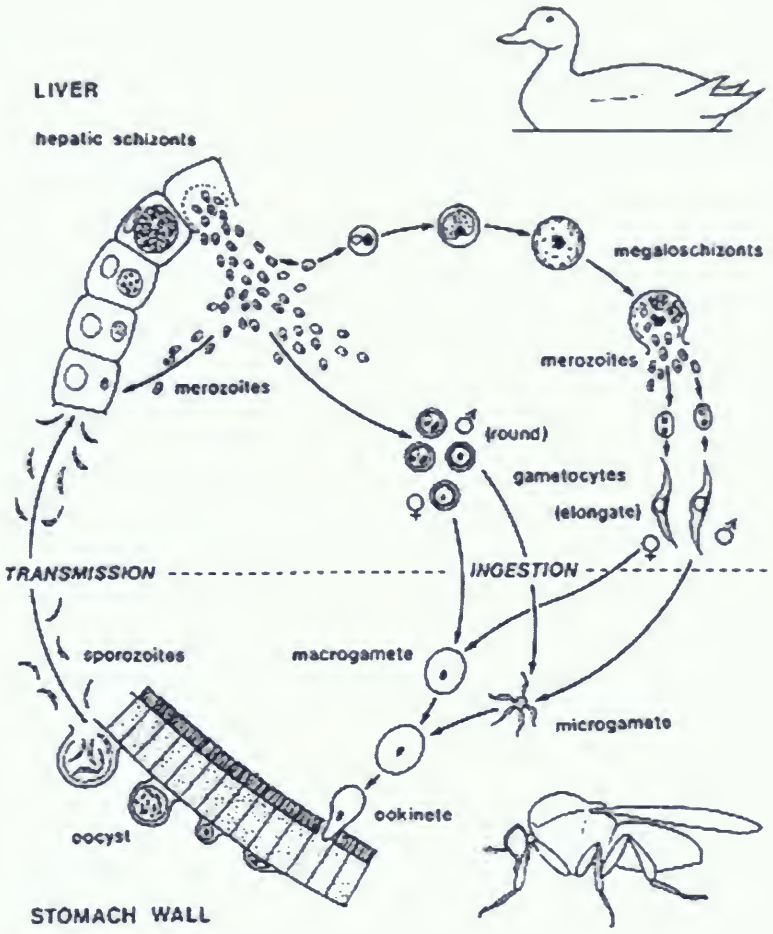


Figure 1: Lifecycle of protozoan parasite *Leucocytozoon simondi* in its black fly and duck hosts (Crosskey 1990)

Study Objectives

Black flies have been observed feeding on both plant nectars and homopteran honeydew. The breakdown products of both of these carbohydrate meals have also been found in individual flies. However, very little work has been done on the effects of these carbohydrates on black fly longevity, fecundity and parasite transmission. By examining the longevity, fecundity and vectoring potential of individual flies fed artificial nectar, artificial honeydew, and distilled water (control) diets we tested the following hypotheses:

(1) the type of diet fed to host seeking flies will affect their survival

(2) the type of diet fed to blood-fed black flies will affect their fecundity

(3) the type of diet fed to *Leucocytozoon*-infected black flies will affect their survival and the number of sporozoites produced and as a result, will affect their vectoring potential.

MATERIALS & METHODS

Study Site

This study was conducted in Algonquin Provincial Park, Ontario, Canada, along the shore of Lake Sasajewun, at the Wildlife Research Station (45° 34' N, 78° 41' W) during the spring of 1998 and 1999 (Figure 2). This site was chosen as the location to collect all of the black flies used in these experiments, as population densities can be very high at this location.

Sampling Host-Seeking Black Flies

Host-seeking black flies were collected by a method known as sweep net sampling. Standard insect nets (30 cm in diameter) were used during these sweep collections. Sweep sampling was done in the evenings between 6:00 pm and 9:00 pm on six collection dates (04, 11, 13, 14, 17, and 20 May, 1999). Sampling started after the investigator had been there for one hour to allow for flies to be attracted to the area. Three 10 minute sweeps were done consecutively, and the flies that were collected were transferred from the insect net into Ziploc® baggies and taken back to the field laboratory. The number of flies collected varied nightly.

Figure 2: Topographic map showing the Sasajewun lake collection site.

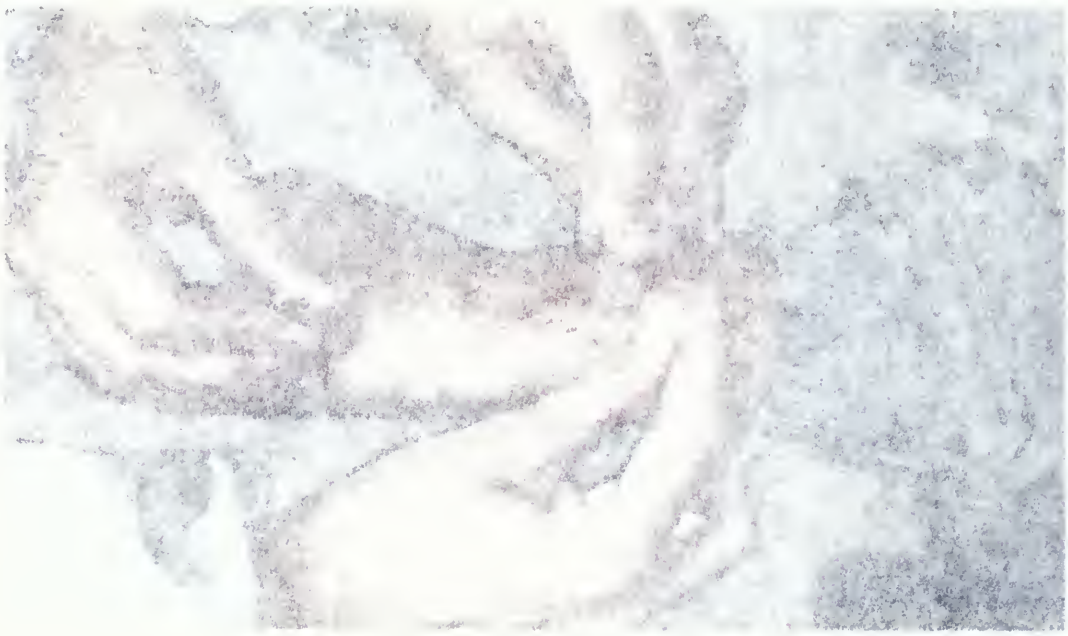
Sampling Blood-Fed Black flies

Two species of black flies were used in the fecundity study. The first was a mammalophilic species, *S. venustum,* which is the most common nuisance pest of humans. The second was an ornithophilic species, *S. rugglesi,* which feeds primarily on waterfowl. The *S. venustum* adults used in these experiments were collected during the 1999 field season and those of *S. rugglesi* were collected during both the 1998 and 1999 field seasons.

Collection of Blood-Fed *S. venustum*

Simulium venustum is one of the main human biters in Algonquin Provincial Park. This species is found in extremely high numbers during the prime part of the black fly season, the middle of May to the end of June. Large numbers of host-seeking flies were attracted to the investigator during the collections of ornithophilic black flies. Blood-fed *S. venustum* were collected after feeding to repletion on the arm of the investigator (Figure 3). Collections were made in the evenings between 6:00 pm and 9:00 pm. Engorged flies were captured in 1.5ml Eppendorf® tubes and taken back to the laboratory.

Figure 3: Collection technique for capturing blood fed *S. venustum* from the investigator's forearm.



Collection of Blood-Fed *S. rugglesi*

Blood-fed *S. rugglesi*, during both the 1998 and 1999 field seasons were collected from infected Pekin ducklings (an avian host of the parasite *Leucocytozoon simondi*) on the shore of Lake Sasajewun using the following protocol.

Avian Host

Sixteen domestic Pekin ducklings (Figure 4) were used as a source of *Leucocytozoon simondi* blood parasites in the 1998 field season. Four of these ducklings, as adults, were kept to ensure a source of *L. simondi* and used with 16 new ducklings in the 1999 field season. These ducklings were all purchased from Frey's Hatchery in St. Jacobs, Ontario. Day old ducklings were transported via truck from the hatchery to the Wildlife Research Station in Algonquin Provincial Park. Young ducklings were then housed in an enclosed metal shed which protected the ducklings from both black flies and potential predators. Initially the ducklings were housed together in a metal cage on a bed of shavings. Once the ducklings were large enough, at approximately 2 weeks of age, they were transferred to metal cages (2 x 1 x 1 m) and kept in smaller groups, four to five individuals per cage. At approximately 4 weeks of age the ducklings were transferred to a mesh-enclosed outdoor pen (4m x 8m). The ducklings had water *ad libitum* and were fed twice daily with Purina® duck starter until 2 weeks of age, after which they were fed Purina® meat builder. Neither of these duck feeds contained any antibiotics. Expandable spiral leg bands (plastic, colour coded), purchased at the Huntsville Co-op, were used to distinguish the individual ducklings from one another (Figure 5). The colour and combination of the leg bands identified individual ducks.

Figure 4: Infected Pekin ducklings used to collect blood fed *S. rugglesi*.

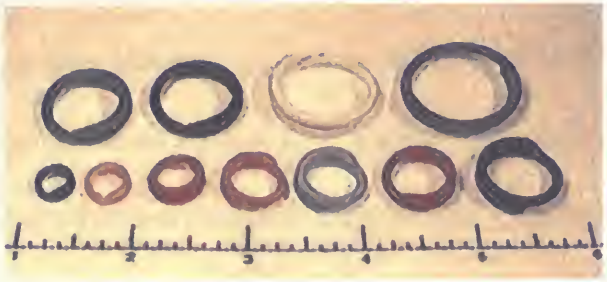
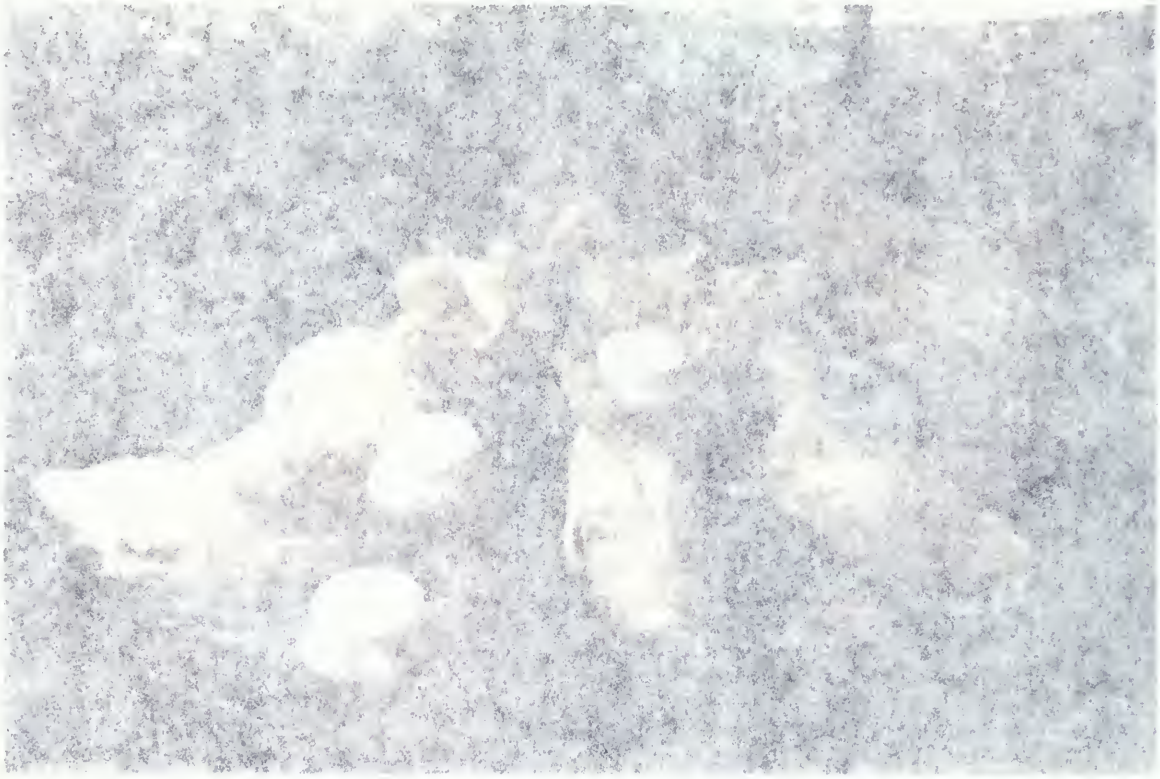


Figure 5: Spiral leg bands used to identify individual ducklings from one another.



Initial Infection of the Avian Host

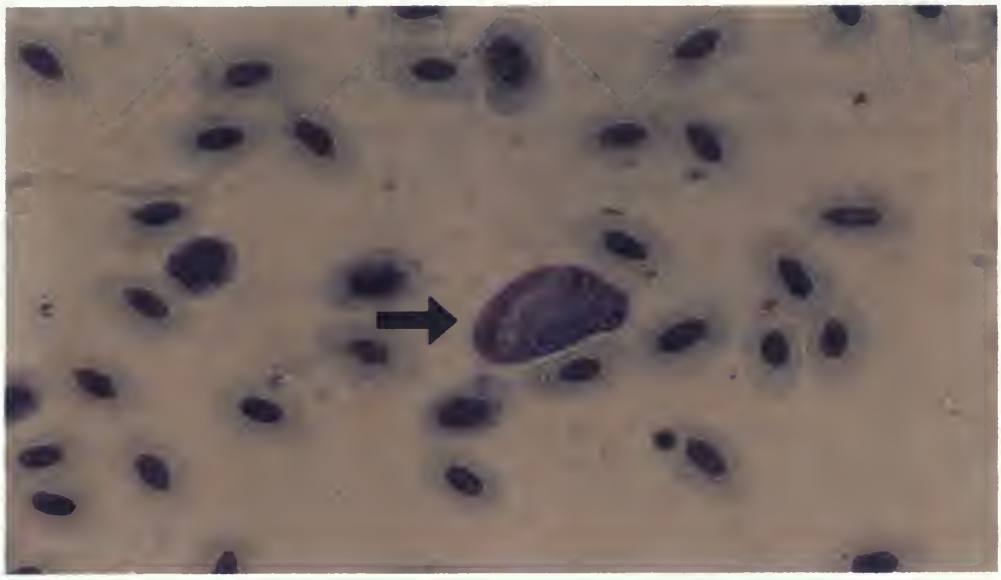
The ducklings were exposed individually to black flies in exposure cages, four to eight exposure cages per evening, to collect flies for the sugar feeding trials. Since there were 16 ducks in total, during the 1998 field season, they were placed on a 4-day rotation to be used in the black fly diet treatment experiments. After the completion of the 1998 field season the 4 adult ducks that were kept had already been infected with *L. simondi* infection. In 1999, therefore, the four adults were placed out daily and the 16 new ducklings were placed on a 4-day rotation. If any of the ducklings displayed signs of infection (e.g. lethargy), they were placed under a heat lamp until they recovered. Only 1 of the ducklings (in 1999) died due to *L. simondi* infection.

Indication of Infectivity Level in the Avian Host

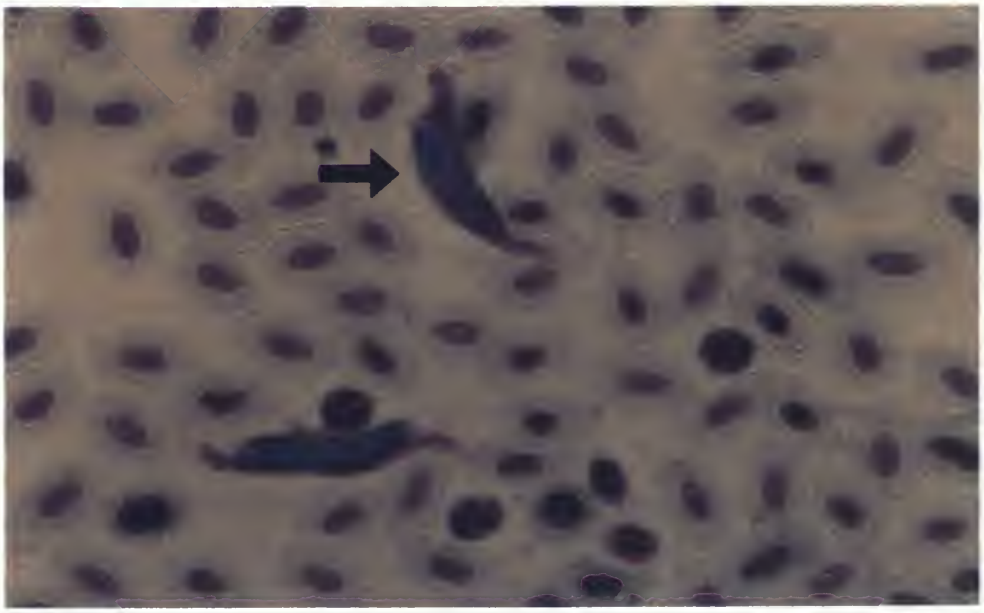
Before the ducklings were exposed to the black flies for the first time, blood smears were made to ensure that the ducklings were not infected. Blood smears were also made of those ducks or ducklings before each nightly exposure. To obtain blood, the femoral vein was punctured using a sterile lancet (Desser & Ryckman 1976) and a heparinized microcapillary tube was then applied to the drop of blood that formed. The blood was then thinly smeared in an s-shape onto a microscope slide and allowed to air dry. Once the slides were dry they were stained using the Diff-Quik® stain set. This stain set contains three steps: (1) a methonaolic fixative which fixes the blood smear; (2) solution 1: Xanthene dye, buffer and sodium azide as preservative which stains the nucleus and membranes pinkish red and; (3) solution II: Thiazine dye mixture and buffer

which stains all other material blue. All slides were made permanent by applying a few drops of Diatex® or Shur/Mount® and covering with a Fisherbrand®

cover slip (24 x 40 mm). From the blood smears, under the high dry field (40x) of a compound microscope, an estimate of the ratio of the number of gametocytes to the number of red blood cells (RBC) was determined for each individual duckling. The average number of round gametocytes (Figure 6a) and elongate gametocytes (Figure 6b) on a given day per 12,000 RBC was recorded.

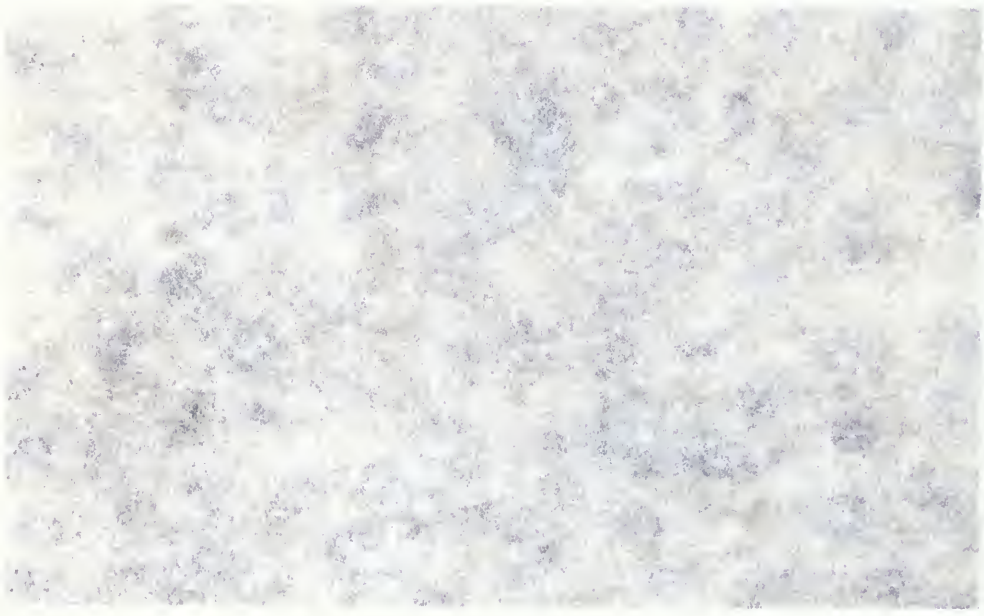


a)



b)

Figure 6: a) *Leucocytozoon simondi* round gametocyte found in the Pekin duckling blood smears; **b)** *L. simondi* elongate gametocytes found in the Pekin duckling blood smears.



Black Fly Experiments with the Avian Host

Ducklings were exposed to black flies on the shore of Lake Sasajewun in the evening between 6:00 p.m. and 9:00 p.m. During this period there were three separate 30 minute exposure periods. Ducklings were exposed for 15 minutes to allow black flies to enter the area and begin feeding (Figure 7a). The ducklings were then covered with fly-proof drop cages (Roller & Desser 1973) for another 15 minutes (Figure 7b), after which time blood-engorged black flies were collected by aspiration. The engorged flies were then taken back to the laboratory.

Diet Treatments

For all experiments, two artificial sugar diets were made. The recipes for these diets were developed based on previous studies that looked at the natural components of floral nectar and homopteran honeydew secretions (Appendix III). The amino acids found in nectar and homopteran honeydew were not present in these artificial diets. Only the carbohydrate components of the sugar meals were used in the artificial diets.

The artificial nectar treatment was made to simulate 10% floral nectar while the artificial honeydew treatment was made to simulate a 20 % honeydew solution (Table 1). This artificial honeydew contained a trisaccharide melezitose. This was chosen because it is a common sugar synthesized by homopterans. The final treatment was a control consisting of distilled water only.

a)

b)

Figure 7: Duck exposure procedure a) exposure to black flies for 15 minutes; b) covering with drop cages.

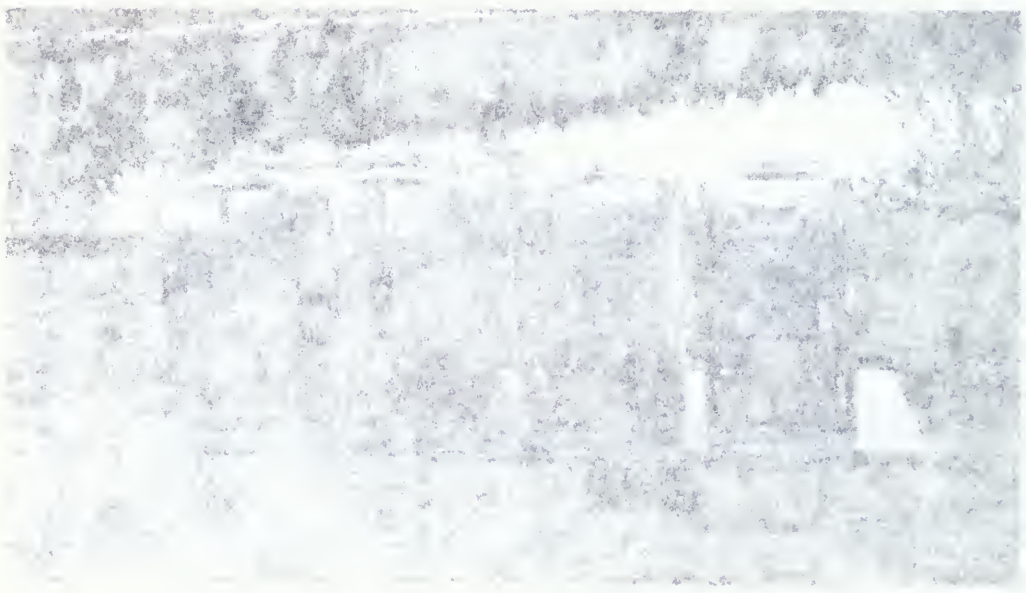


Table 1: Artificial diet treatment recipes used to feed experimental black flies

Artificial Nectar - AN	**Artificial Honeydew – AH**
3.5 g glucose	2.0 g glucose
4.0 g sucrose	1.5 g sucrose
2.5 g fructose	2.5 g fructose
	2.0 g melezitose
in 100 ml of distilled water	in 40 ml of distilled water

Black Fly Housing

In the laboratory, the flies in all experiments were placed individually in a modified version of the apparatus used by Hunter *et al.* (1994) to rear museum-quality specimens (Figure 8). Each female, host-seeking or blood-fed, was placed inside a 20 ml glass scintillation vial (Fisher Brand). There was a disk of polyethylene (Plastazote®) foam cut to fit the bottom of the vial. A small hole made with a cork borer was made in the Plastazote® to support a smaller (1/8 dram) shell vial containing one of the three diet treatments (artificial nectar, artificial honeydew, distilled water) and a plug of absorbent cotton. The shell vials were replaced daily with fresh diet solutions and plugs to avoid bacterial and fungal build-up. Changing these vials daily also reduced the chance of losing experimental flies to desiccation during the experiment. Once the female was placed into the vial the screw cap was placed on loosely to avoid condensation. The vials were then placed in a cardboard box in a cupboard to provide a relatively dark undisturbed environment at 21.0 ^{o}C ± 2.7 and 21.0 ^{o}C ± 2.6, for the 1998 and 1999 field seasons, respectively (Appendix IV).

Duration on Diet Treatments

Host-seeking flies were maintained on their treatment diets until they died, at which point the number of days surviving were recorded for survival data.

All of the blood-fed flies were on the other hand, maintained on their diet treatment for 11 days. On the 11th day they were dissected for analysis of mature oocysts

(fecundity experiments) and salivary gland sporozoites (parasite transmission experiments). The 11th day was chosen to ensure that: 1) the oocysts had reached maturity; and 2) the sporozoites had migrated to the salivary glands. Any flies that died between day 5 and day 11 were also dissected and used in the analysis. However, flies that died before day 5 were not dissected since oocysts were not be fully developed and sporozoites would not have migrated to the salivary glands.

Figure 8: Black fly housing apparatus used, modified 20 ml glass scintillation vial.

Black Fly Dissections

Salivary Gland Dissections

On day 11 when flies appeared fully gravid, salivary glands and mature oocysts were removed. Counts of the *L. simondi* sporozoites (Desser and Ryckman, 1976) were made.

Under a dissecting microscope, in a drop of distilled water on a slide, forceps were used to pull the head away from the thorax of the black fly, thus exposing the salivary glands. The glands were then placed on a dry microscope slide and teased apart to make a thin film. The slide was then air dried and was stained immediately using the Diff-Quik® stain set. The slides were made permanent by placing 2 drops of Diatex®, directly over the smear, and then covering with a Fisherbrand® cover slip (24 x 40 mm). The fly bodies were stored in 70% ethanol until the mature oocysts were removed.

Mature Oocyst Dissections

Under a dissecting microscope, each fly's abdomen was gently torn open using fine forceps so that the mature eggs were exposed. The mature oocytes were removed from the abdomen and spread across the surface of a microscope slide. The mount was then made permanent by placing a drop of Shur-Mount® directly on the eggs and covering them with a Fisherbrand® cover slip. The number of mature oocytes on each slide was then counted and recorded. Ten eggs, chosen randomly on each slide, were measured using the eyepiece micrometer. The average sums of the 10 measurements were converted

into millimeters (mm).

Identification of Black Flies

Black flies after they died were identified using the adult black fly keys of Davies *et al.* (1962). The two species of black flies used in the survival experiments were: 1) *Prosimulium fuscum/mixtum*, an early season species; and 2) *Simulium venustum*, which appears later in the season. Both species are mammalophilic. *S. venustum* was also the species of black fly used in the fecundity experiments. In both the longevity and fecundity experiments, the other species in the *Simulium venustum/verecundum* complex (*S. truncatum, S. rostratum, S. verecundum*) were not included in the experimental data analyses. The ornithophilic species, *Simulium rugglesi* was used in the both the fecundity and parasite transmission experiments.

Data Analysis

Initially, all data were tested using the Wilk-Shapiro test for normal distribution (Zar 1984). The results indicated that the data were normally distributed. Parametric one-way ANOVA analyses and *post-hoc* Tukey (HSD) comparison of means analyses (Zar 1984) were then performed on these data using Statistix© for Windows version 2.0 (Analytical Software, 1998). The ANOVA analyses resolved whether there were any significant differences among treatments. If differences were found between the treatments the comparison of means analysis pointed to where the differences were. In some cases paired-sample t-tests were performed, to examine comparisons between years

and between species again using Statistix© (Analytical Software, 1998).

Linear regressions were also performed on the wing measurement data, the egg sum data and egg length data using Excel 97 for Windows. The reported values give both the slope of the line of best fit as well as the R^2 values for each diet treatment.

Chi-square analyses were performed on the survival time of flies less than or greater than 10 days on each diet. This test was chosen to examine whether there was a significant difference in the length of time that flies survived on each of the diet treatments. Since all but one of the flies maintained on distilled water died before 10 days, the Chi-square analysis was performed on the two sugar diet treatments only. Yates corrected values were reported at $\alpha = 0.05$ and $df = 1$.

Finally, the infective outputs (IO) of the flies were calculated. The infective output is the number of sporozoites found in the fly's salivary glands divided by the number of gametocytes that were in the duck on the day in which the infected fly fed (IO = no. sporozoites / no. gametocytes). The GT2-Method (Sokal and Rohlf 1995) was then used to determine whether there were significant differences between the average infective outputs (IO) of flies maintained on the three diet treatments.

RESULTS

Effects of Sugar Feeding on Black Fly Survival

Wing Length Measurements

Wing measurements were made from 60 *P. fuscum/mixtum* and 67 *S. venustum* flies (Table 2). The average wing length of *P. fuscum/mixtum* flies (3.18 ± 0.23 mm) was much greater than for *S. venustum* (2.66 ± 0.26 mm) for all treatments (Table 2; $t = 7.27$, $df = 25$, $p < 0.0001$). The average wing length of a fly does not change once it has reached maturity. Therefore, one-way ANOVA was used to ensure that flies, of both species, had been placed on the diet treatments randomly with respect to wing lengths. The results showed that *P. fuscum/mixtum* and *S. venustum* females were assigned to all diet treatments randomly, since their wing lengths did not differ with respect to treatment (Table 2; $F_{(2,57)} = 1.84$; $p = 0.168$ and $F_{(2,64)} = 0.98$; $p = 0.384$, respectively).

Survival of Host-Seeking Females

Survival experiments were conducted using 84 *P. fuscum/mixtum* and 199 *S. venustum* host-seeking flies. The survival of host-seeking females was measured as the duration in days that these flies survived on one of three sugar meal diets, after being collected in the physiological state of host-seeking (but not having been allowed to blood feed) (Table 3). Differences were found among the treatments with *P. fuscum/mixtum* host-seeking females (Table 3; $F_{(2,81)} = 8.92$; $p = 0.0004$). A *post hoc* Tukey (HSD) comparison of means test showed that *P. fuscum/mixtum* maintained on the two sugar

diets (artificial honeydew and artificial nectar) survived longer (5 - 8 days longer) than those maintained only on distilled water (Table 3; $p < 0.05$). There was a difference among treatments with *S. venustum* host-seeking females (Table 3; $F_{(2,196)} = 112.84$; $p = 0.0001$). *S. venustum* flies lived longer (22 – 23 days longer) when maintained on either artificial honeydew or artificial nectar than those maintained on distilled water alone (Table 3; $p < 0.05$).

Table 2: Wing lengths (in mm ± SD) from a sub-sample of host-seeking *P. fuscum/mixtum* and host-seeking *S. venustum* maintained on one of three sugar meal diets (artificial honeydew - AH; artificial nectar -AN; distilled water - dH_20) during the 1999 field season at the Wildlife Research Station, Algonquin Provincial Park. Mean values followed by the same letter within a column are not significantly different.

	P. fuscum/mixtum			*S. venustum*		
Treatment	**Wing length**		**n**	**Wing length**		**n**
AH	3.26 ± 0.18	a	19	2.61 ± 0.34	b	16
AN	3.12 ± 0.24	a	20	2.62 ± 0.26	b	17
dH_20	3.18 ± 0.26	a	21	2.70 ± 0.22	b	34
one-way ANOVA	$F_{(2,57)} = 1.84$; $p = 0.168$			$F_{(2,64)} = 0.98$; $p = 0.384$		

Table 3: The average survival (in days ± SD) of host-seeking *P. fuscum/mixtum* and host-seeking *S. venustum* maintained on one of three diets (artificial honeydew - AH; artificial nectar -AN; distilled water - dH_20) during the 1999 field season at the Wildlife Research Station, Algonquin Provincial Park. Mean values followed by the same letter within a column are not significantly different as measured by a *post hoc* Tukey (HSD) comparison of means test.

	P. fuscum/mixtum			*S. venustum*		
Treatment	**Survival (in days)**		**n**	**Survival (in days)**		**n**
AH	10 ± 8.2	a	27	27 ± 11.1	c	66
AN	13 ± 8.3	a	29	26 ± 11.8	c	70
dH_20	5 ± 2.8	b	28	4 ± 2.3	d	63
one-way ANOVA	$F_{(2,81)} = 8.92$; $p = 0.0004$			$F_{(2,196)} = 112.84$; $p = 0.0001$		

Effects of Sugar Feeding on Black Fly Fecundity

Estimation of Fly Size

S. venustum, 1999

The average wing lengths of a sub-sample of *S. venustum* used for the fecundity study were examined (Table 4). There were no significant differences among treatments (Table 4; $F_{(2,67)} = 0.55$; $p = 0.587$). Therefore these flies had been assigned randomly to diet treatments.

S. rugglesi, 1998 & 1999

The average wing lengths of all *S. rugglesi* used in the fecundity experiments were measured (Table 5). To ensure that the *S. rugglesi* collected in 1998 and 1999 had been assigned randomly to diet treatments a one-way ANOVA was used. In 1998 and 1999 the *S. rugglesi* were assigned randomly to diet treatments, since wing lengths across treatments are similar (Table 5; $F_{(2,78)} = 0.03$, $p = 0.957$ and $F_{(2,247)} = 0.65$; $p = 0.528$, respectively).

The *S. rugglesi* used in the fecundity experiments were smaller in 1998 than those used in 1999 as evidenced by a mean wing length in 1998 of 2.03 ± 0.09 mm versus 2.15 ± 0.21 mm in 1999 (Table 5; $t = 5.44$, $df = 80$, $p < 0.0001$).

Table 4: Wing lengths (in mm ± SD) of blood-fed *S. venustum* fed on one of three diets (artificial honeydew - AH; artificial nectar - AN; distilled water - dH_2O) during the 1999 field season at the Wildlife Research Station, Algonquin Provincial Park. Mean values followed by the same letter are not significantly different.

Treatment	Wing length		n
AH	2.67 ± 0.26	a	24
AN	2.66 ± 0.27	a	26
dH_2O	2.60 ± 0.23	a	20
one-way ANOVA	$F_{(2,67)} = 0.55$, $p = 0.587$		

Table 5: Wing length (in mm ± SD) of blood-fed *S. rugglesi* fed on one of three diets (artificial honeydew - AH; artificial nectar -AN; distilled water - dH_20) during the 1998 and 1999 field seasons at the Wildlife Research Station, Algonquin Provincial Park. Mean values followed by the same letter within a column are not significantly different.

	1998 Field Season			1999 Field Season		
Treatment	**Wing length**		**n**	**Wing length**		**n**
AH	2.03 ± 0.08	a	27	2.16 ± 0.21	b	85
AN	2.03 ± 0.09	a	30	2.15 ± 0.27	b	90
dH20	2.04 ± 0.10	a	24	2.12 ± 0.22	b	75
one-way ANOVA	$F_{(2,78)} = 0.03$; $p = 0.957$			$F_{(2,247)} = 0.65$; $p = 0.528$		

Fecundity of Blood-Fed *S. venustum*

The fecundity of blood-fed *S. venustum* was measured by the number of mature eggs or oocytes produced on each of the three diet treatments (Table 6). Differences were found among treatments for the numbers of eggs produced (Table 6; $F_{(2,133)} = 5.70$, $p = 0.004$) and for the average egg lengths (Table 6; $F_{(2,133)} = 38.14$, $p = 0.0001$). Females produced more eggs (approximately 57 more eggs) when maintained on sugars, either artificial honeydew or artificial nectar, than those maintained on distilled water (Table 6; $p < 0.05$). Females also produced larger eggs (approximately 0.5 mm larger) when maintained on sugar meals (either artificial honeydew or artificial nectar), than on distilled water (Table 6; $p < 0.05$).

Fecundity of Blood-Fed *S. rugglesi*, 1998 & 1999

S. rugglesi fecundity was also determined by examining the number of eggs produced and the average egg lengths of individual flies maintained on one of three diet treatments (Tables 7 and 8). In 1998, differences were found in *S. rugglesi* fecundity, with respect to both the average number of eggs produced and the average egg lengths (mm), among the diet treatments (Table 7; $F_{(2,93)} = 5.82$, $p = 0.004$; $F_{(2,93)} = 4.61$, $p = 0.01$ respectively). *S. rugglesi* maintained on artificial honeydew produced more eggs per batch (approximately 70 eggs more) than those maintained on distilled water (Table 7; $p < 0.05$). However, *S. rugglesi* maintained on artificial honeydew did not produce significantly more eggs than *S. rugglesi* maintained on artificial nectar. The *S. rugglesi* maintained on artificial nectar did not produce significantly more eggs than the *S.*

rugglesi maintained on distilled water. *S. rugglesi* maintained on artificial nectar produced larger eggs (approximately 0.5 mm larger) than those maintained on distilled water (Table 7; $p < 0.05$). However, *S. rugglesi* maintained on artificial nectar did not produce larger eggs than those maintained on artificial honeydew. *S. rugglesi* maintained on artificial honeydew did not produce larger eggs than those maintained on distilled water. These results suggest that *S. rugglesi* maintained on artificial honeydew produce more eggs but the flies maintained on artificial nectar produce larger eggs relative to the distilled water treatment.

In 1999, the results differed slightly in that there were no differences between sugar treatments for the numbers or size of eggs produced by *S. rugglesi* (Table 8). There were differences among treatments for the number of eggs produced (Table 8; $F_{(2,237)} = 8.62$, $p = 0.0003$) and for the lengths (in mm) of the eggs (Table 8; $F_{(2,237)} = 23.50$, $p = 0.0001$). *S. rugglesi* maintained on sugars, (AH and AN), produced more eggs (approximately 45 more eggs) that were larger (approximately 0.2 mm larger) than those maintained on distilled water alone (Table 8; $p < 0.05$).

Table 6: The average number of eggs produced and the average egg length (in mm ± SD; n = 11) of blood-fed *S. venustum* fed one of three sugar meal diets (artificial honeydew - AH; artificial nectar -AN; distilled water - dH_20) during the 1999 field season at the Wildlife Research Station, Algonquin Provincial Park. Mean values followed by the same letter within a column are not significantly different as measured by a *post hoc* Tukey (HSD) comparison of means test.

Treatment	Average number of eggs produced		n	Average egg length (mm)		n
AH	176 ± 103	a	45	0.23 ± 0.03	c	45
AN	183 ± 101	a	49	0.23 ± 0.04	c	49
dH_20	123 ± 62	b	42	0.18 ± 0.03	d	42
one-way ANOVA	$F_{(2,133)} = 5.70$; $p = 0.004$			$F_{(2,133)} = 38.14$; $p = 0.0001$		

Table 7: The average number of eggs produced and the average egg length (in mm ± SD; n = 11) of blood-fed *S. rugglesi* fed on one of three sugar meal diets (artificial honeydew - AH; artificial nectar -AN; distilled water - dH_20) during the 1998 field season at the Wildlife Research Station, Algonquin Provincial Park. Mean values followed by the same letter within a column are not significantly different as measured by a *post hoc* Tukey (HSD) comparison of means test.

Treatment	Average number of eggs produced		n	Average egg length (mm)		n
AH	254 ± 80	a	35	0.18 ± 0.02	c,d	35
AN	229 ± 71	a,b	40	0.19 ± 0.10	c	40
dH_20	184 ± 75	b	21	0.14 ± 0.02	d	21
one-way ANOVA	$F_{(2,93)} = 5.82$; $p = 0.004$			$F_{(2,93)} = 4.61$; $p = 0.01$		

Table 8: The average number of eggs produced and the average egg length (in mm ± SD; n = 11) of blood-fed *S. rugglesi* fed on one of three sugar meal diets (artificial honeydew - AH; artificial nectar -AN; distilled water - dH_20) during the 1999 field season at the Wildlife Research Station, Algonquin Provincial Park. Mean values followed by the same letter within a column are not significantly different as measured by a *post hoc* Tukey (HSD) comparison of means test.

Treatment	Average number of eggs produced		n	Average egg length (mm)		n
AH	167 ± 81	a	83	0.19 ± 0.02	c	83
AN	155 ± 82	a	89	0.18 ± 0.02	c	89
dH20	116 ± 68	b	68	0.17 ± 0.03	d	68
one-way ANOVA	$F_{(2,237)} = 8.62$; $p = 0.0003$			$F_{(2,237)} = 23.50$; $p \leq 0.0000$		

Effect of Egg Batch Size on Average Egg Length

S. venustum

For a given diet treatment the number of eggs that *S. venustum* produced did not affect the average length of those eggs, as the slopes of the regression lines were close to zero (Figure 9). However, the diet treatment on which the flies were maintained affected the average length of eggs produced, with flies maintained on sugar meals (either artificial honeydew or artificial nectar) producing longer eggs than those produced by flies maintained on distilled water.

S. rugglesi 1998

The number of eggs produced by *S. rugglesi*, collected during the 1998 field season, did not appear to affect the average length of eggs produced (slope of the regression lines were close to zero), with the exception of the flies maintained on distilled water (Figure 10). As the egg batch size increased for the flies fed on distilled water the length of their eggs also increased, such that the slope of the regression line was greater than zero (but not significantly so). However, the eggs produced by the *S. rugglesi* maintained on the artificial sugar meals were longer than those produced by *S. rugglesi* maintained on distilled water.

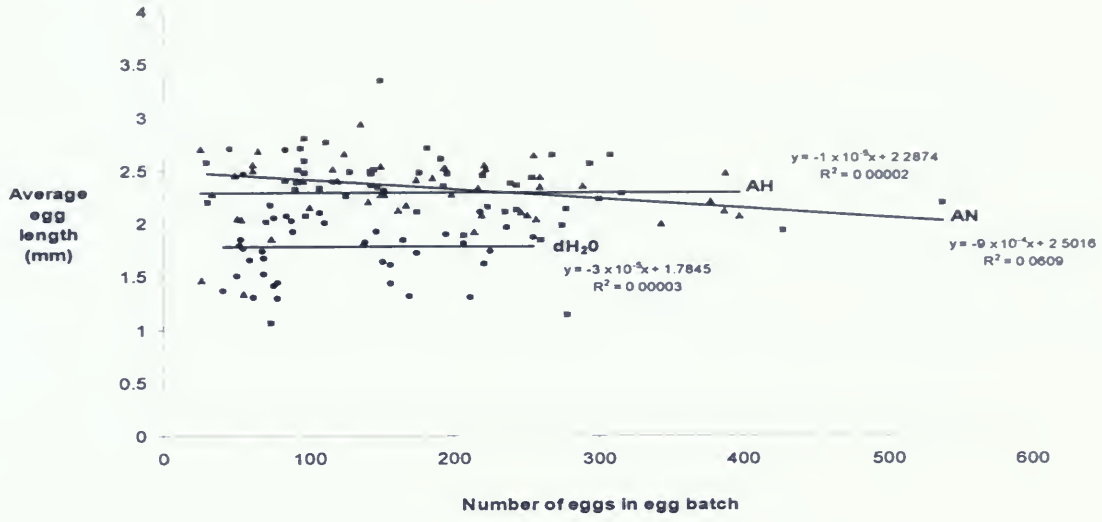


Figure 9: Average egg lengths from egg batches of various sizes, produced by *S. venustum*, maintained on artificial honeydew - AH (triangle), artificial nectar - AN (square) or distilled water - dH20 (circle), during the 1999 field season. Each data point is the average of 10 measurements

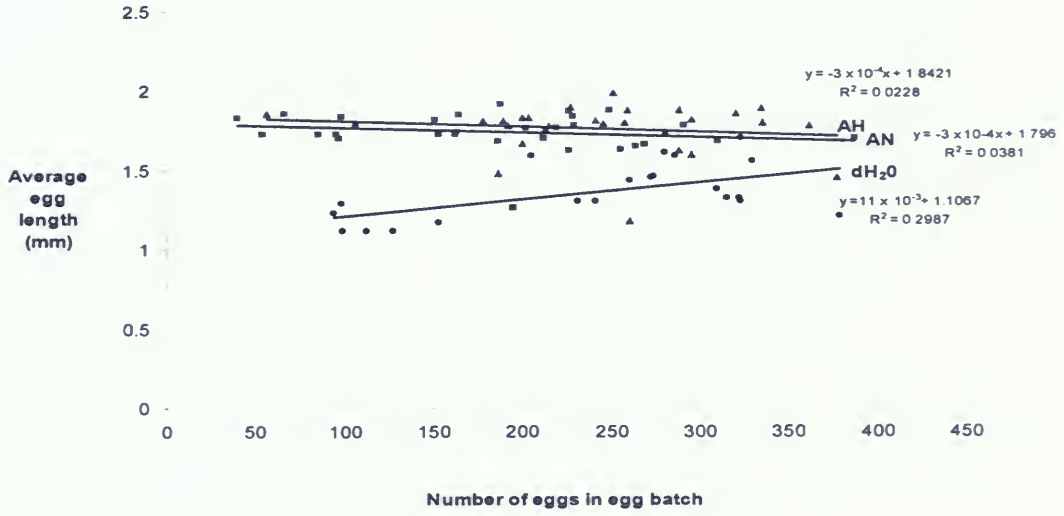


Figure 10: Average egg lengths from egg batches of various sizes, produced by *S. rugglesi*, maintained on artificial honeydew - AH (triangle), artificial nectar - AN (square) or distilled water - dH20 (circle), during the 1998 field season. Each data point is the average of 10 measurements

S. rugglesi 1999

Generally, the *S. rugglesi* collected in 1999 followed the same pattern as those collected in 1998 (Figure 11). For all treatments the slopes of the regression lines were close to zero suggesting that there was no effect of egg batch number on length of eggs produced and *S. rugglesi* maintained on artificial sugar meals produced eggs that were slightly longer than those produced by the flies maintained on distilled water.

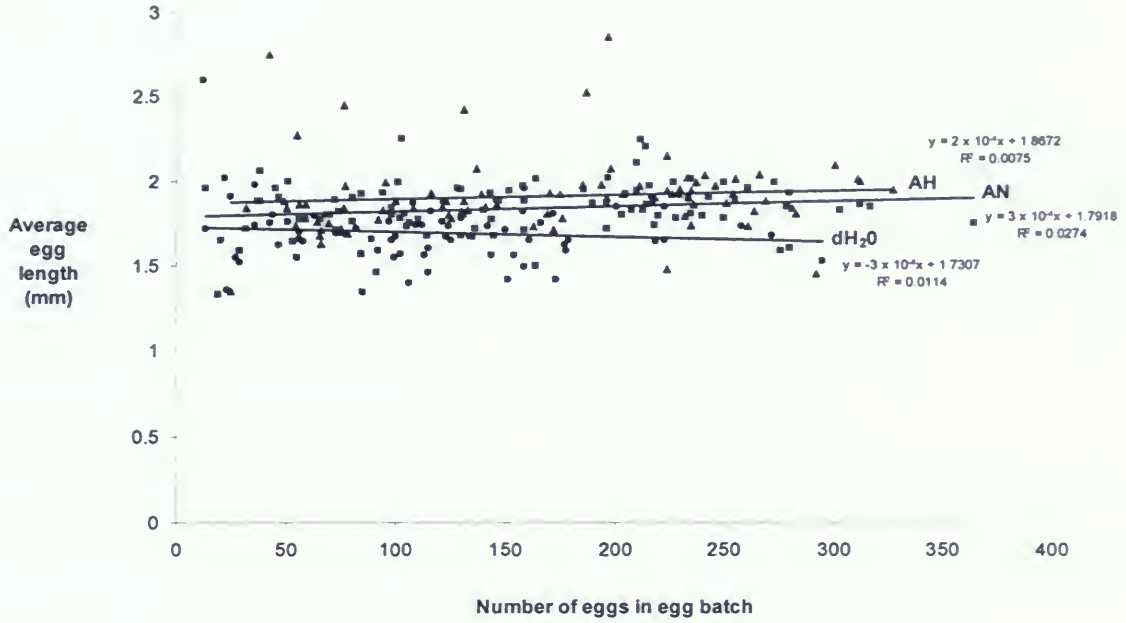


Figure 11: Average egg lengths from egg batches of various sizes, produced by *S. rugglesi*, maintained on artificial honeydew - AH (triangle), artificial nectar - AN (square) or distilled water - dH20 (circle), during the 1999 field season. Each data point is the average of 10 measurements

Diet Treatment Effects on Survival

The length of time that blood-fed *S. venustum* and *S. rugglesi* (1998 and 1999 flies) survived (up to 11 days-dissection day) on one of the three diet treatments was determined. Flies that died before they were 10 days old were placed in the less than or equal to 10 day category ($\leq$ 10 days). The greater than 10 day category (> 10 days) contained the flies that survived for at least 10 days but were killed on day 11 for investigation of the salivary glands, egg number and size.

S. venustum survival data for the two sugar diets (Appendix V) was tested using a 2 x 2 Chi-square analysis. No differences in fly survival occurred between diets when *S. venustum* females were maintained on the two artificial sugar diets. When the sugar data were pooled and Chi-square analysis used to compare sugar diets to the distilled water, the majority (80%) of *S. venustum* females survived more than 10 days ($\geq$ 10 days) on the sugars but not on distilled water (Appendix V; $X^2 = 74.71$, df = 1, $p < 0.05$). One hundred percent of the *S. venustum* maintained on distilled water died on or before day 10 (Appendix V).

The survival data for *S. rugglesi* for the two field seasons was examined separately (1998 Appendix VI; 1999 Appendix VII). Chi-square analysis showed that there were no differences between fly survival, during either field season, when females were maintained on artificial honeydew or artificial nectar. Therefore, the sugar data were pooled and Chi-square analysis used to compare sugar diets to the distilled water diet. More than 80% of the *S. rugglesi* collected in 1998 and 1999 survived more than 10 days

on sugar diets as compared to the distilled water diets (Appendix VI; $X^2 = 54.74$, df = 1, $p < 0.05$; and Appendix VII; $X^2 = 128.41$, df = 1, $p < 0.05$ respectively). In 1998, 95% of the *S. rugglesi* maintained on distilled water died on or before day 10 (Appendix VI). In the following year (1999), 100% of the *S. rugglesi* fed distilled water died on or before day 10 (Appendix VII).

Infectivity of Pekin Ducklings

Over the two field seasons, 1998 and 1999, 35 ducklings were infected naturally with *L. simondi* (Table 9). In 1998, only 31% of the ducklings were able to transfer the *L. simondi* to *S. rugglesi*, and in 1999 47% of the ducklings did so successfully. A Chi-square analysis showed that the transfer rate of the parasite from the host to the vector did not differ between the two field seasons (Table 9; $X^2 = 0.39$, df = 1, $p = 0.53$). The average transfer rate of *L. simondi* to *S. rugglesi* was 39% for the 2 years.

Infectivity Levels of *L. simondi* in *S. rugglesi*

During the 1998 and 1999 field seasons 353 blood-fed female *S. rugglesi* were collected after feeding naturally on the ducklings (Table 10). Of those females collected, 21 became infected with *L. simondi*. Eight of those females were maintained on artificial honeydew, 9 on artificial nectar and 4 on distilled water. Chi-square analysis showed that total numbers of infected black flies collected did not change between the two collection years (Table 10; $X^2 = 2.16$, df = 1, $p = 0.14$); therefore, these data were pooled.

The percentage of these blood-fed females containing reproductive stages (sporozoites) of *L. simondi* for a given year was regarded as the infectivity level of *L. simondi* and was 9% in 1998 and 5% in 1999. The average infectivity level of *L. simondi* over the 2 years was 7%.

Table 9: The total number of Pekin ducks that became infected naturally with *L. simondi* and were able to transfer the parasite to *S. rugglesi* during the 1998 and 1999 field seasons ($X^2 = 0.39$, df = 1, p = 0.53).

	1998	**1999**
No transfer to *S. rugglesi*	11	10
Transfer to *S. rugglesi*	5	9
Total number ducks infected	16	19

Table 10: The total number of infected and uninfected blood-fed *S. rugglesi* that were collected from 16 infected Pekin ducklings in 1998 and from 19 infected Pekin ducklings in 1999 ($X^2 = 2.16$, df = 1, p = 0.14).

	1998	**1999**
Infected flies	10	11
Uninfected flies	99	233
Total flies collected	109	244

Infective Output

The average infective outputs (IO = no. sporozoites / no. gametocytes) for the 21 infected flies (1998 Appendix VIII; Appendix IX) were calculated (Table 11). The flies that were maintained on distilled water had the highest infective output. The flies maintained on artificial honeydew were second, with the flies maintained on artificial nectar the lowest.

The GT2-method (Sokal and Rohlf 1995) was used to determine whether there were significant differences between the average infective outputs of the flies maintained on the three diets (Figure 12). The 95% comparison intervals for the means of the infective outputs of *S. rugglesi* were calculated and the values were ranked according to the magnitude of their means. The mean of the flies that fed on distilled water ranked first, mean of the flies that fed on artificial honeydew ranked second and the mean of the flies that fed on the artificial nectar ranked third. However, there were no significant differences between these mean infective outputs because the 95% comparison intervals overlap in all cases (Figure 12).

Table 11: The average infective output (IO = no. sporozoites / no. gametocytes) ± SE of infective black flies fed on one of three sugar meal diets (artificial honeydew - AH; artificial nectar - AN; distilled water dH_20) during the 1998 and 1999 field seasons at the Wildlife Research Station, Algonquin Provincial Park.

Treatment	Average Infective Output	n
AH	2.39 ± 0.72	8
AN	2.10 ± 1.07	9
dH_20	4.55 ± 1.28	4

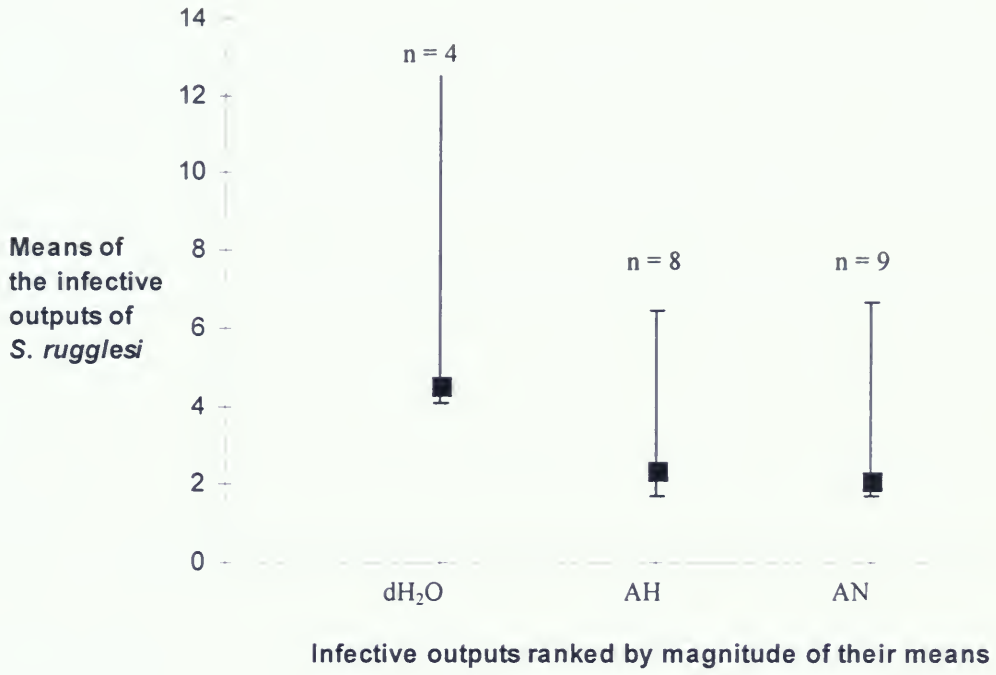


Figure 12: 95% comparison intervals, calculated using the GT2-method, for the means of the infective outputs of *S. rugglesi* ranked according to the magnitude of their means. Means whose intervals overlap are not significantly different.

Survival of Infected flies

The blood-fed flies that ingested *L. simondi* lived longer on artificial honeydew and artificial nectar as opposed to distilled water (Table 12). Of the eight infected *S. rugglesi* that were maintained on artificial honeydew, seven (87%) survived more than 10 days. Eight of the nine *S. rugglesi* (88%) maintained on artificial nectar survived more than 10 days. Chi-square analysis showed no differences (Table 12; $X^2 = 0.02$, df = 1, p = 0.89). None of the 4 flies that were maintained on distilled water survived 10 days.

Table 12: Total number of blood-fed *S. rugglesi* (1998 and 1999 field seasons) that fed on Pekin ducklings when the round gametocytes of *L. simondi* were reproductively viable. All of these flies were maintained on one of three sugar meal diets (artificial honeydew - AH; artificial nectar - AN; distilled water - dH_20) and killed on day 11 for dissections. Flies maintained on distilled water did not survive until day 11 ($X^2 = 0.02$, df = 1, p = 0.89).

	Treatments		
	AH	**AN**	**dH_2O**
Infected flies	7	8	0
Uninfected flies	56	51	0
Totals	63	59	0

DISCUSSION

Survival of Host-Seeking Females

Flies break down the carbohydrates and utilize the nutrients for basic survival and energy needs e.g., flight. After a certain length of time the flies deplete their fat body reserves and must find and use other sources of nutrients (Beier 1998; Brenner and Cupp 1980; Jahan and Hurd 1997; Lyimo and Koella 1992). These other sources of nutrients include both floral nectar and honeydew. Prior to this study the effect of nectar versus honeydew had never been examined in longevity studies using wild caught host-seeking black flies. Since honeydew is thought to contain both simple and complex carbohydrates it is thought to offer more energy to its insect visitors. Suggesting that flies maintained on artificial honeydew should survive longer than flies on artificial nectar, however, this was not the case. Results showed flies, regardless of species, maintained on artificial sugar meals survived much longer then those maintained on distilled water alone. There were no significant differences between artificial honeydew and artificial nectar. This demonstrates the importance of sugar diets in the basic survival of black flies, but the exact composition of the diets might not be critical when fed *ad libitum*.

Simulium venustum has a longer season and is a later species than *P. fuscum/mixtum*. *Prosimulium* species come out in late April or early May and are usually almost gone by the end of June. *S. venustum* females on the other hand, start to emerge and become nuisance pests in the latter half of May and early June into mid July. But, *P. fuscum/mixtum* did not survive as long as *S. venustum*.

A reason for the differences in survival between these species could have to do with their nutrient use for egg development. *P. fuscum/mixtum* is autogenous for its first gonotrophic cycle, emerging with partially or fully developed eggs, as well as a fair amount of stored nutrient in the fat body. They are known to oviposit 6 to 7 days after the peak of emergence without a blood meal (Davies *et al.* 1962). Shortly after the first oviposition the females are attracted to mammals to obtain a blood meal for the second gonotrophic cycle, but only about 10% of them lay a second batch of eggs. This suggests that the *P. fuscum/mixtum* flies collected during this study were in their second gonotrophic cycle or greater and nearing the end of their lifespan. *S. venustum* on the other hand are anautogenous, feeding on mammals and occasionally birds to provide nourishment for the first gonotrophic cycle. It has been estimated that 20% of parous flies will survive to complete a second ovarian cycle (Davies *et al.* 1962). Newly emerged *S. venustum* contain undeveloped eggs and little stored nutrient. These flies are thought to live 2-3 weeks completing a minimum of two gonotrophic cycles. This suggests that the *S. venustum* that were collected were in their first or second gonotrophic cycle and therefore much younger than the *P. fuscum/mixtum*. As a result the *S. venustum* flies survived longer than the *P. fuscum/mixtum* flies.

Effects of Sugar Feeding on Black Fly Fecundity

Fecundity studies prior to this one demonstrated that carbohydrates were used by females for egg production (Anderson 1987; Hunter 1977; Lavoipierre 1961; Mokry 1980). This study is not only in agreement with previous studies that carbohydrate meals are important for egg development but that the type of sugar meal, whether it be artificial

nectar or artificial honeydew, may influence the strategy adopted to produce those eggs if flies are starved, small, or have poor larval reserves.

Estimation of Fly Size

Measurements of the average wing lengths of the black flies that were collected during the 1998 (*S. rugglesi*) and the 1999 (*S. rugglesi* and *S. venustum*) field seasons were used for average fly size for a species over the season. In addition to confirming that flies were assigned randomly to diet treatments, these measurements showed size variation between *S. rugglesi* adults collected in 1998 and 1999. Those collected in 1999 were larger, than flies collected in 1998. One possible reason for this difference could be seasonal variations (Colbo 1982). During the 1998 field season black fly habitat close to the study area faced severe flooding. This flooding may have caused habitat changes and ultimately changes in available nutritional resources.

Fecundity of Blood-Fed *S. venustum*

When *S. venustum* adults were maintained on artificial sugar meals their fecundity was substantially better than those maintained on distilled water alone. Fecundity was measured as the number of mature eggs produced by an individual blood-fed female. When sugar diets are digested they are used for energy to assist breakdown of the blood meal for nutrients used in development of eggs (Brenner and Cupp 1980). If there are not enough nutrients provided by a sugar meal the flies must use part of the blood meal for energy rather than for egg nutrients. Therefore, the flies maintained on distilled water were using a smaller portion of their blood meal in the development of their eggs, and as

a result had fewer eggs that were smaller in size.

Fecundity of Blood-Fed *S. rugglesi*, 1998 & 1999

Generally, *S. rugglesi* collected during the 1998 and 1999 field seasons had substantially higher fecundity when maintained on either artificial sugar diet as opposed to distilled water alone. The fecundity data for *S. rugglesi* collected during the 1998 field season were however, very interesting.

The 1998 flies were small females with potentially poor nutrient reserves. These flies appeared to be using the two sugar meals differently. When maintained on the complex sugar meal of artificial honeydew *S. rugglesi* produced more eggs of smaller size relative to those maintained on the simple sugar meal of artificial nectar. When they are maintained on distilled water, regardless of their size, *S. rugglesi* still produce fewer smaller eggs.

The blood-fed *S. rugglesi* collected in 1999 were larger than those collected in 1998. The size of the 1999 flies suggests that they had more nutrient reserves. Results show that the 1999 *S. rugglesi* were not using their two sugar meals differently.

Effect of Egg Batch Size on Average Egg Length

The number of eggs matured by a female can vary within a species even though the dimensions and shape of those eggs are similar among individuals within a species (Davies and Peterson 1956; Chutter 1970). The *S. venustum* and *S. rugglesi* fecundity data suggest that changes in egg batch size do not always affect the egg length within a

batch. One of the factors that might be contributing to the variability of egg batch size could be due to the number of oocytes present at emergence (Chutter 1970). Black flies that emerge with a low number of oocytes may mature a lower proportion of their oocytes then females with high oocyte counts at emergence, however, the dimensions and shape of those eggs will be similar.

Diet Treatment Effects on Survival

Black flies need to obtain and utilize carbohydrates for basic energetic needs (Brenner and Cupp 1980). Therefore when flies are provided only distilled water they need to use internal carbohydrate reserves. Hence, it is expected they will have lower survival rates. Results show that *S. venustum* and *S. rugglesi* flies survive significantly longer when fed artificial sugar diets (artificial honeydew and artificial nectar) compared to a distilled water diet. Generally, most energy derived from carbohydrates is expended in flight. The flies used in these experiments were not able to fly very much. Therefore, the flies maintained on distilled water must have utilized a large portion of their internal carbohydrates in blood digestion and egg maturation.

Infectivity of Pekin Ducklings

During the 1998 16 ducklings were exposed and became infected. In 1999, 4 of the infected ducks kept from the 1998 season were exposed and a low rate of transmission to 16 new ducklings occurred. Light parasitemias were detected in all the ducks and only one of the infected ducks died (in 1999), presumably from the infection. *Leucocytozoon simondi* infections in the ducks during this study were lower than those

previously recorded in Algonquin Park (Bennett & Fallis 1960; Fallis *et al.* 1956; Roller & Desser 1973).

Transmission rates between ducks coincide with the abundance of *S. rugglesi*. In 1998, there were few *S. rugglesi* feeding on the ducks and as a result light infections occurred. The 1999 season was better although the transmission to other ducks was still quite low. There were more flies feeding in 1999 but not as many as had been previously recorded in the literature (Bennett 1960, 1963; Bennett and Fallis 1960; Desser 1967; Fallis and Anderson 1956; Fallis et al. 1951; Khan and Fallis 1970; Shewell 1955). Perhaps placement of the ducks in the water rather than on the beach might have increased the level of *L. simondi* infection in the ducks.

Infectivity Levels of *L. simondi* in *S. rugglesi*

Protozoan parasites move around the blood stream of the host. If the host has a light infection then there are few reproductively viable gametocytes moving through the bloodstream to be ingested by the vector (Desser 1967). *Leucocytozoon simondi* infections were light in the ducks therefore there was a 39 % successful transfer of *L. simondi* from infected ducks to 7% of *S. rugglesi* feeding on those infected ducks. Sporozoite rates have not been previously recorded in studies of black flies infected with *Leucocytozoon* spp., however, this observed sporozoite rate of 7% is comparable to rates reported in studies of mosquitoes infected with *Plasmodium* spp. (Hogg & Hurd 1997).

Survival & Infective Output of Vectoring Flies

The majority of the *S. rugglesi* that were vectoring *L. simondi* were flies maintained on artificial sugar diets. However, these flies did not harbour the highest number of sporozoites; rather, it was the flies maintained on distilled water. This suggests that there might be something going on in the midgut of the flies affecting the early stages of parasite development. Zieler *et al.* (2000) suggests that midgut carbohydrates are involved in malaria parasite (*P. gallinaceum*) ookinete binding, lectins bind to a specific ligand and inhibit ookinete adhesion. If midgut carbohydrates are involved in *L. simondi* development then *S. rugglesi* maintained on distilled water should have a better vectoring capacity since there are fewer carbohydrates to compete for binding sites with the developing gametocytes on the midgut walls.

The survival data for these vectoring flies show that none of the flies maintained on the distilled water live long enough to complete one gonotrophic cycle and find their next blood meal host. To be an efficient vector of *L. simondi* the flies must take their next blood meal hence *S. rugglesi* maintained on distilled water have no vectoring ability. Only the flies maintained on the two sugar meals have the ability to vector *L. simondi*.

CONCLUSION

Black fly feeding frequency, longevity and flight range, as well as the size and rate of digestion of both blood and sugar help determine the fly's importance as vectors of parasites. The present results substantiate that black flies have the ability to vector *L. simondi* from a natural host population and show that this ability is affected by sugar diets.

It is clear that the survival, fecundity and vectoring potential of individual black flies is affected by their artificial sugar diets, but that, compositions and concentrations of sugar diets only seem potentially important when flies are under stress i.e. starved, small or have poor larval reserves. This study demonstrated that individual black flies maintained on a sugar diets, will survive longer, have higher fecundity and hence have vectoring capacity unlike those maintained on water alone. However, flies maintained on distilled water were able to harbour almost twice as many infective stages of *Leucocytozoon*, even if not surviving long enough to vector.

In conclusion, it is apparent that sugar feeding is necessary for survival, fecundity and vectoring ability in black flies but the type of sugar meal, artificial honeydew or artificial nectar, does not have an affect when fed *ad libitum.*

FUTURE RESEARCH

In this study there were no differences found between type of artificial sugar diets, suggesting that concentration and composition of the diets might not be that important when fed *ad libitum*. Future research should focus on more complex sugars in the artificial honeydew diet, i.e., stachyose. Such sugars might be digested differently.

In addition to looking at the carbohydrates in sugar meals it would be interesting to look at the amino acids and their roles and effects. Mosquitoes provide essential nutrients to the parasite oocyst through the hemolymph. Concentrations of amino acids, such as valine, histidine and methionine in the mosquito hemolymph change oocyst infection develop (Beier 1998). This suggests that more than just carbohydrates in sugars could be playing roles in parasite development. This also suggests that parasites can use nutrients provided by the vector through the hemolymph. The next step would be to look at whether these nutrients affect longevity, fecundity and parasite transmission.

Additionally, future research should focus on the midgut physiology of interaction with parasites and viruses of black flies since the midgut is the first barrier a parasite must pass when it enters the insect (Zeiler et al. 2000). Very little is known about the interactions of the midgut with carbohydrates and parasites. In fact, certain carbohydrates may affect the early stages of parasite development. This suggests that the midgut might play a very important role in parasite transmission. Studies should examine molecular information on midgut carbohydrates and proteins that serve as points of attachment and invasion by parasites, viruses and other pathogens.

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APPENDIX I Studies in which the carbohydrates of nectar were analyzed.

Barnes *et. al.* 1995 (HPLC)
Ericaceae (heaths and heathers)
Nectar carbohydrates
-sucrose
-fructose
-glucose

Van Handel *et. al.* 1972 (chromatography)
Various plant species (extrafloral hibiscus, loosestrife, mangrove etc.)
Nectar carbohydrates
-sucrose
-fructose
-glucose
*no evidence for presence of maltose, raffinose, or other oligosaccharides

Baskin and Bliss 1969 (paper chromatography)
Orchidaceae (extrafloral orchids)
Nectar carbohydrates
- fructose
- sucrose (mostly)
- glucose
- raffinose
- unknown

APPENDIX II Studies in which the carbohydrates of phloem and honeydew were analyzed.

Tarczynski *et al.* 1992 (HPLC)
Bemisia tabaci
Honeydew carbohydrates
- fructose
- sucrose
- glucose
- raffinose
- stachyose
- trehalulose

Gossypium hirsutum (cotton)
Phloem carbohydrates
- sucrose
- raffinose series oligosac.; raffinose
- stachyose, verbascose and ajugose
- polyols; mannitol, sorbitol

Byrne and Miller 1990 (HPLC)
Bemisia tabaci
Honeydew carbohydrates
- sucrose
- glucose
- fructose
- stachyose
- raffinose
- galactose
- melezitose
- trehalulose

Poinsettia and Pumpkin
Phloem carbohydrates
- sucrose - both
- glucose - both
- fructose - both
- stachyose - pumpkin only
- raffinose - pumpkin only
- galactose - pumpkin only

Hussain *et. al.*, 1974 (paper chromatography)
Myzus persicae (aphid)
Honeydew carbohydrates
- fructose
- glucose
- sucrose
- trehalose
- unknown oligosaccharides
- melezitose

Raphanus sativus (radish)
Phloem carbohydrates
- fructose
- glucose

Mittler, 1958 (paper chromatography)
Tuberolachnus salignus (aphid)
Honeydew carbohydrates
- sucrose
- fructose
- glucose
- melezitose

Salix actifolia (willow)
Phloem carbohydrates
- sucrose

Auclair, 1963 (Mostly by paper chromatography)

Additional accounts of melezitose presence in honeydew

Lachnus muravensis Arnhart	larch
Lachnus robus L.	oak (approx. 46% of honeydew)
Lachnus pitchtae Mordwilko	fir
Lecanium spp.	spruce
aphid spp.	*Tilia spp.* (approx. 40% of honeydew)
aphid spp.	*Populs nigra* (approx. 40% of honeydew)
Tuberolachnus salignus (Gmelin)	willow
Coccus hesperidium L.	lemon plants
C. pseudomagnoliarum (Kuwana)	orange trees
Eucallipterus tiliae L.	lime tree
Planococcus citri (Risso)	lemon and grapefruit
Saissetia oleae (Bernard)	orange tree

*modified from Burgin (1996)

APPENDIX III Sugars found in honeydew, floral and extrafloral nectar

Type of Sugar	Source
Monosaccharides	
Glucose	-various floral nectars (main sugar) -various extrafloral nectars (main sugar) -honeydew
Fructose	-various floral nectars (main sugar) -various extrafloral nectars (main sugar) -honeydew
Galactose	-extrafloral nectar -honeydew
Mannose	-traces in floral nectar and fruits
Rhamnose	-extrafloral nectar
Disaccharides	
Sucrose	-various floral nectars (main sugar) -various extrafloral nectars (main sugar) -honeydew
Trehalulose	-honeydew (insect synthesized)
Maltose	-coccid honeydew -floral nectar
Melibiose	-floral nectar -eucalyptus exudates (manna)
Trisaccharides	
Raffinose	-primarily in honeydew (plant derived and insect synthesized) -some floral nectars
Melezitose	-primarily in honeydew (insect synthesized) -rare in extrafloral nectars

Erlose	-honeydew
Tetrasaccharides	
Stachyose	-honeydew

*modified from Wackers 2001

APPENDIX IV Laboratory temperatures taken inside the cupboard where the experimental flies were kept, Wildlife Research Station Algonquin Provincial Park, for the 1998 and 1999 field seasons.

Date	1998 Field season Temperature °C	1999 Field season Temperature °C
05 May	N/A	21.5
06 May	N/A	21.5
07 May	N/A	19.5
08 May	N/A	19.3
09 May	N/A	19.3
10 May	N/A	19.5
11 May	N/A	19.9
12 May	N/A	21.3
13 May	N/A	20.0
14 May	N/A	19.8
15 May	N/A	21.0
16 May	N/A	19.5
17 May	25.0	20.5
18 May	21.0	23.5
19 May	24.0	17.8
20 May	21.0	19.9
21 May	20.0	17.5
22 May	24.0	18.0
23 May	21.0	16.0
24 May	19.0	18.0
25 May	21.0	17.5
26 May	19.0	16.0
27 May	18.0	15.4
28 May	18.0	21.8
29 May	22.0	21.0
30 May	18.0	24.5
31 May	20.0	22.0
01 June	15.0	22.5
02 June	15.0	22.0
03 June	10.0	18.0
04 June	17.0	19.5
05 June	20.0	20.0
06 June	20.0	22.0
07 June	21.0	21.0

08 June	21.0	23.0
09 June	21.0	23.5
10 June	20.0	21.0
11 June	21.0	26.5
12 June	21.0	24.0
13 June	24.0	25.5
14 June	23.0	20.0
15 June	N/A	17.0
16 June	N/A	18.0
17 June	N/A	19.0
18 June	N/A	21.0
19 June	N/A	16.0
20 June	N/A	17.8
21 June	N/A	20.5
22 June	N/A	22.0
23 June	N/A	21.5
24 June	N/A	21.5
25 June	N/A	21.0
26 June	N/A	21.0
27 June	N/A	22.0
28 June	N/A	24.0
29 June	N/A	21.5
30 June	N/A	19.5
01 July	N/A	20.5
02 July	N/A	19.5
03 July	N/A	23.0
04 July	N/A	26.0
05 July	N/A	24.0
06 July	N/A	24.5
07 July	N/A	24.5
08 July	N/A	24.5
09 July	N/A	21.5
10 July	N/A	21.5
Average	**20.0**	**20.8**

APPENDIX V The length of time (in days) blood-fed *S. venustum* survived on one of three diet treatments (artificial honeydew – AH; artificial nectar – AN; distilled water – dH_20) during the 1999 field season. The >10 category contained flies killed by the investigator on day 11 for experimental purposes.

Diet treatment	Survival days			Total number of individuals
	<5	5-10	>10	
AH	3	6	36	**45**
AN	0	10	39	**49**
dH_20	9	33	0	**42**

APPENDIX VI The length of time (in days) blood-fed *S. rugglesi* survived on one of three diet treatments (artificial honeydew – AH; artificial nectar – AN; distilled water – dH_20) during the 1998 field season. The >10 category contained flies killed by the investigator on day 11 for experimental purposes.

Diet treatment	Survival days			Total number of individuals
	<5	5-10	>10	
AH	0	6	29	**35**
AN	0	3	37	**40**
dH_20	1	19	1	**21**

APPENDIX VII The length of time (in days) blood-fed *S. rugglesi* survived on one of three diet treatments (artificial honeydew – AH; artificial nectar – AN; distilled water – dH_20) during the 1999 field season. The >10 category contained flies killed by the investigator on day 11 for experimental purposes.

Diet treatment	Survival days			Total number of individuals
	<5	5-10	>10	
AH	0	17	66	**83**
AN	0	17	72	**89**
dH_20	0	68	0	**68**

APPENDIX VIII During the 1998 field season 10 infected *S. rugglesi* were collected from 16 ducklings and placed on one of four sugar meal diets (artificial honeydew - AH, artificial nectar - AN, natural honeydew - H, distilled water - dH_2O). The number of sporozoites found in the salivary glands of each fly (2 glands/fly) and the number of gametocytes/12,000 RBC for each duck on which an individual fly fed, was recorded. A ratio (no. sporozoites in fly/no. gametocytes in duck) was calculated to represent the total infective output of an individual fly.

Treatment	No. of sporozoites	No. of gametocytes	Ratio
AN	1	10	**0.1**
AN	2	2	**1**
AN	5.3	4	**1.3**
AN	1	2	**0.5**
AN	14	22	**0.6**
AN	20	2	**10**
AN	8	10	**0.8**
AH	12	2	**6**
AH	2	4	**0.5**
AH	1.3	1	**1.3**

APPENDIX IX During the 1999 field season 11 infected *S. rugglesi* were collected from 20 ducklings and placed on one of three sugar meal diets (artificial honeydew - AH, artificial nectar - AN, distilled water - dH_2O). The number of sporozoites found in the salivary glands of each fly (2 glands/fly) and the number of gametocytes/12,000 RBC for each duck on which an individual fly fed, was recorded. A ratio (no. sporozoites in fly/no. gametocytes in duck) was calculated to represent the total infective output of an individual fly.

Treatment	No. of sporozoites	No. of gametocytes	Ratio
AN	13	3	**4.3**
AN	1	5	**0.2**
AH	5	1	**5**
AH	1.3	1	**1.3**
AH	4	3	**1.3**
AH	8	3	**2.7**
AH	4	4	**1**
dH_2O	6	1	**6**
dH_2O	14	2	**7**
dH_2O	4	1	**4**
dH_2O	7	6	**1.2**