THE QUANTITY OF TOTAL LIPIDS IN *CULEX TARSALIS* COQUILLETT (DIPTERA: CULICIDAE) UNDER LABORATORY AND FIELD CONDITIONS IN MANITOBA

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Lynn Manaigre

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Dedicated to:

my father and mother,

Maurice and Yolande Manaigre

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Abstract

The most characteristic feature of overwintering insects is the accumulation of a large lipid reserve prior to the overwintering period. These reserves of metabolizable energy ensure that the insect will survive prolonged periods of metabolic activity without feeding. Culex tarsalis Coquillett overwinters as an inseminated, nulliparous adult. The objective of this study was to identify the various environmental factors affecting lipid accumulation and depletion in the summer generations and in overwintering females.

The total lipid content of adult Cx. tarsalis females was determined using Van Handel's (1985) colorimetric assay. The use of soybean oil as a standard to determine total lipid content provided an accurate and reliable representation of total lipids in mosquitoes. The total lipid content of newly emerged, fed and starved Cx. tarsalis females indicated that lipids increased with sucrose feeding. The baseline lipid level (ie. starved to death), representing all non-nutritional and structural lipids was 104 μg total/female.

The relationship between temperature, development and lipid content was investigated to determine the duration of larval development at different constant temperatures, the

upper and lower thresholds of development and the relationship between temperature and the lipid content of newly emerged females. These results indicated that the low temperature threshold for Cx. tarsalis development was 14°C and the upper threshold was 34°C or above. At low temperatures (ie. 14 and 17°C), an increase in development time and larger quantities of lipid were observed at emergence. At higher temperatures (ie. 25 to 34°C), a decrease in development time and decreased quantities of lipid were observed at emergence. Therefore, temperature significantly affected development time and total lipid content.

The relationship between environmental temperature and photoperiod on total lipid content was investigated in 1986 to determine the period of the summer or fall season in which lipid accumulation occurred in natural populations. These results indicate that total lipid content in field population was not closely related to air temperature and development time. Therefore, the possible role of photoperiod was proposed.

The field population of Cx. tarsalis females was also sampled in 1987 at Glenlea, Manitoba to determine if the fall generation of females had a higher lipid content than summer generations. These data indicate that lipid levels were higher in July than in August. The field population of females was sampled weekly using SSAM traps (attractants=

CO_2 and light). Unfortunately, the attraction of females by these attractants represents a cohort of unknown age and physiological condition, and lipid correlations cannot be determined. The increased availability of nectar sources in mid-summer likely produced larger lipid levels in July.

The effect of different experimental photoperiods and temperatures on lipid accumulation was investigated in 1987 in controlled environment conditions. These results indicate that females accumulated significantly larger quantities of total lipid at 16L:8D than at 12L:12D at both 21 and 26.5°C. Although long photoperiod did affect lipid levels, it appeared of limited advantage to the overwintering female.

The rate of lipid depletion in an experimental overwintering population was investigated in 1986 and 1987 to determine the relationship between overwintering survival and lipid reserves. The low survival of females in 1986 was attributed to an excess accumulation of mold in the overwintering cages. In 1987, the lipid content of the last January survivors indicated that females did not die of starvation.

CHAPTER I

Introduction

Lipids, including triglycerides comprise a large group of structurally heterogeneous compounds that assume a number of disparate physiological functions in insects (Downer 1978; 1985). In general, the amount of lipid present in an insect varies between developmental stages, and is influenced by numerous internal and external factors such as nutrition, temperature, sex, starvation, and diapause (Beenakkers 1983).

One characteristic feature of overwintering insects is the accumulation of a large triglyceride reservoir prior to the overwintering period. Many studies propose that triglyceride accumulation is modified by temperature and photoperiod to ensure overwintering survival. In addition, increased levels of unsaturated fatty acids are believed to influence the permeability and fluidity of biological membranes to decrease the rate of substrate transport, particularly from the fat body. A thorough understanding of the role of glycerides in insect bioenergetics and the various metabolic interactions that contribute to the synthesis and degradation of lipids is thus necessary to

understand the physiological requirements and metabolic processes of overwintering insects.

Culex tarsalis Coquillett overwinters as an inseminated, nulliparous adult. Numerous studies have focused on the overwintering habitat and survival of the females (Keener 1952; Rush et al. 1958; Rush 1962; Bellamy and Reeves 1963; Shemanchuk 1965; Kliewer et al. 1969; Hudson 1977; Mitchell 1979; Nelson 1980); however, few studies have investigated the physiological requirements for overwintering survival.

In order to identify the various environmental factors affecting lipid accumulation and depletion in the summer generations and in overwintering Culex tarsalis Coquillett females, the objectives of this study were:

1. To determine the period of the summer or fall season in which lipid accumulation occurred in natural populations.
2. To determine if there was a variation in lipid content throughout the summer, using experimental field populations.
3. To determine the effect of different experimental photoperiods and temperatures on lipid accumulation, under controlled environment conditions.
4. To determine the rate of lipid depletion in an experimental overwintering population.

CHAPTER II

Literature Review

Introduction

Lipids comprise a large group of structurally heterogeneous compounds that are characterized by their solubility in nonpolar solvents, such as ether or chloroform (Gilby 1965; Gilbert 1967; Lehninger 1982). Lipids include free fatty acids, fatty acid esters of glycerol, sphingosine, phosphoglycerides, waxes, terpenes, steroids, prostaglandins, lipoproteins and glycolipids (Downer 1985). In general, insect lipids function as essential and integral components of cell membranes and appear to be involved in most cellular activity such as energy production and storage, protein synthesis, and cellular respiration (Fast 1964, Keeley 1978, Marinotti and Bianchi 1986).

In insects, the greatest concentration of lipid is found in three tissues: the fat body, the hemolymph and the cuticle (Downer and Matthews 1976). The dominant lipid class in the insect fat body is clearly triglycerides (Chino and Gilbert 1965). Functionally, the fat body may be considered analogous to the vertebrate liver and adipose

tissue, serving not only for the storage of lipid, protein and carbohydrate, but also as an organ of intermediary metabolism (Kilby 1963; Patton 1963). Intermediary metabolism denotes the cellular processes that take place between the introduction of the energy source and the liberation of energy (ie. the chemical degradation and synthesis of specific compounds such as lipid, protein and carbohydrate) (Patton 1963). The primary lipid component of insect hemolymph is diglyceride, with triglyceride and free fatty acids also present in smaller quantities (Gilbert 1967; Gilbert and O'Connor 1970; Gilbert and Chino 1974; Chino 1981). The main functions of insect hemolymph are the transport of nutrient substrates to sites of utilization and the delivery of metabolic wastes to the excretory system. Cuticular lipids will not be discussed since phospholipids are not directly involved in the accumulation and depletion of overwintering lipid reserves.

The most characteristic feature of overwintering insects is the accumulation of a large lipid reserve prior to the overwintering period. Overwintering is a type of diapause, or a period of developmental arrest which ensures that the active stages of morphogenesis are completed in favourable environmental conditions (Downer and Matthews 1976; Beenakkers et al. 1981). In most diapausing insects only one stage, characteristic of a species, will enter

diapause. The various factors affecting overwintering survival include the accumulation of an adequate quantity of lipid reserve, the selection of a suitable hibernaculum, and the development of cold hardiness (Danks 1987).

Lipid Composition

Mono-, di- and triglycerides are esters of the alcohol glycerol with one, two or three fatty acid molecules, respectively (Lehninger 1982). The analyses of total lipid extracts from a variety of insect species indicate that the most abundant lipid class is triglycerides (Chino and Gilbert 1965; Downer 1981, 1985). Triglycerides are nonpolar, hydrophobic molecules since they contain no electrically charged or highly polar functional group (Lehninger 1982). Triglycerides differ from one another in the identity and position of the three esterified fatty acids, however, all glycerides undergo hydrolysis when acted upon by the enzyme lipase (Chapman 1982).

Lipid reserves, or triglycerides, have several advantages over carbohydrate reserves, or glycogen (Chapman 1982; Downer 1985). These advantages include a higher caloric content per unit weight of substrate, the liberation upon oxidation of two times more metabolic water than carbohydrate, and the capacity for storage in an anhydrous form. Therefore, lipid tends to be the primary source of

metabolic energy that is accumulated in insects which undergo prolonged periods of metabolic inactivity without feeding (eg. embryogenesis, pupation, migration and diapause) (Downer and Matthews 1976; Downer 1985). The pattern of distribution and the absolute quantity of triglyceride within an insect at any particular time is subject to the principles of supply and demand; thus, triglyceride levels fluctuate according to prevailing physiological requirements (Downer 1981; 1985).

The characteristic building-block components of most lipids are long-chain organic acids having from 4 to 24 carbon atoms and a single carboxyl group (Lehninger 1982; Christie 1982). The long, non-polar hydrocarbon tail of fatty acids may be fully saturated (ie. containing only single bonds) or unsaturated (ie. containing one or more double bonds). Fatty acids differ from one another in chain length and in the presence, number and position of their double bonds. In cells and tissues, fatty acids are generally present in a covalently bound form, from which they can be released by chemical or enzymatic hydrolysis. In insects, as in other forms of life, the major proportion of the fatty acid complement is represented by eight fatty acids (Fast 1964; 1970; Gilbert 1967; Thompson 1973). These include the saturated fatty acids, myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0); the

monounsaturated fatty acids, myristoleic acid (C14:1), palmitoleic acid (C16:1), and oleic acid (C18:1); and the polyunsaturated fatty acids, linoleic acid (C18:2) and linolenic acid (C18:3) (Thompson 1973; Downer 1985). Since insects cannot synthesize polyunsaturated fatty acids, these acids are only detected when they are present in the diet (Fast 1964; Thompson 1973).

Lipid Digestion and Transport

The principal lipid class that is digested in the insect gut is triglycerides (Downer 1978). The digestion of triglycerides involves the hydrolysis of long-chain fatty acid esters to produce smaller molecules of diglycerides, monoglycerides and free fatty acids (Gilbert 1967; Chapman 1982). It is theorized that the products of lipolysis enter the hemolymph in the form of diglyceride (Gilbert 1967). Since insect hemolymph does not have the capacity to synthesize triglycerides, the absorbed diglycerides are reconstructed into triglycerides in the fat body.

Diglyceride is largely transported in association with two classes of lipoproteins: diglyceride-carrying lipoprotein I (DGLP-I) and diglyceride-carrying lipoprotein II (DGLP-II) (Chino 1981). On the basis of density, insect DGLP-I and DGLP-II are comparable to the high density lipoprotein and the very high density lipoprotein of the

mammalian lipoprotein system (Chino et al. 1969; Beenakkers et al. 1981). Diglyceride-carrying lipoprotein I and diglyceride-carrying lipoprotein II have been renamed lipoprotein I (LP-I) and lipoprotein II (LP-II), respectively (Gilbert and Chino 1974). It is now evident that LP-I has multiple functions, such as transporting diglyceride, cholesterol, and hydrocarbons from sites of storage, absorption, or synthesis to sites where they are utilized as metabolic fuels, precursors for triglyceride and phospholipid synthesis, or as structural components of the cell membrane and cuticle (Havel et al. 1980; Chino 1981). Gilbert and Chino (1974) propose that LP-I is predominantly synthesized in the larval fat body and released into the hemolymph. In the hemolymph, it functions both as a carrier of lipid during the insect's life and as a regulatory agent in eliciting the release of diglyceride from the fat body (Gilbert and Chino 1974; Chino et al. 1977; Chino 1981). The intricate mechanisms involved in "loading" and "unloading" of the lipoprotein have yet to be determined. LP-II, or the female-specific vitellogenin, does not accept diglyceride from the fat body (Downer 1978). The loading of this fraction with lipid must either occur before its release from the fat body or from other sources.

To study the relationship between hemolymph and fat body glycerides, Beenakkers and Gilbert (1968) examined the

fatty acid composition of pupal and adult Hyalophora cecropia (L.). The difference in the fatty acid composition of fat body and hemolymph diglycerides suggests that glyceride release does not occur at random, but is restricted to neutral lipids with a specific fatty acid composition (Beenakkers and Gilbert 1968). The alternative explanation is that the fat body may contain two different glyceride pools, only one of which is freely accessible to the hemolymph. The overall difference in fatty acid composition between the fat body and hemolymph glycerides supports the idea of chemical and/or structural compartmentalization in the fat body (Beenakkers and Gilbert 1968; Gilbert and O'Connor 1970; Gilbert and Chino 1974; Municio et al. 1980). This hypothesis is in general agreement with studies of mammalian adipose tissue. In mammalian adipose tissue, it is believed that there is an active compartment in which glycerides are in direct interchange with the fatty acids of the medium, and a storage compartment where the bulk of cellular lipids are located (Gilbert and Chino 1974). Therefore, Beenakkers and Gilbert (1968) hypothesize that in insects, triglycerides in the storage compartment of the fat body are hydrolysed, and the resulting products (ie. presumably monoglycerides and free fatty acids) are transported to the active compartment where glycerides are resynthesized. This is in contrast to

the mammalian adipose tissue where only free fatty acids are released. This two-compartment theory is consistent with other data suggesting that diglyceride release is an endergonic process (ie. energy is required for glyceride synthesis in the active compartment) and the release of free fatty acids is a passive process involving simple diffusion (Beenakkers and Gilbert 1968; Gilbert and O'Connor 1970; Gilbert and Chino 1974).

Lipid Utilization and Synthesis

Fate of Glycerol

The complete hydrolysis of diglyceride yields glycerol and free fatty acids (Downer 1978; 1985). Although some tissues, particularly flight muscle, can oxidize glycerol at a slow rate, most of the glycerol that is produced by lipolysis is phosphorylated to glycerol-3-phosphate by the enzyme glycerol kinase (Bailey 1975; Downer 1985).

Fatty Acid Oxidation

In insects, the energy contained within a fatty acid molecule is made available to a cell by the sequential removal of two-carbon units in the form of acetyl-S-coenzyme A (acetyl-S-CoA) (Beenakkers et al. 1981; Downer 1985). The units of acetyl-S-CoA undergo condensation with oxaloacetate to form citrate, which is subsequently oxidized to CO_2 and

H_2O in the tricarboxylic acid (TCA) cycle (Bailey 1975; Downer 1985). The sequential shortening of the fatty acid chain is called ß-oxidation. It is now evident that in addition to furnishing acetyl-S-CoA to the TCA cycle, ß-oxidation also produces the reduced coenzymes $FADH_2$ and $NADH_2$, which are oxidized through the electron transport chain to yield additional molecules of ATP (Downer 1985). The initial activation of fatty acid oxidation is catalyzed by microsomal fatty acid thiokinase, whereas the enzymes of ß-oxidation reside within the inner membrane of the mitochondria (Beenakkers et al. 1981). Transport of fatty acyl CoA across the mitochondrial membrane is facilitated, in many species, by the formation of the readily permeable carnitine ester, fatty acyl carnitine (Downer 1978; 1985; Beenakkers et al. 1981). This reaction is catalyzed by the enzyme carnitine acyl transferase I. On the other side of the mitochondrial membrane, the fatty acyl carnitine is reconverted to carnitine and fatty acyl-CoA by the enzyme carnitine acyl transferase II.

Although carnitine is an obligatory cofactor for ß-oxidation in some species, it is not universally required among all insects (Downer 1978; 1985; Beenakkers et al. 1981). Insects that are primarily carbohydrate-utilizers use carnitine to facilitate the rapid oxidation decarboxylation of pyruvate to acetyl-S-CoA, whereas insects

that are primarily lipid-utilizers either require carnitine for fatty acid transport across the mitochondrial membrane (ie. Manduca sexta (L.), Locusta migratoria (L.)), or are carnitine independent for fatty acid oxidation (ie. Prodenia eridania (Cramer), Autographa gamma (L.)) (Downer 1985). Since the permeability of membranes for long-chain fatty acids increases with temperature, Downer (1978) postulates that the differing usage of carnitine in lipid-utilizing species may be related to temperature. The measurement of fatty acid oxidation rates by isolated mitochondria at various temperatures in different species is now required to understand fatty acid oxidation in lipid-utilizing insects (Keeley 1981).

Ketone Body Formation

Although fatty acid oxidation normally proceeds to completion without the accumulation of intermediates (ie. fatty acids oxidized to CO_2 and H_2O via the TCA cycle), the ketone bodies, acetoacetate and D-3-hydroxybutyrate may also be formed under certain conditions (Downer 1978). In general, ketone bodies serve a glucose-sparing role and provide a major source of energy for the insect brain and nervous system during periods of starvation (Downer 1985).

Fatty Acid Synthesis

In insects, long-chain saturated and monounsaturated fatty acids are synthesized from acetyl-CoA via two distinct systems, the cytoplasmic or malonyl system and the mitochondrial system (Fast 1970). The cytoplasmic fatty acid synthase system is primarily responsible for de novo synthesis of saturated fatty acids (ie. acetyl-CoA to palmitate) whereas the mitochondrial system results in the elongation of preformed fatty acids by successive additions of acetyl-CoA (eg. C16 to C18) (Downer 1978).

It is now evident that the fatty acid synthase system catalyses the following reaction,

acetyl-CoA + 7 malonyl-CoA + 14 NADPH + 14 H^+---------->

1 palmitic acid + 7 CO_2 + 8 CoA + 14 $NADP^+$ + 6 H_2O

in which one molecule of acetyl-CoA and seven molecules of malonyl-CoA yield one molecule palmitic acid (C16:0) (Lehninger 1970; 1982). Chain growth during fatty acid synthesis starts at the carboxyl group of the primer molecule, acetyl-CoA, and proceeds by successive additions of acetyl residues at the carboxyl end of the growing fatty acid chain (Lehninger 1970). Each successive acetyl residue is derived from two of the three carbon atoms of malonyl-CoA. The third carbon atom of malonyl-CoA is lost as CO_2. The reducing power in this sequence is NADPH.

The seven enzymes involved in fatty acid biosynthesis

are intimately associated with an acyl carrier protein (ACP) (Gilbert 1967; Lehninger 1982). This ACP functions as the carrier of fatty acyl intermediates from one enzyme active site to the next via the formation of thiol esters with the prothetic group, 4'phosphopantetheine. The formation of the 4C-butyryl intermediate, butyryl-S-synthase, completes the first of seven cycles en route to the end product of the fatty acid synthase complex, palmitoyl-ACP (Lehninger 1970). Free palmitic acid can then be released by hydrolysis. Although it is evident that this pathway results in the production of fatty acids containing an even-number of carbon atoms, a small percentage of "odd-numbered" fatty acids have also been reported in insects (Thompson 1973; Downer 1985). This observation can be explained by the occasional substitution of propionyl-CoA for the primer molecule, acetyl-CoA, to which are added successive two-carbon units via condensations with malonyl-CoA (Lehninger 1970).

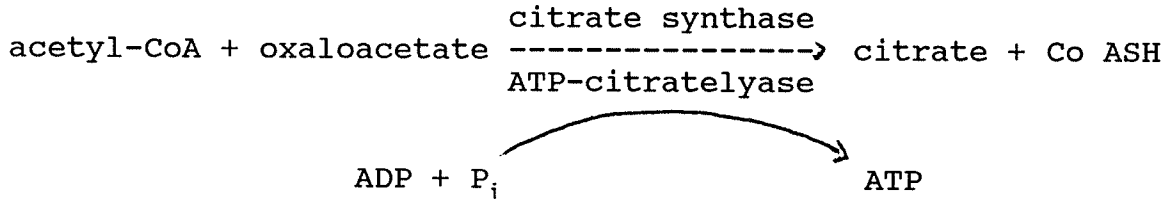
The elongation of palmitic acid (C16:0) to stearic acid (C18:0), and desaturation reactions (ie. palmitic and stearic acids desaturated to yield palmitoleic and oleic acids, respectively), are the primary functions of the mitochondrial fatty acid synthase system (Fast 1970). The fatty acid elongation system is believed to proceed by exactly the same pathway as palmitate synthesis; however,

double bonds are introduced into the fatty acid chain by an oxidative reaction that is catalized by fatty acyl-CoA oxygenase (Lehninger 1982). This system requires ATP, DPNH, TPNH and acetyl-CoA. In fact, Stephen and Gilbert (1969) confirmed that H. cecropia can elongate and desaturate existing fatty acids, and that palmitate appears to be the primary substrate for chain elongation. Similar results are observed in Anthonomus grandis Boheman (Lambremont and Blum 1963; Lambremont et al. 1964), Bombyx mori (L.)(Sridhara and Bhat 1965), and Musca autumnalis De Geer (Pitts and Hopkins 1965; Valder et al. 1969; Gonzalez-Buitrago et al. 1979). Although most studies indicate that insects can elongate and desaturate existing saturated fatty acids, it is now known that they cannot elongate unsaturated fatty acids or introduce a second or third double bond in any fatty acid (Stephen and Gilbert 1969; Gilbert and O'Connor 1970).

The overall rate of fatty acid biosynthesis is primarily determined by the rate of the following acetyl-CoA carboxylase reaction,

$ATP + acetyl\text{-}CoA + CO_2 + H_2O \rightarrow malonyl\text{-}CoA + ADP + Pi + H^+$

(Lehninger 1970; 1982). The major stimulatory modulator of the regulatory enzyme, acetyl CoA carboxylase, is citrate. Whenever the citrate level in the mitochondria rises, citrate moves out of the mitochondria via the acetyl group shuttle:



and becomes both the precursor of cytosolic acetyl-CoA and the allosteric signal for the activation of acetyl-CoA carboxylase. This indicates that the citric acid cycle is amply supplied with fuel and that excess acetyl-CoA is to be stored as fat (ie. increase malonyl-CoA, increase fatty acid synthesis). On the other hand, when there is an overproduction of palmitoyl-CoA (end product of fatty acid synthesis and immediate precursor of triglycerides), cytosolic citrate serves as an allosteric signal that inhibits acetyl-CoA carboxylase (ie. decrease malonyl-CoA, decrease fatty acid synthesis). This information clearly demonstrates that the availability of malonyl-CoA is dependent upon the activity of acetyl-CoA carboxylase; hence, this enzyme may play an important role in determining fatty acyl chain length (Downer 1985).

Glyceride Synthesis

In insects, as in other forms of life, there are two major biosynthetic pathways for the formation of glyceride in the microsomal fraction of the fat body; the monoacylglycerol pathway and the α-glycerophosphate pathway (Downer 1978; 1985). The two major precursors in the

α-glycerophosphate pathway are fatty acyl-CoA and glycerol-3-phosphate (Lehninger 1970; 1982). Glycerol-3-phosphate can be derived from two different sources. Its normal precursor is dihydroxyacetone phosphate, which is generated during glycolysis by the action of cytosolic NAD-linked, glycerol-3-phosphate dehydrogenase:

dihydroxyacetone phosphate + NADH + H^+ ⟷

glycerol-3-phosphate + NAD^+

The second source of glycerol-3-phosphate is free glycerol, a product of glyceride degradation (lipolysis). This reaction is catalyzed by the enzyme glycerol kinase:

ATP + glycerol ----------→ glycerol-3-phosphate + ADP

The first step in the synthesis of glyceride is the acylation of glycerol-3-phosphate by two molecules of fatty acyl-CoA to yield phosphatidic acid (Lehninger 1970; 1982). Phosphatidic acid then undergoes hydrolysis by phophatidic phosphohydrolase to yield 1,2-diglyceride. This molecule reacts with a third molecule of fatty acyl-CoA to yield triglyceride by the action of diglyceride acyltransferase. In the alternative pathway, 2-monoacylglycerol is directly acylated by monoacyl-glycerol acyltransferase to yield 1,2-diglyceride. Phosphatidic acid is no longer an intermediate in the monoacylglycerol pathway. Downer (1985) proposes that the two pathways for the synthesis of glycerides serve two different physiological functions in the insect: the

monoacylglycerol pathway is primarily concerned with the synthesis of diglycerides for release during periods of high energy demand, whereas the α-glycerophosphate pathway functions in the synthesis of storage triglycerides. This proposal is based on the fact that substrate is present in low concentrations during periods of high energy demand, and free fatty acyl-CoA is incorporated into the monoacylglycerol pathway for the synthesis of energy-supplying diglycerides (Downer 1978; 1985). Alternatively, during periods of rest and/or absorption, glycerol-3-phosphate is present in appreciable quantities to participate in the formation of storage triglycerides via the α-glycerophosphate pathway. This hypothesis certainly provides a useful model for the control of carbohydrate and lipid metabolism in insects; however, further investigations are required before the model can be applied to all insect species.

Municio et al. (1975a; 1975b) and Gonzalez-Buitrago et al. (1979) investigated the difference in the regulation of glyceride synthesis during Ceratitis capitata larval and pharate adult stages of development. These studies demonstate that the major factor contributing to developmental differences in triglyceride metabolism is the differing mitochondrial acyltransferase activity in the various stages of insect development. That is, all fatty

acids are more efficiently incorporated by larval homogenates than by pharate adult homogenates. Also, fatty acids are primarily incorporated into triglycerides by larval homogenates whereas free fatty acids largely predominate in pharate adult homogenates (Municio et al. 1971; 1975a; 1975b; Gonzalez-Buitrago et al. 1979). From these results, it is evident that the larval stage of development possesses the highest fatty acid incorporating ability and that free fatty acids preside in pharate adult homogenates.

Endocrine Regulation of Lipid Metabolism

Lipid metabolism in insects is directly and/or indirectly affected by a number of hormones which include the adipokinetic hormone (AKH), the hypolipemic hormone, and the juvenile hormone (JH) (Downer 1978; 1985; Beenakkers 1983).

Adipokinetic hormone is a neutral hyperlipemic factor which is synthesized and stored in the glandular lobes of the corpora cardiaca (CC) (Candy 1981; Downer 1985). Following flight activity in Schistocerca gregaria (L.), Candy (1981) confirmed that the CC produced an AKH which stimulated a three to four-fold increase in the concentration of hemolymph diglycerides. Similar results are observed in Locusta migratoria (L.), Manduca sexta (L.)

(Ziegler and Schulz 1986) and Tenebrio molitor (L.) (Bailey 1975). In light of these observations, it is proposed that AKH interacts with a specific adenylate cyclase receptor on the plasma membrane of the fat body to elevate intracellular levels of cyclic nucleotide (Candy 1981). The elevated concentrations of cAMP can then activate a protein kinase to enhance diglyceride mobilization from the fat body. This activation sequence is equivalent to the hormone-sensitive lipase system in mammalian adipose tissue. Unfortunately, a hormone-sensitive lipase in insect fat body has not yet been discovered; hence, the proposed mechanism of action of AKH remains questionable.

In addition to the stimulation of lipid release from the fat body, it is proposed that AKH may have a direct effect on the process of diglyceride-carrying lipoprotein formation (Beenakkers 1983). This observation certainly suggests a possible mechanism by which the supply, storage, and/or consumption of fatty acids may be regulated; however, this proposed mechanism remains debatable due to inconclusive evidence (Chino 1981; Beenakkers 1983).

In contrast to the proposed AKH activity in S. gregaria, studies by Downer (1972) and Steele (1976) on the American cockroach, Periplaneta americana (L.) suggest the occurrence of a hypolipemic factor. The site of synthesis of this hormone appears to be the storage lobe of the CC.

Downer (1972) demonstrated that the injection of cockroach CC extract into locust hemolymph produced a hyperlipemic response in the locust, and that the injection of locust CC extract into cockroach hemolymph produced a hypolipemic response in the cockroach. It appears, therefore, that the same or similar substances are producing opposite effects in the cockroach and the locust (Downer 1972; Steele 1976). From these observations, it is suggested that the differential response to the CC extracts reflects the physiological differences between these two species. The locust is primarily a lipid-utilizer to ensure migratory flight, and the adipokinetic hormone stimulates the release of diglyceride from the fat body. In contrast, the cockroach is primarily a carbohydrate-utilizer to permit terrestrial locomotion, and the same CC factor facilitates the absorption and storage of dietary lipid by the fat body. As a result, the specificity of the observed responses in S. gregaria and P. americana resides at the site of action - the fat body.

The corpus allatum synthesizes juvenile hormone, which serves a number of apparently unrelated physiological functions in development and reproduction (Downer 1972; 1978). These processes include the modulation of the developmental sequence, the determination of diapause and/or migratory behaviour, the functioning of accessory sex

glands, the expression of various types of polymorphism, and the regulation of reproductive behaviour (Keeley 1978; Downer 1985). Steele (1976) considered the various effects of JH on fat body lipogenesis and concluded that JH has a dual effect on lipid metabolism. That is, in the presence of JH, lipid syntheis is suppressed and protein synthesis is stimulated. In the absence of JH, the apoprotein moieties that normally facilitate the release and transport of lipid from the fat body are not produced. This results in an accumulation of lipid in the fat body. The reduced rate of protein synthesis also produces numerous non-lipid precursors (ie. carbohydrate and amino acids) for conversion to storage triglycerides (Steele 1976). The accumulation of lipid following allatectomy has been confirmed in several species, including Drosophila melanogaster Meigen, Calliphora erythrocephala (Meigen), P. americana, Phormia regina (Meigen), S. gregaria and L. migratoria (Beenakkers 1983).

Unfortunately, fat body hypertrophy following allatectomy is not universally observed in all insect species (Van Handel and Lum 1961; Downer 1978; 1985). Van Handel and Lea (1965; 1970) found that in Aedes taeniorhynchus (Wied.), Aedes aegypti (L.) and Aedes sollicitans (Walker), removal of the CA or the median neurosecretory cells (MNC), greatly increased the storage

capacity of the fat body for glycogen at the expense of triglycerides. These observations, in addition to the lack of *in vitro* systems and purified neurohormones, prevent a more complete understanding of the action of JH in lipid metabolism.

Overwintering of Adult Mosquitoes

Most insects overwinter in sheltered sites, such as beneath bark or snow, in hollows, caves, or in leaf litter (Danks 1978). One of the most characteristic features of overwintering insects is the accumulation of nutrients prior to the overwintering period (Downer and Matthews 1976; Beenakkers *et al*. 1981; Danks 1987). Other features may include a complete arrest of reproductive activity and a suspension of feeding (Reisen 1987).

Culex tarsalis, the primary vector of Western Equine Encephalitis (WEE), overwinters as an inseminated, nulliparous female (Schaefer and Miura 1972; Reisen *et al*. 1983; 1986a; 1986b). Since *Cx. tarsalis* females overwinter as nulliparous females, it is now evident that females are not winter reservoirs of WEE.

The overwintering sites of *Cx. tarsalis* include animal burrows in Southern Alberta (Shemanchuk 1965; Hudson 1977, 1978), rockpiles in Washington (Rush *et al*. 1958), food storage cellars in Nebraska (Keener 1952), mines in Colorado

(Blackmore and Winn 1956) and in Nevada (Chapman 1961), badger burrows in Alberta (Shemanchuk 1965; Hudson 1977), and talus slopes in Oregon (Rush 1962), Washington (Harwood 1962) and in Utah (Trent 1960). In warmer temperate latitudes, reproductive diapause may be achieved with or without the suspension of carbohydrate feeding (Reisen 1987).

In addition to the above sites, overwintering mosquitoes have been found in many other locations. Anopheles earlei was found in a shed in Southern Manitoba (Shemanchuk 1965) and in empty buildings in Alaska (Hopla 1970). Culex territans was found in the nests of the tree squirrel (Tamiasciurus sp.) in Alaska (Hopla 1965) and in clumps of grass in Alaska (Hopla 1965). Culiseta alaskaensis was found in the burrows of the artic ground squirrel (Spermophilus sp.) in Alaska (Hopla 1970). Culiseta inornata was found in the nests of the wood rat (Neotoma sp.) in Riverside County, California (Ryckman and Arakawa 1952). Dow et al. (1976) conclude that successful hibernation appears to depend initially on an adequate reserve of energy, and secondly, on finding a hibernaculum which remains cool enough so that the accumulated energy reserve will not be expended in futile activity.

Currently, there is very little information on overwintering survival in relation to temperature and cold-

hardiness in adult mosquitoes. All information is confined to temperature records from overwintering sites. McLeod and McLintock (1947) found 3 living and 3 dead An. earlei in a shed in Manitoba where the temperature was -23°C. Trent (1960) collected Cx. tarsalis from rockpiles in Utah, and recorded temperatures of -2°C to +3°C. Rush (1962) recorded similar overwintering temperatures of -4°C to +3°C for Cx. tarsalis in Oregon. In another study, Mail and McHugh (1961) found that both Cx. pipiens and Cx. tarsalis collected in January in Utah survived better at -2°C and 0°C than at +3°C and +8°C . Hudson (1977) monitored the temperature in various overwintering sites in Central Alberta. The ten-day mean temperatures in winter (November-March) were -3°C to -13°C in rockpiles, -1°C to -5°C in an animal burrow, +3°C to -23°C in the outdoor air and continuously above 0°C in root cellars. From these studies, it appears that Culex females will survive better at, or slightly below 0°C, and that animal burrows provide an ideal overwintering site.

Insects that are capable of enduring prolonged temperatures of 0°C or colder are classified as either freeze tolerant or freeze intolerant (Baust 1982; Duman 1982; Baust and Rojos 1985; Lee et al. 1987). Insects that survive to temperatures below -40°C have low freezing points attributable to high solute concentrations, and are freeze-

tolerant. Freeze tolerant species can survive the formation of extracellular and possible intracellular ice by producing low molecular weight antifreeze/cryoprotective agents (ie. glycerol, sorbitol and glucose) and high molecular weight proteins, or thermal hysteresis factors (THF) (Salt 1961; Baust 1982). Insects that have a freezing point only a degree or two below 0°C are freeze intolerant species. Freeze intolerant species must adapt to conditions by producing antifreeze compounds to lower their supercooling point and/or selecting a thermally buffered overwintering site (Duman 1982). For freeze intolerant species, the supercooling point represents the lethal limit of low temperature. Although water loss or dehydration is considered obligatory to concentrate solutes and lower the freezing point in overwintering insects, this mechanism confers a minimum degree of cold tolerance (Salt 1961). Hudson (1977) found that the supercooling points were lowest in mosquitoes overwintering in exposed sites (ie. rockpiles), higher in those overwintering in a cellar above 0°C, and highest in the species which do not overwinter as adults (ie. Aedes vexans). The seasonal mean supercooling point of An. earlei and Cx. territans collected from rockpiles from November to March was -23°C and -26.1°C, respectively. From April to May, the mean supercooling point for the above species was -15.1°C and -26.1°C,

respectively. Since the minimum temperature in a rockpile was -10°C, the above supercooling points of An. earlei and Cx. territans suggest that these species spend most of the winter supercooled. The median lethal time of wild-caught females at -5°C was 137 days for Cx. territans (ie. approximately 4.5 months) and 20 days for Cs. inornata (Hudson 1977). The higher supercooling point (-11.3°C) and poor survival of Cs. inornata suggests that this species may only overwinter in well-insulated sites.

A number of factors including acclimation, cold tolerance, selection of hibernaculum and the accumulation of lipid reserves are known to influence the survivability of overwintering insects. DeGeer, Pitts and Hopkins (1965) investigated the lipid composition of the face fly, Musca autumnalis, and found that hibernating flies contained about seven times more lipid than non-hibernating flies, and that short photoperiod and low temperatures appear to be the primary stimulus for fat body hypertrophy (Stoffolano and Matthysse 1967). A larger percentage of unsaturated fatty acids is also characteristic of hibernating flies (Valder et al. 1969; Krafsur et al. 1985).

In the boll weevil, Anthonomus grandis Boheman, Lambremont et al. (1964) found that diapausing adults contained 18-25% body fat (75% to 85% triglycerides), whereas non-diapausing adults contained 6-10% body fat (40-

60% triglycerides). Lambremont and Earle (1961) and Earle and Newson (1964) also found that diapause is induced by 11-hour photoperiods and that the response to photoperiod is modifiable by diet and temperature. Furthermore, about 62% of the fatty acids in the boll weevil contain at least one double bond; these unsaturated acids occurring mainly in the C18 series (Lambremont and Blum 1963). The remaining 38% of the fatty acids are saturated, with palmitic acid accounting for 30% of the total. A similar accumulation of lipid by overwintering adult beetles is reported in Henosepilachna vigintioctopunctata Fabricius (Kono 1982), Odontopus calceatus (Say) (Heinrichs 1969), Leptinotarsa decemlineata Say (De Kort 1981), Coccinella septempunctata L. (El-Hariri 1966), Adalia bipunctata (L.) and Prolylea quatuordecimpunctata (L.).

In Cx. tarsalis females, Harwood and Halfhill (1964), Takahashi and Harwood (1964) and Anderson and Harwood (1966) found that short photoperiod (ie. 8 hours) and low temperatures initiated fat body hypertrophy at the expense of ovarian development . Similar results were observed in Culex pipiens pipiens L. (Buxton 1935), Culex apicalis Adams (Schaefer and Washino 1974), Culiseta inornata (Williston) (Barnard and Mulla 1977; 1978), Culiseta annulata Schrank (Ramsdale and Wilkes 1985), Anopheles atroparvus van Thiel, Anopheles freeborni Aitken (Schaefer and Washino 1974),

and Anopheles earlei Vargas (Hudson 1977; Gallaway and Brust 1982). Schaefer and Washino (1970) found that approximately 78 percent of total lipids in overwintering Cx. tarsalis females were triglycerides. The remaining percentage of total lipid content was composed of free fatty acids. Harwood and Halfhill (1964), Takata and Harwood (1964), Harwood and Takata (1965) and Schaefer and Washino (1970) also found that females reared at 22°C and a short photoperiod contained more unsaturated fatty acids than females at 30°C . Similar results are observed in C. pipiens pipiens (Buffington and Zar 1968) and A. freeborni (Hayashiya and Harwood 1968; Schaefer and Washino 1969). From this data, it is evident that prolonged exposure to temperature extremes affects the quantity and saturation of lipids, and that these conditions significantly affect the ability of an insect to survive cold conditions.

The accumulation of lipid reserves and the unsaturation of fatty acids in many overwintering insects is well-documented. However, there is very little information on the overwintering biology of northern populations of Cx. tarsalis, where females must survive lower temperature extremes and a longer period of inactivity. Therefore, studies with respect to the seasonal and developmental timing of lipid accumulation in Cx. tarsalis females, the depletion of total lipids in females during overwintering,

and the relationship of total lipid content to overwintering survival are required.

CHAPTER III

Material and Methods

3.1 The Measurement of Wet and Dry Weight

The weight of individual female mosquitoes was measured on a microbalance (Cahn Electrobalance®). The females were killed at -10°C and weighed immediately to obtain a "wet weight" record. The females were then placed in individual cuvettes and freeze-dried. Window screening was secured to each tray prior to freeze-drying to prevent the loss of specimens. Dry weight measurements were obtained using the microbalance.

3.2 The Colorimetric Assay

The quantity of total lipid in adult females of Cx. tarsalis was determined using a colorimetric assay (Van Handel 1985). Individual freeze-dried females were extracted with 0.5 ml chloroform-methanol (1:1) in a borosilicate glass mortar and pestle tissue grinder. The supernatants were transferred into a clean test tube, and the tubes were placed in a heating block at 100°C to evaporate the solvent. Upon evaporation, 0.2 ml of

sulphuric acid was added, and the mixture was heated for 10 minutes. The addition of sulphuric acid converted the unsaturated lipids to water-soluble sulphonic acid derivatives, and a reddish colour developed with the addition of 4.8 ml of vanillin-phosphoric acid reagent. The reagent consisted of 600 mg vanillin dissolved in 100 ml hot water. The mixture was cooled, and 400 ml of 85% phosphoric acid was added. The intensity of colour was used to determine the quantity of lipid in the sample. The absorbance was read in a Spectrophotometer (Bausch & Lomb, Spectrophotometer #501®) at 525 nm. Optical densities measured at 490 nm were found to be unsastifactory due to the decreasing reliability of the estimate at high concentrations of lipid. Samples with an optical density reading greater than 1.00 were diluted five fold before a reading was attempted. A more detailed description of the analysis is given in Appendix 1.

3.3 The Standard Regression Line

The analysis of total lipids in mosquitoes is facilitated by the use of a standard reagent to quantify total lipid content. Van Handel (1985) found that over 90% of the total lipids in Ae. aegypti consisted of triglycerides and phospholipids. The remaining lipid profile consisted of free fatty acids, sterols and traces of

hydrocarbons. D'Alonza et. al. (1982) reported that purified soybean oil contains 97.9% triglycerides. The remaining lipid profile is composed of 1.0% diglyceride and 1.1% "other" glycerides. The high percentage of triglycerides in soybean oil makes this substance a suitable standard for mosquito lipids.

Van Handel (1985) verified the validity of soybean oil as a standard for mosquito lipids using 1 mg/ml total lipid from sucrose-fed A. aegypti. This solution of total lipids gave optical densities identical to those obtained from the soybean oil standard. As a result, the total lipid content per mosquito can be calculated from a calibration line, using soybean oil standard. The calibration line for this study was obtained using three replicates of 25, 50, 100 and 150 μl of refined soybean oil standard (Fisher Scientific). A linear regression was produced each time a new batch of vanillin reagent was produced to ensure the reproducibility of the results was maintained throughout the analyses. In fact r^2 was maintained at or above 0.990.

3.4 The Reaction Stability

The stability of the colorimetric assay was determined using a blank sample, a diluted sample, and an undiluted sample of soybean oil standard. The samples were measured in the spectrophotometer at 5 minute intervals over a 30

minute period. These results indicated that the colour reaction was most stable between 20 and 25 minutes from time zero (ie. commencement of the reaction). Furthermore, a 1:5 dilution did not appear to affect the stability of the reaction. Similar results were observed by Van Handel (1985), Knight et. al. (1972), and Frings and Dunn (1970). On the basis of these results, all optical densities were read between 20 and 25 minutes from time zero.

3.5 The Determination of the Weight Equation

The weight of individual females was determined using the linear equation of the regression line, $y = ax + b$ (a = the slope of the line, or the change in y for a one-unit change in x and, b = the y-intercept or the value of y when $x = 0$). If the equation is transposed as follows, $y = ax + b$ and $y - b = ax$, then $(y - b)/a = x$. Since y = % absorbance (A) and b, or the y-intercept, equals zero, the equation to determine the total lipid content of individual females is $x = A/a$.

3.6 The Lipid Content of Fed and Starved Ae. aegypti

The analysis of total lipid content using the colorimetric assay has shown that total lipids in female Ae. aegypti increase when females are fed a sugar solution and decrease when females are starved (Van Handel 1985). In

order to ensure that the colorimetric assay would replicate published data, the total lipid content of three groups of female Ae. aegypti were analyzed. The three groups consisted of females fed 10% sucrose for 3 days, 10% sucrose for 7 days and those that were starved. A fourth group consisting of adults within 24 hours of emergence was not obtained. For each group, first instar larvae were reared in dechlorinated water at 25°C and 16Light:8Dark. Each group consisted of 3 plastic pans with 100 larvae per pan, and larvae were fed excess liver powder. The starved to death condition was defined when one-half of the original population had died. The assay of the remaining mosquitoes that were starved to death provided the baseline that included all structural and non-nutritional lipids.

3.7 The Lipid Content of Newly Emerged, Fed and Starved Cx. tarsalis

The total lipid content of 4 groups of Cx. tarsalis females was assayed to determine if total lipids also increased with sucrose feeding. The 4 groups sampled consisted of females at adult emergence, females fed 10% sucrose for 3 days, 10% sucrose for 7 days, and starved to death. In each group, first instar larvae were reared in dechlorinated water at 25°C and 16L:8D. Each group consisted of 3 plastic pans with 100 larvae per pan, and

larvae were fed excess liver powder.

3.8 Colony Maintenance

Adult Cx. tarsalis females were obtained from a colony established in 1988. A total of 606 egg rafts were collected from 12 oviposition pools located at Glenlea, Manitoba. All egg rafts were collected 3 times a week between July and August 1988, and transported to the University of Manitoba to permit hatching at 25°C and 16L:8D. The first instar larvae were identified to species according to Dodge (1966), and Culex tarsalis larvae were pooled. The larvae were reared in 21 X 32 X 7 cm. plastic pans at 25°C and 16L:8D, and fed finely screened liver powder, ad libidum. The surface film on the larval medium was removed daily. Prior to pupation, the pans were placed in a 2.5 X 1 X 1 m polyvinyl cage with a zippered, walk-in entrance. The adults were maintained in the cage until approximately 50% mating resulted (10 to 14 days). A customized dimming device on the room light simulated one hour of dusk and one hour of dawn each day. For blood feeding, females were transferred to a 30 X 30 X 30 cm cage containing restrained Japanese quail (Coturnix coturnix). The oviposition site consisted of a styrofoam cup which was filled with dechlorinated water, and contained a submerged piece of sod.

3.9 The Effect of Different Constant Temperatures on the Rate of Larval Development and on Lipid Synthesis

The relationship between temperature, development and lipid content was investigated to determine 1) the duration of larval development at different constant temperatures, 2) the upper and lower thresholds of development in Culex tarsalis, and 3) the relationship between temperature and lipid content of newly emerged females.

The experimental temperatures consisted of 12°C, 14°C, 17°C, 20°C, 25°C, 30°C, 32°C, and 34°C at 16L:8D. At each temperature, 300 first instar larvae were reared at 100 larvae per pan, and fed excess liver powder. The surface film on the larval medium was removed daily. The rate of pupation and the percent survival was recorded for each experiment. The emergence of adults at for the 14°C and 17°C treatments was facilitated by the transfer of pupae to 20°C. To determine if rearing temperature affected lipid synthesis in Cx. tarsalis, the adults were maintained for a maximum of 24 hours in 30 X 30 X 30 cm transparent plastic cages. Adults were given water only, and a sample of 20 females were removed for lipid analysis.

3.10 The Effect of Temperature and Photoperiod on Wet, Dry and Total Lipids in Cx. tarsalis

Six rearings were conducted in outdoor conditions at

the University of Manitoba in 1986 to determine the period of the summer or fall season in which lipid accumulation occurred. The 6 rearings were commenced on June 25, July 7, 23 and 28, and August 7 and 18. In each rearing, a minimum of 500 larvae (100 larvae/pan) was obtained from 15, 101, 24, 110, 34 and 79 egg rafts, respectively. All egg rafts were collected from oviposition pools at Glenlea, Manitoba, and transported to the laboratory to ensure hatching. The first instar larvae were reared in 32 X 21 X 7 cm plastic pans in outdoor conditions and fed excess liver powder. The adults were fed water only, and samples were taken at 12 hour intervals. A total of 20 females per replicate were weighed to obtain wet and dry weight, and analyzed for total lipid content. Air temperature was recorded on a weekly basis using a thermograph, located at the University of Manitoba Campus. Photoperiod (sunrise to sunset and 2 X civil twilight) was calculated from the United States Naval Observatory Data given in Beck (1980).

3.11 The Field-Collection of Cx. tarsalis

The study site selected for the field collection of Cx. tarsalis egg rafts was the Glenlea Research Station, located approximately 20 km. south of Winnipeg, Manitoba, Canada on Highway 75. Egg rafts were collected using the method described by Brust (1990).

To compare laboratory-reared and field-collected females, 3 battery powered SSAM traps were operated at Glenlea, Manitoba. The attractants were carbon dioxide and a light source. A motorized fan drew the females into a mesh collecting bag, once they were attracted to the trap. Females were collected 3 times per week, and a total of 20 females per week were analyzed for total lipid content.

3.12 The Effect of Different Constant Temperatures and Photoperiod on the Accumulation of Total Lipids

Four experiments were conducted in 1987 at different temperature and photoperiod conditions to determine the effect of temperature and photoperiod on total lipid content. These experiments included controlled environment chambers maintained at 21 and 26.5°C, each with 12L:12D or 16L:8D. A total of five 24-hour sampling intervals (ie. 24, 48, 72, 96, and 120 hours) were used in all experiments. Two additional experiments were conducted at the University of Manitoba Campus to determine the importance of carbohydrates, or nectar-feeding in nature, to lipid accumulation. In each experiment, adults were either offered water only or 10% sucrose. This treatment provided a comparison between un-fed and sucrose-fed females.

A total of 4 replicates per experiment were conducted on July 17, July 3, August 5 and August 24, 1987. In each

replicate, a minimum of 600 larvae per experiment was obtained from 38, 21, 40 and 25 egg rafts, respectively. All egg rafts were collected from oviposition pools at Glenlea, and transported to the laboratory to ensure hatching. First instar larvae were reared in 32 X 21 X 7 cm plastic pans at 100 larvae per pan, and fed excess liver powder. The adults were fed 10% sucrose, and samples were taken at 24-hour intervals.

3.13 Preparation of the Cx. tarsalis Overwintering Site

Eighty postholes were dug on the south and west dikes at Glenlea, Manitoba to provide simulated overwintering hibernacula for Cx. tarsalis females. Each posthole consisted of an excavated hole measuring 90 cm deep and 15 cm wide. The collapse of the walls was prevented by the insertion of a cardboard tube (Sonotube®) into each posthole. Random samples of experimental females were taken from 20 postholes at Glenlea, Manitoba and from 10 postholes at the University of Manitoba, Fort Garry Campus to determine the rate of lipid depletion in the overwintering Cx. tarsalis population. The overwintering cages consisted of a small cylindrical cage constructed of plywood and window screening. An extension rod was attached to each cage to permit the vertical movement of the cage within each posthole. Once the cage was inserted into the hole, a piece

of insulation and a plywood sheet were placed over the hole, and secured with a brick.

3.14 Production of the Overwintering Population of *Cx. tarsalis*

In 1986, 256 egg rafts were collected at Glenlea, Manitoba, between August 13 and August 18, 1986. The first instar larvae were identified to species, and reared in outdoor conditions at 500 larvae per 32 X 21 X 7 cm. plastic pan, for a total of 24 pans. The pupae were moved into the laboratory at 20°C and outdoor photoperiod on September 04 to facilitate emergence. Adult emergence occurred between September 06 and September 20. Between September 06 and 20, the newly-emerged adults were transferred back to outdoor conditions every third day. Between September 23 and October 09, 1986, the adults were gradually acclimatized to 15 and 10°C, 0L:24D and fed 10% sucrose. On October 09, 1986, the ambient temperatures were approximately 10°C and decreasing; therefore, the females were counted and placed into field conditions with water wicks only.

In 1987, 213 egg rafts were collected at Glenlea, Manitoba, between August 07 and August 22. Larvae were reared outdoors at 500 larvae per pan, for a total of 24 pans. Pupae were moved to the laboratory at 20°C and outdoor photoperiod on September 09, and the adults emerged

between September 10 and September 24. Between September 10 and 24, the newly-emerged adults were transferred regularly to outdoor conditions. The adults were gradually acclimatized to 15 and 10°C 0L:24D between September 25 and October 22. The adults were placed into field conditions without a water source on October 22, 1987, when ambient temperatures were 6°C and decreasing. Samples throughout 1986 and 1987 were taken at monthly intervals. A minimum of 3 replicates per month were obtained, and 20 living females per month were retained for the determination of total lipids. The sampled females were protected from subzero air temperatures in winter by wrapping the cardboard tube liner and cage in insulation, and the tube and cage were transported to the laboratory. The percent survival was recorded for all samples.

3.15 Statistical Analysis

The SAS Institute Inc. statistical package was used to determine the analysis of variance for all experiments. The Multiple-Comparison procedures used to determine the difference between mean data points were Duncan's and Scheffe's Multiple Range Tests.

CHAPTER IV

RESULTS

4.1 The Standard Regression Line

The results obtained from the calibration line using refined soybean oil are illustrated in Figure 1. The slope of the calibration line was a = 0.004 μg lipid. The corresponding regression equation was x = A/0.004 (r^2 = 0.994)(S.E. 0.0001 μg)($p \leq 0.001$). This equation was used in all experiments to determine the micrograms of total lipid per female mosquito.

4.2 The Precision of the Technique

To determine the precision or reproducibility of my technique, I replicated the standard regression line each time a new batch of vanillin reagent was produced. My results indicate that the slope of the line is reproducible with r^2=0.985.

4.3 The Lipid Content of Fed and Starved Ae. aegypti

The data from Table 1 indicates that 114 μg. total lipid per female was the baseline lipid level which includes all non-metabolizable lipids, and that total lipid content

Figure 1: The calibration line for soybean oil at 525 nm. The slope of the line was calculated as 0.004 μg lipid.

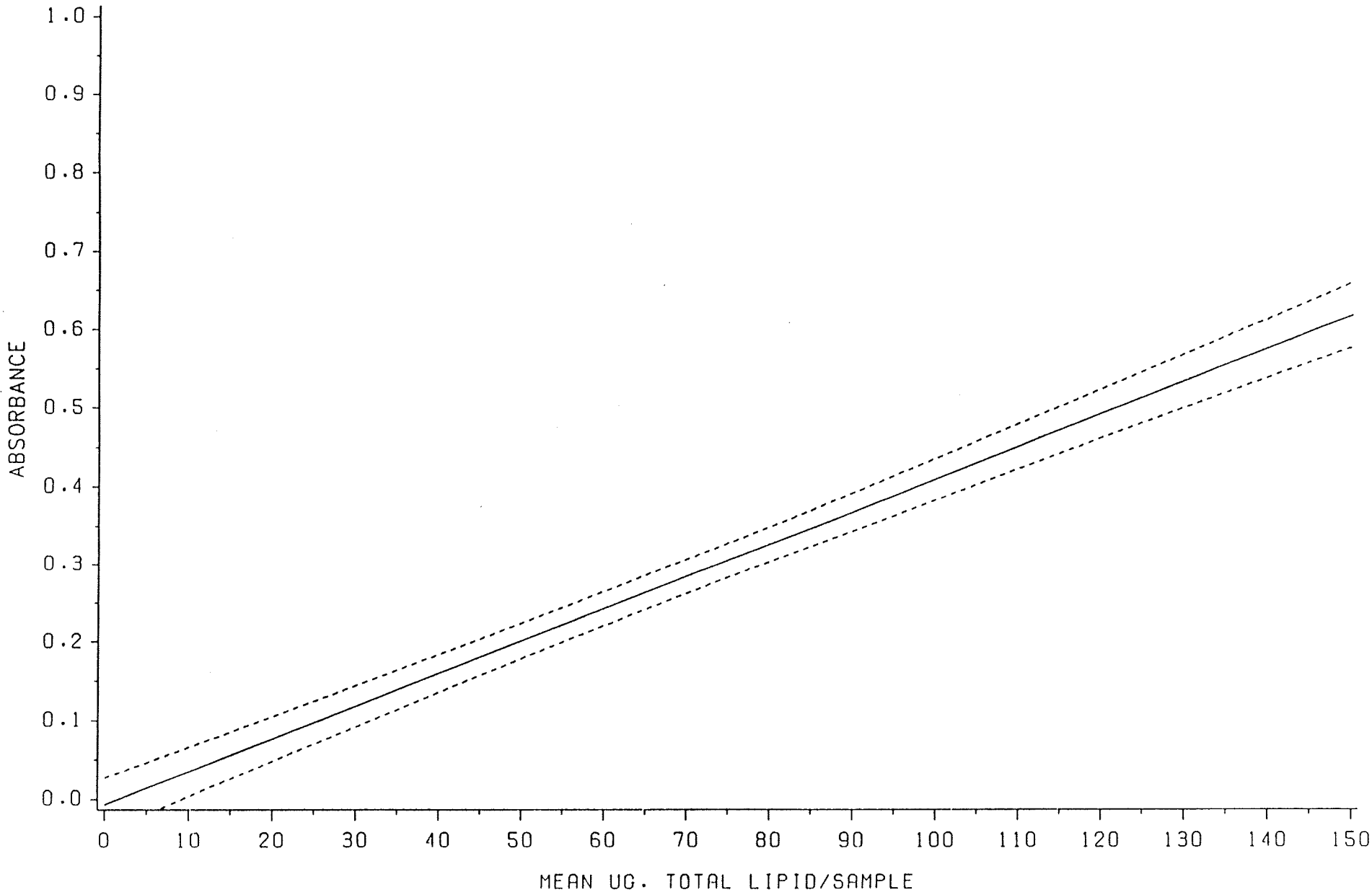

ABSORBANCE
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
MEAN UG. TOTAL LIPID/SAMPLE

Table 1. Total Lipid Content in Aedes aegypti (mean μg. lipid/female ± S.E.) when subjected to 3 different feeding regimens.

Condition	N	Lipid Content/Female (μg)
10% sucrose for 7 days	12	550.1 ± 16
10% sucrose for 3 days	29	495.8 ± 16
Starved to death	5	113.8 ± 13

Table 2. Total lipids in Culex tarsalis (mean μg. lipid/female ± S.E.) when subjected to 4 different feeding regimens.

Condition	N	Lipid Content/Female (μg)
10% sucrose for 7 days	15	726.5 ± 33
10% sucrose for 3 days	15	453.2 ± 22
Adult emergence	15	178.9 ± 5
Starved to death	15	104.0 ± 3

was significantly different between the starved to death condition and those fed 10% sucrose (Scheffe $p \leq 0.05$). There was no significant difference in lipid content between mosquitoes fed 10% sucrose for 3 days and those fed 10% sucrose for 7 days (Scheffe $p \geq 0.05$).

4.4 The Lipid Content of Newly Emerged, Fed and Starved *Cx. tarsalis*

The baseline lipid concentration for starved *Cx. tarsalis* females was 104.0 µg. total lipid (Table 2). A significant difference in total lipid content was observed between adult emergence, 3 days 10% sucrose and 7 days 10% sucrose (Scheffe $p \leq 0.05$). However, the total lipid content at adult emergence and the starved to death condition was not significantly different (Scheffe $p \leq 0.05$). The µg. lipid/female approximately doubled from adult emergence to 3 days on 10% sucrose, and again by 7 days on 10% sucrose; therefore, it was concluded that lipid content increased with sucrose feeding.

4.5 The Effect of Different Constant Temperatures on the Rate of Larval Development and the Accumulation of Total Lipids in *Cx. tarsalis*

The rate of larval development to 50% pupation was

determined for 8 different rearing temperatures (Fig. 2). Fifty percent pupation was defined as when one-half of the original population had pupated. The low temperature threshold for Cx. tarsalis development was 14°C, and the upper temperature threshold was 34°C or above. Below 14°C, successful development to pupation did not occur. The percent survival was recorded at each temperature, and it appears that the optimum temperature for development with respect to survival occurred between 20°C and 30°C. At 20°C, 70% survival was observed and 80% survival was observed at 30°C. A decrease in survival was recorded at 25°C (ie. 52% survival); however, it is unlikely that this was temperature related. At both 32 and 34°C, 5 days were required for 50% pupation with 59.7% survival and 41.3% survival, respectively.

To determine the quantity of total lipid produced at each temperature, the μg total lipid was determined on a per mg dry weight basis (Fig. 3). The concentration of lipids was highest at temperatures of 14 and 17°C, and lowest at temperatures of 25°C and above. There appeared to be a direct relationship between development time (Fig. 2) and the concentration of lipids in newly emergent females (Fig. 3). A significant difference in total lipid content occurred between 17°C and 20°C and between 20°C and 25°C (Scheffe $p \leq 0.05$). There was no significant difference

Figure 2. The relationship of temperature to larval development to 50% pupation.

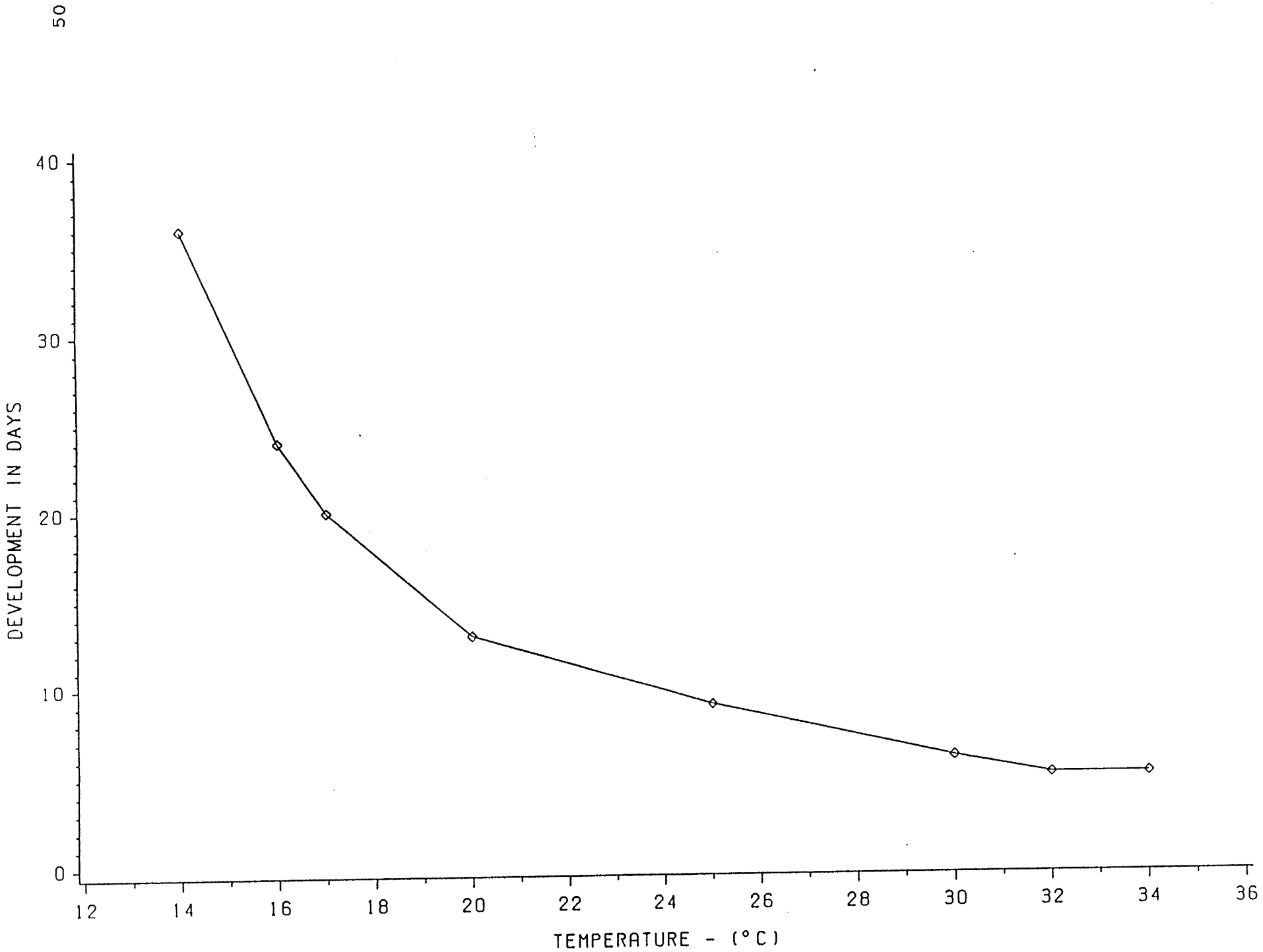
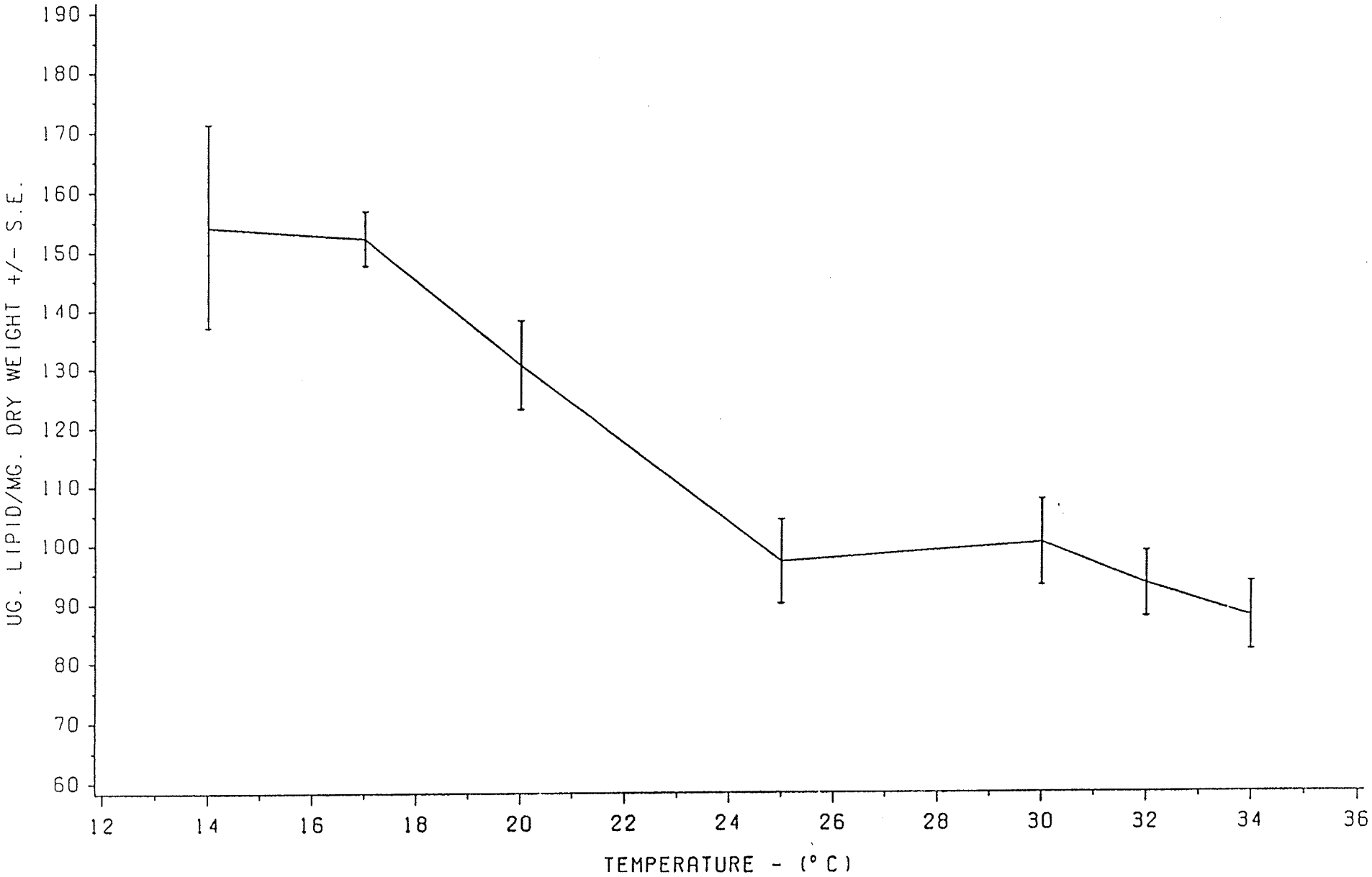

50
40
30
20
10
0
DEVELOPMENT IN DAYS
12
14
16
18
20
22
24
26
28
30
32
34
36
TEMPERATURE - (°C)

Figure 3: The concentration of lipids (mean ug. lipid/mg. dry weight at emergence) for 7 different temperatures.


UG. LIPID/MG. DRY WEIGHT +/- S.E.
190
180
170
160
150
140
130
120
110
100
90
80
70
60
12
14
16
18
20
22
24
26
28
30
32
34
36
TEMPERATURE - (° C)

between 14 and 17°C, and no significant difference between 25, 30, 32 and 34°C.

4.6 The Effect of Temperature and Photoperiod on the Wet and Dry Weights of Female Cx. tarsalis

A series of 4 rearings for wet weight and 6 rearings for dry weight were conducted under natural conditions at the University of Manitoba Campus in 1986. In Figure 4, the mean mg wet weight ± S.E. is plotted with mean air temperature (°C) for each rearing. These data illustrate the indirect role of water in mosquito physiology at different mean temperatures. Significant differences in wet weight occurred between all dates (Scheffe $p \leq 0.05$). These data suggest that mean air temperatures indirectly affected the quantity of physiological water available to regulate homeostasis.

The mean mg dry weight ± S.E. was plotted along with mean air temperature (°C) for each date (Fig. 5). The dry weight of females on July 07 was significantly greater than any other date, and the dry weight on August 18 was significantly lower than any other date (Scheffe $p \leq 0.05$).

Figure 4: The mean emergence wet weight of 20 newly emerged field-reared females ± S.E. (——) and mean air temperature (---) for the previous week at the University of Manitoba Campus, 1986.

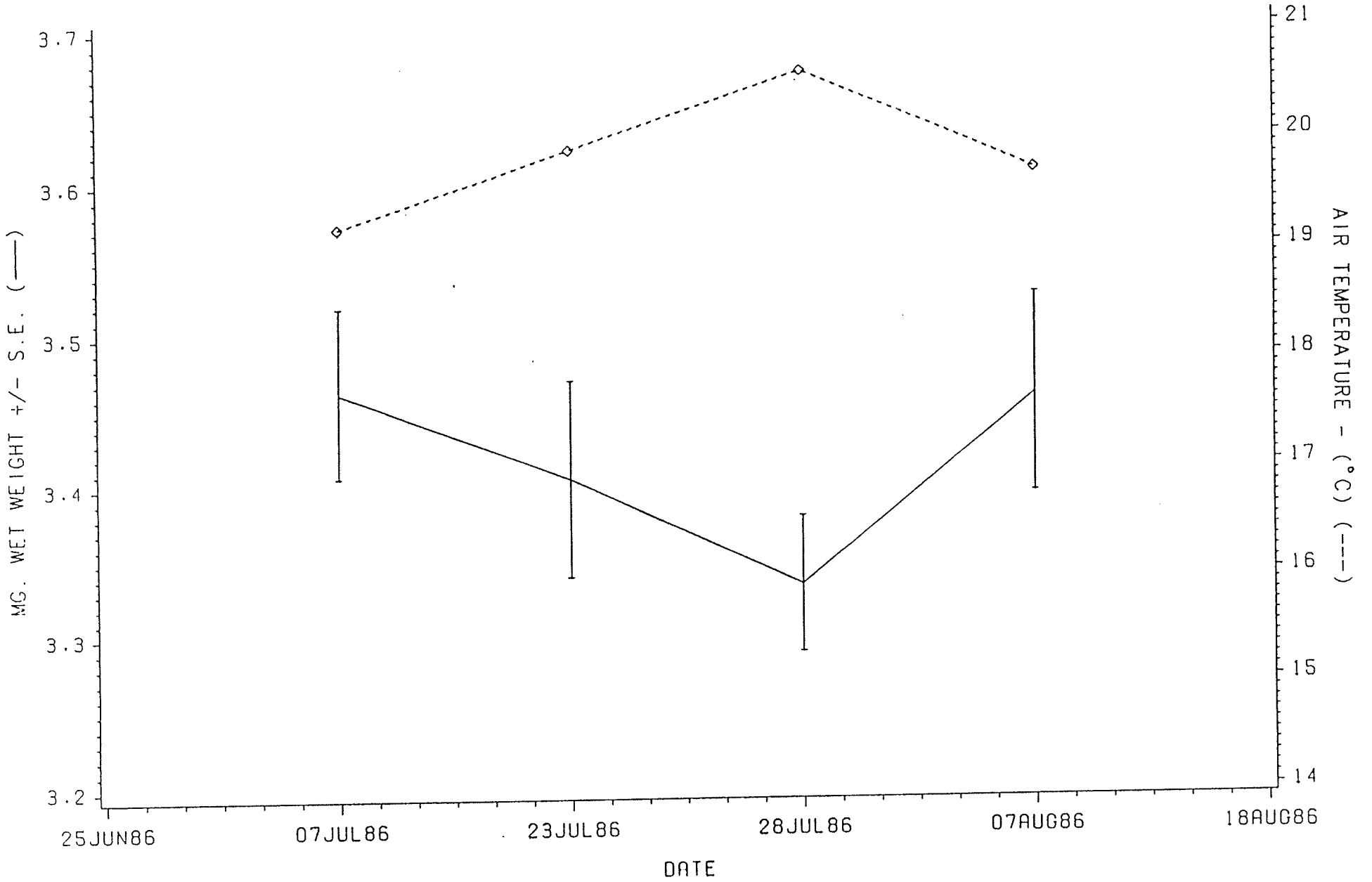
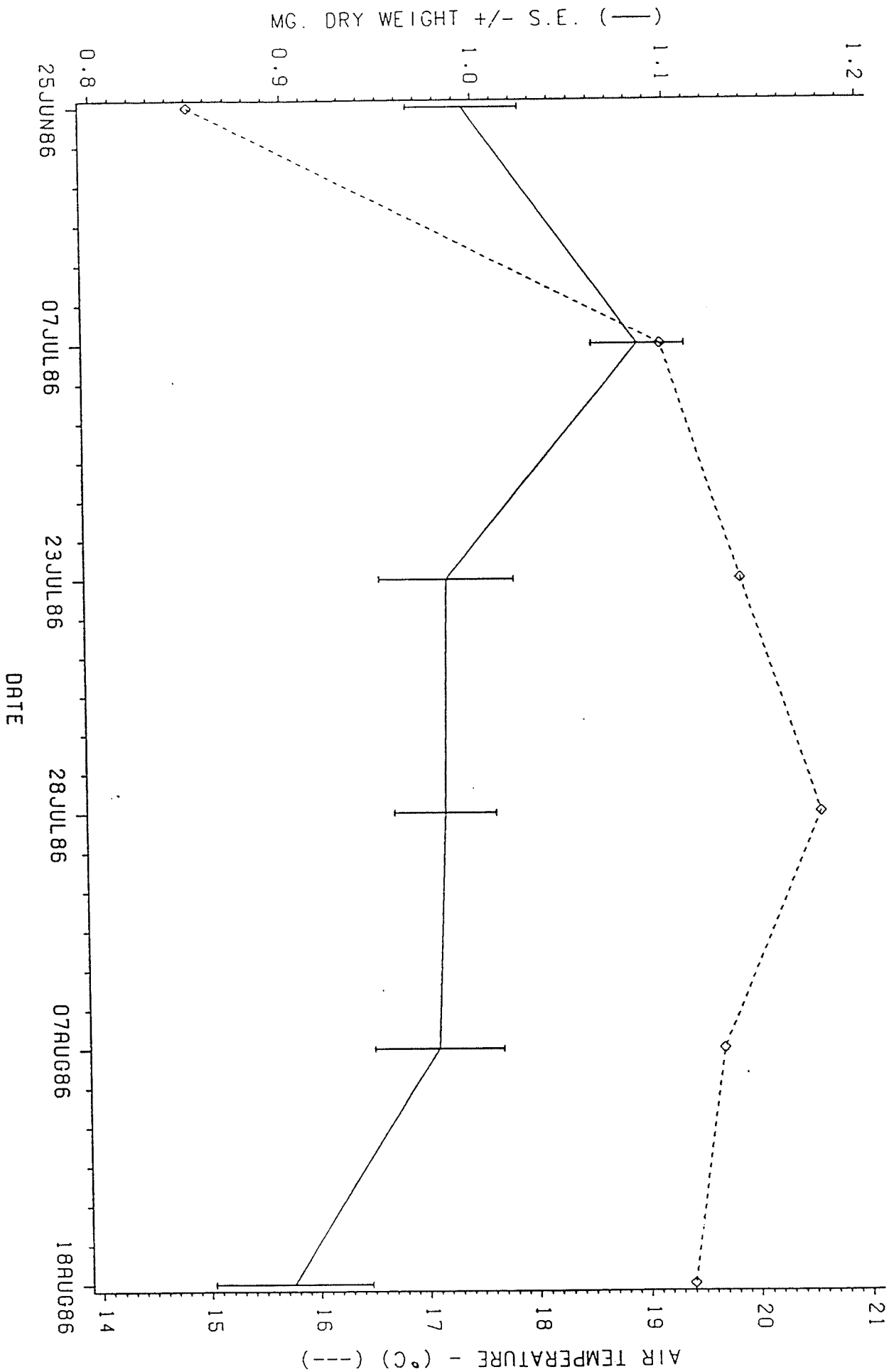

MG. WET WEIGHT +/- S.E. (——)
3.7
3.6
3.5
3.4
3.3
3.2
AIR TEMPERATURE - (°C) (---)
21
20
19
18
17
16
15
14
25JUN86
07JUL86
23JUL86
28JUL86
07AUG86
18AUG86
DATE

Figure 5: The mean emergence dry weight of 20 newly emerged field-reared females ± S.E. (——) and mean air temperature (---) for for previous week at the University of Manitoba Campus, 1986.


MG. DRY WEIGHT +/- S.E. (——)
0.8
0.9
1.0
1.1
1.2
25JUN86
07JUL86
23JUL86
28JUL86
07AUG86
18AUG86
DATE
14
15
16
17
18
19
20
21
AIR TEMPERATURE - (°C) (---)

4.7 The Effect of Temperature and Photoperiod on Total Lipid Content in Cx. tarsalis

The concentration of lipids ± S.E. was plotted with mean air temperature for each date (Fig. 6). The lipid levels (μg/mg dry weight) on July 07 and 23 were significantly greater than those of June 25, and July 28 to August 07 (Scheffe $p \leq 0.05$). The lipid level (μg/mg dry weight) on August 18 was significantly lower than earlier levels (Scheffe $p \leq 0.05$). The lipid levels per female on June 25, July 7, 23, 28 and August 7 and 18 were 126, 158, 151, 135, 129 and 86 μg lipid/female, respectively. Since the starved to death lipid level was at 104.0 μg total lipid per female, these results indicate that lipid is not accumulated prior to adult emergence in the fall.

4.8 Lipid Levels of Natural Populations of Cx. tarsalis from Glenlea, Manitoba

Female Cx. tarsalis were sampled throughout 1987 to determine whether lipid levels in natural populations increased in the fall, as compared to those populations collected in early summer. A total of 20 field-collected females/week from Glenlea, Manitoba, were analyzed for total lipid content. In Figure 7, the μg total lipid/female and mean air temperature (°C) were plotted for each weekly sample. A significant difference in total lipid content

Figure 6: The mean concentration of lipid (μg. lipid/mg. dry weight) (——) of 20 newly emerged field-reared females ± S.E. and mean air temperature (---) for the previous week and daily photophase (sunrise to sunset + civil twilight) at the University of Manitoba Campus, 1986.

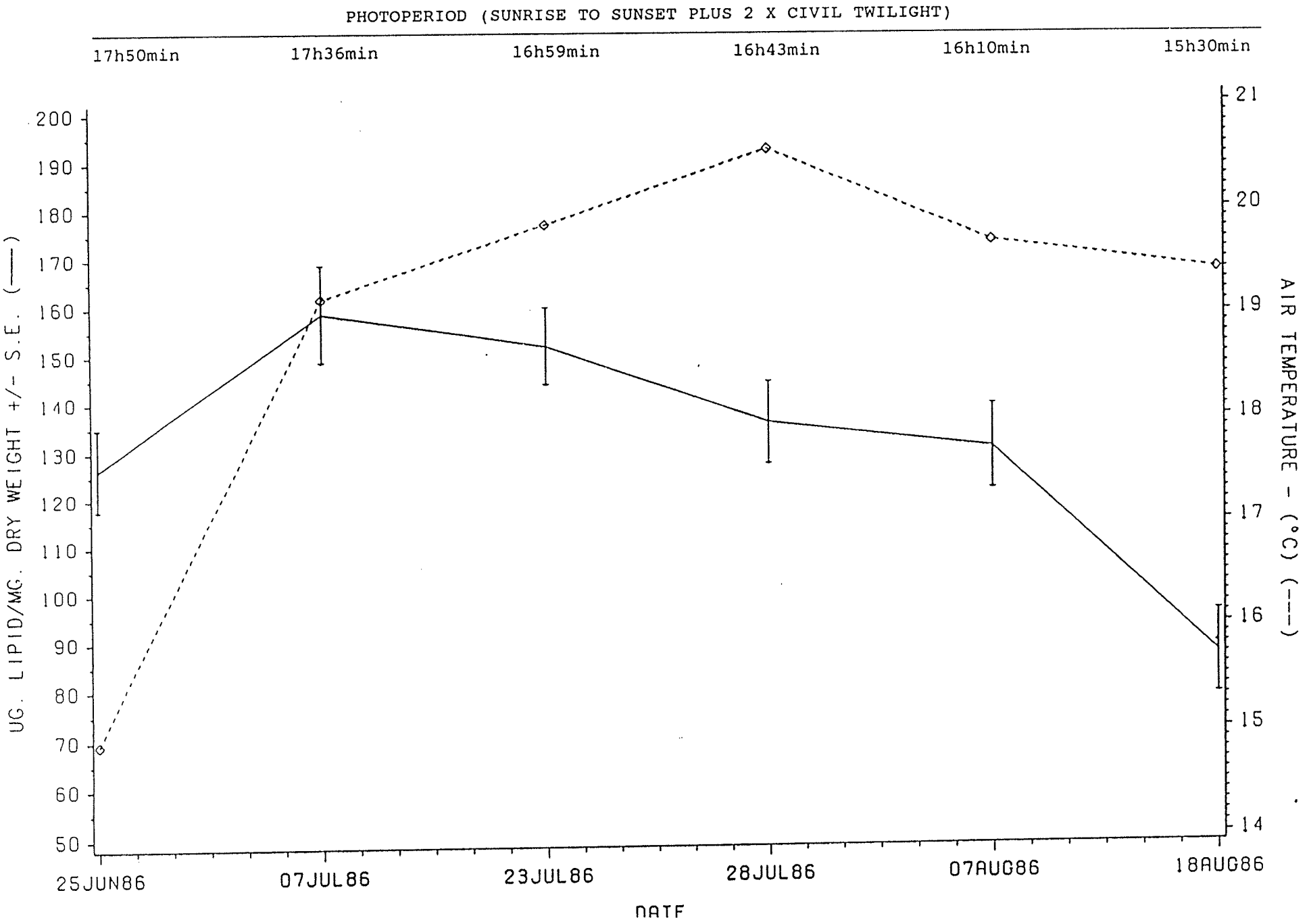
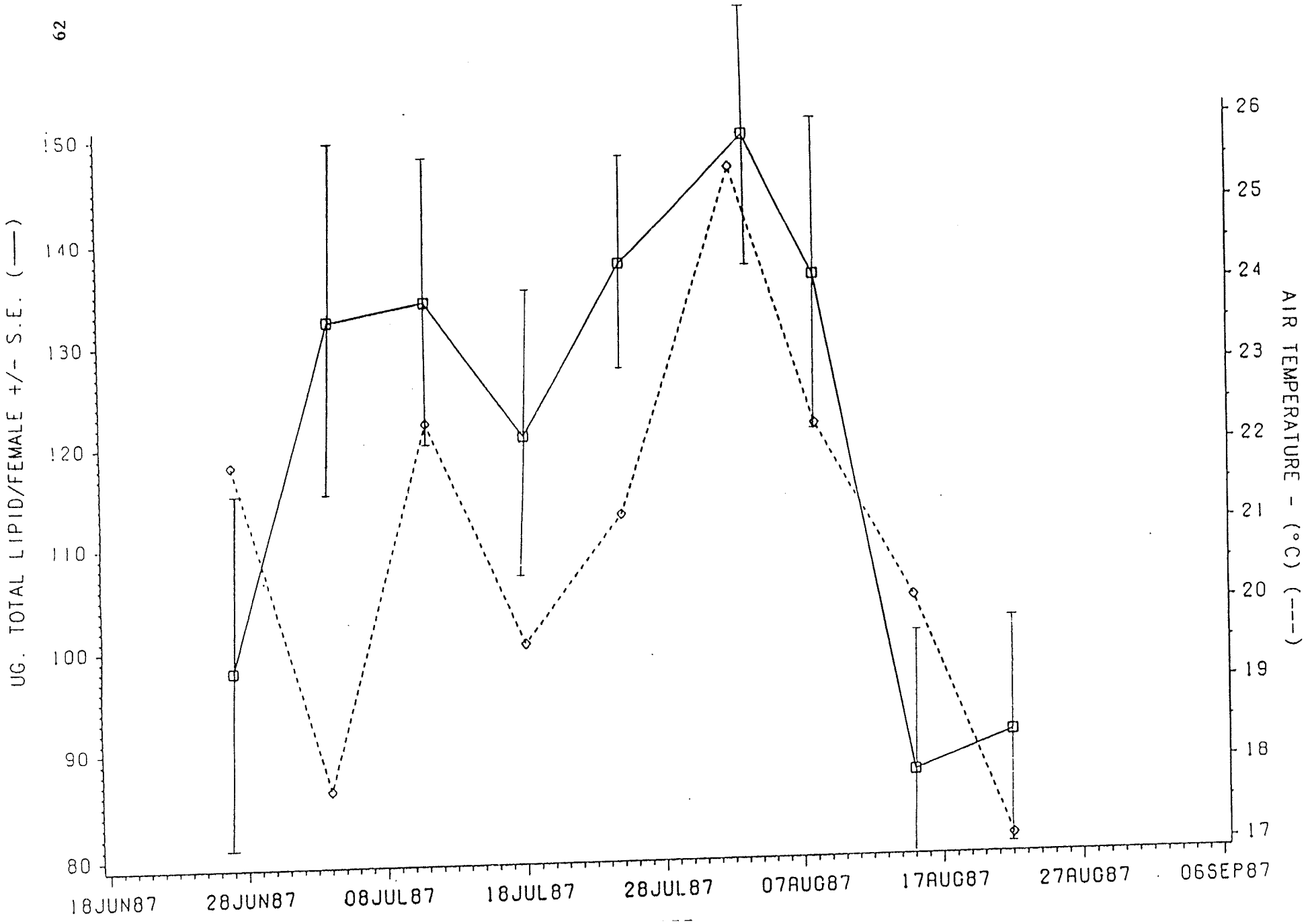

PHOTOPERIOD (SUNRISE TO SUNSET PLUS 2 X CIVIL TWILIGHT)
17h50min
17h36min
16h59min
16h43min
16h10min
15h30min
UG. LIPID/MG. DRY WEIGHT +/- S.E. (——)
AIR TEMPERATURE - (°C) (---)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
21
20
19
18
17
16
15
14
25JUN86
07JUL86
23JUL86
28JUL86
07AUG86
18AUG86
DATE

Figure 7: The mean amount of lipid (μg lipid/female) of 20 wild caught females (——) and mean air temperature (---) for the week previous at Glenlea, Manitoba, 1987.


UG. TOTAL LIPID/FEMALE +/- S.E. (——)
AIR TEMPERATURE - (°C) (---)
150
140
130
120
110
100
90
80
26
25
24
23
22
21
20
19
18
17
18JUN87
28JUN87
08JUL87
18JUL87
28JUL87
07AUG87
17AUG87
27AUG87
06SEP87

occurred between June 27 and July 4, and between all samples from July 11 to August 22 (Duncan $p \leq 0.05$). Although this suggests that 2 peaks in lipid accumulation occurred throughout the season, no significant differences in lipid content were identified with Scheffe ($p \leq 0.05$). The statistical results were identical to the above data when the females were compared on a dry weight basis (μg total lipid/mg dry weight ± S.E., Fig. 8)

4.9 The Effect of Photoperiod on Total Lipids, at Constant Temperatures of 21 and 26.5°C

There were no significant difference in total lipid content in females which had been reared at a 12L:12D photophase at 21°C, from 24 to 120 hours following emergence (Scheffe $p \geq 0.05$) (Fig. 9). At 21°C and 16L:8D, total lipid content was significantly lower at 24 than at 48 hours; however, no significant difference was observed between 48, 72, 96 and 120 hours (Scheffe $p \geq 0.05$). Total lipids were significantly higher at 21°C 16L:8D than at 12L:12D at 72, 96 and 120 hours following emergence (Fig. 9).

There were no significant differences in total lipid content when females were exposed to 12L:12D and 16L:8D at 26.5°C (Fig. 10). However, total lipids were significantly higher at 26.5°C 16L:8D that an 12L:12D at 72 hours

Figure 8: The mean amount of lipid (μg. lipid/mg. dry weight) of 20 wild caught females (——) and mean air temperature (---) for the week previous at Glenlea, Manitoba, 1987.

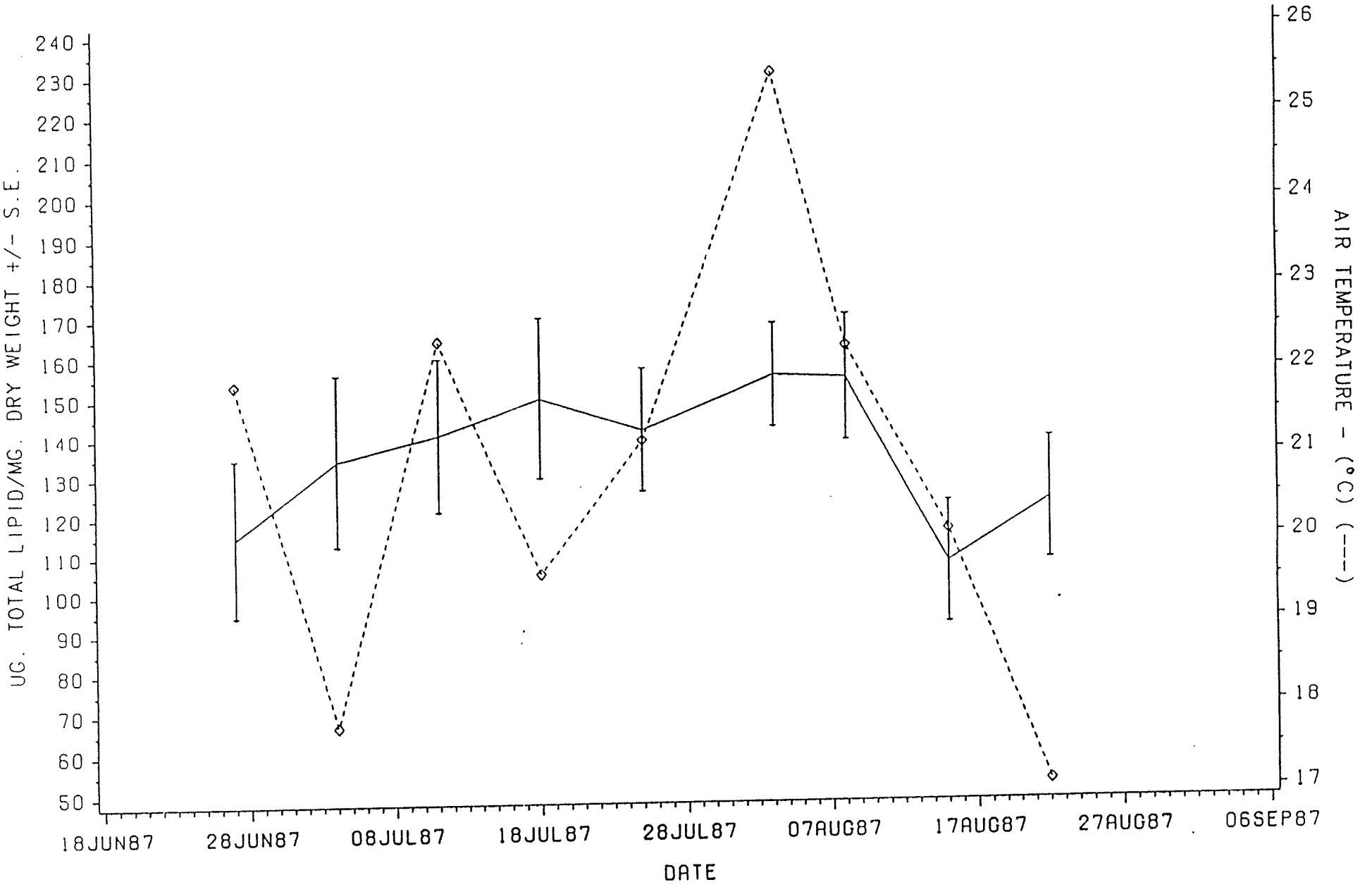

UG. TOTAL LIPID/MG. DRY WEIGHT +/- S.E.
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
AIR TEMPERATURE - (°C) (---)
26
25
24
23
22
21
20
19
18
17
18JUN87
28JUN87
08JUL87
18JUL87
28JUL87
07AUG87
17AUG87
27AUG87
06SEP87
DATE

Figure 9: The accumulation of lipid at 21°C and 12L:12D (——) and 16L:8D (---). Upon emergence, adults were given a 10% sugar solution. Each mean represents a sample of 10 females and S.E.

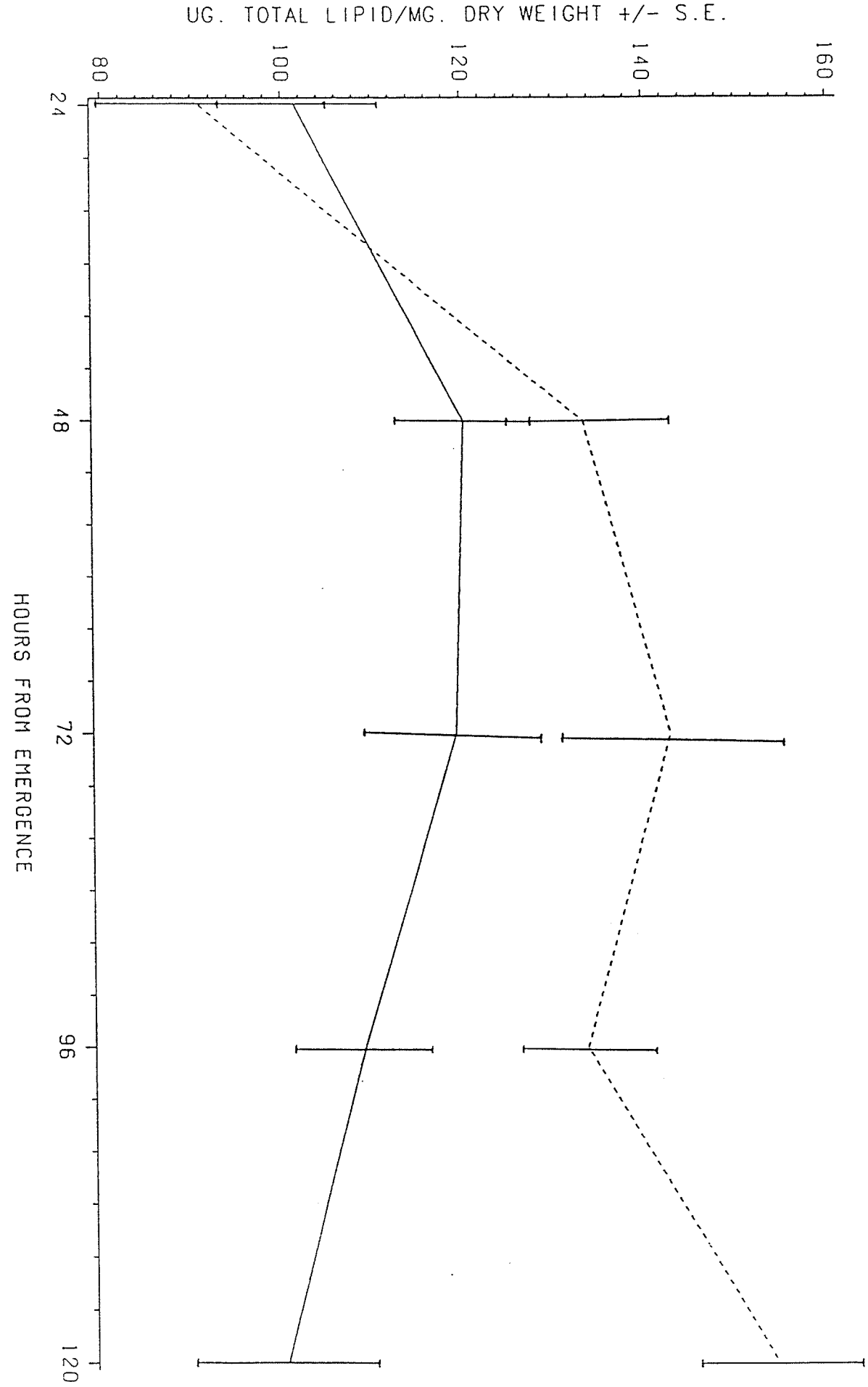
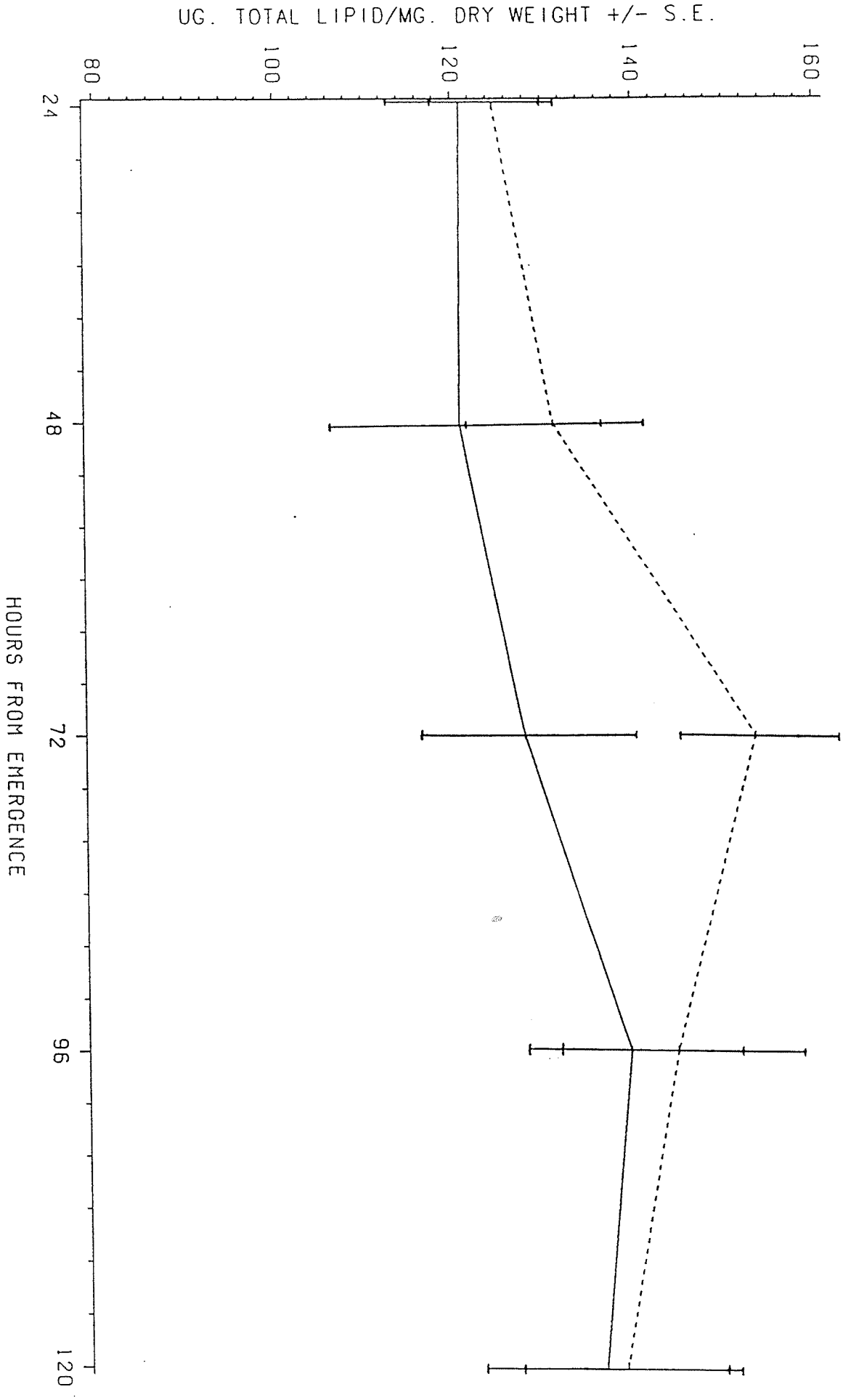

UG. TOTAL LIPID/MG. DRY WEIGHT +/- S.E.
80
100
120
140
160
24
48
72
96
120
HOURS FROM EMERGENCE

Figure 10: The accumulation of lipid at 26.5°C and 12L:12D (——) and 16L:8D (---). Upon emergence, adults were given a 10% sugar solution. Each mean represents a sample of 10 females and S.E.


UG. TOTAL LIPID/MG. DRY WEIGHT +/- S.E.
80
100
120
140
160
24
48
72
96
120
HOURS FROM EMERGENCE

following emergence (Scheffe $p \leq 0.05$).

4.10 The Effect of Sucrose Feeding on Total Lipid Content

The concentration of lipid per unit dry weight ($\mu g/mg \pm$ S.E.) for females given water only in outdoor conditions was higher at 24 hours than at 48 hours (Scheffe $p \leq 0.05$) (Figure 11). The 48, 72, 96 and 120 hour time periods were not significantly different (Scheffe $p \geq 0.05$). A significant increase in total lipid was observed between 24 and 48 hours, when females were given 10% sucrose (Scheffe $p \leq 0.05$). However, no significant difference in lipid content was observed between 48, 72, 96 and 120 hours following emergence. There was a significantly greater quantity of total lipids in females given 10% sucrose than those given water only, at all sampling intervals.

4.11 The Rate of Lipid Depletion in the Overwintering *Cx. tarsalis* Population in Manitoba

Random samples of experimental female *Cx. tarsalis* were taken at monthly intervals during the winter months of 1986 and 1987 to determine the rate of lipid depletion in the overwintering population (Table 3). In 1986, only 100 females were recovered for lipid analysis. The accumulation of excess mold in the overwintering cages in 1986 prohibited further sampling. The decrease in total lipid (μg lipid/mg

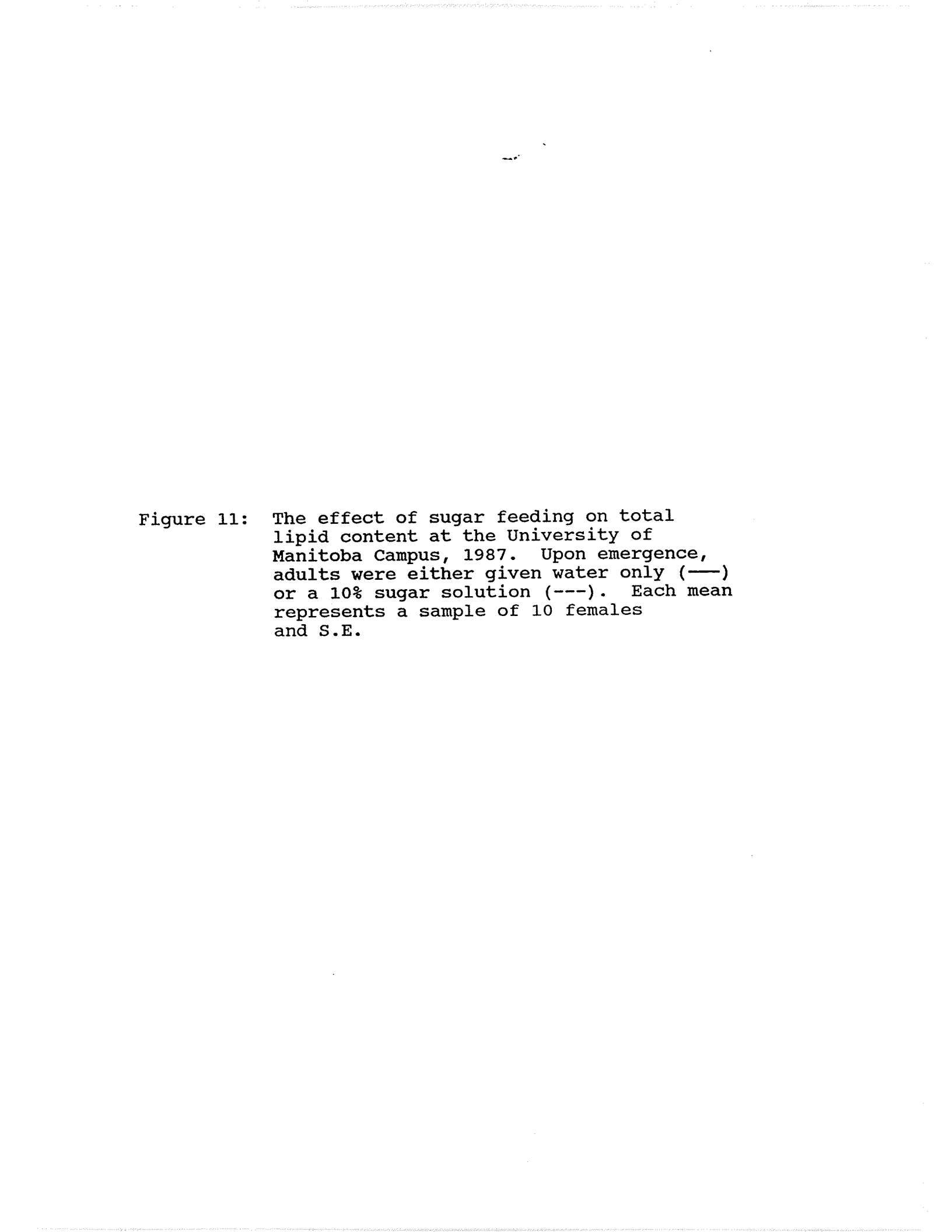


Figure 11: The effect of sugar feeding on total lipid content at the University of Manitoba Campus, 1987. Upon emergence, adults were either given water only (——) or a 10% sugar solution (---). Each mean represents a sample of 10 females and S.E.

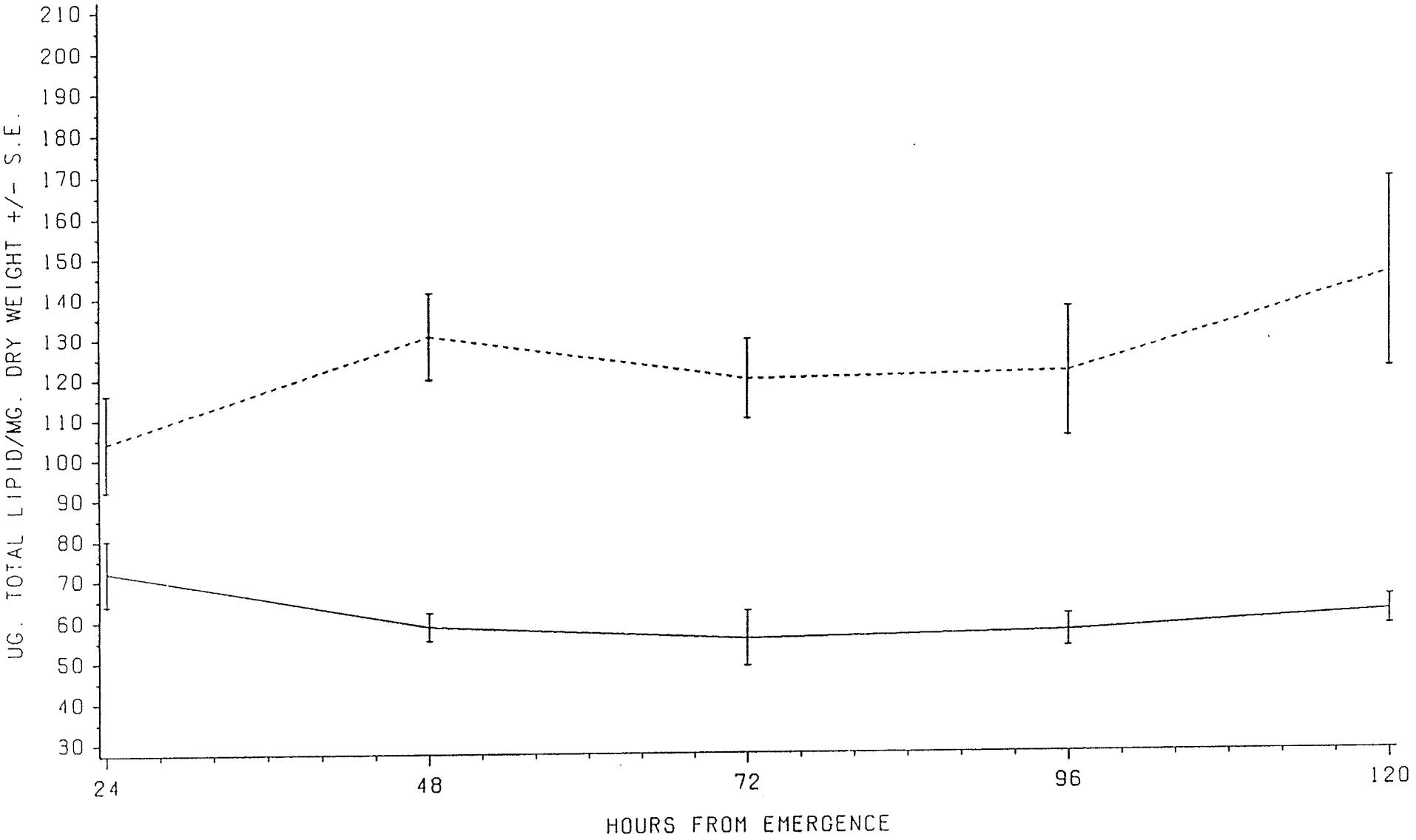

UG. TOTAL LIPID/MG. DRY WEIGHT +/- S.E.
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
24
48
72
96
120
HOURS FROM EMERGENCE

Table 3. The total lipid content of overwintering Culex tarsalis females (mean µg per female, mean µg lipid/mg dry weight ± S.E.) at the University of Manitoba Campus and Glenlea, Manitoba in 1986 and 1987.

Date	N	µg/female ± S.E.	µg/mg dry wt ± S.E.	% survival
Nov 17, 1986	60	158.22 ± 7	163.26 ± 7	49
Dec 17, 1986	40	146.48 ± 10	158.71 ± 9	2
Oct 22, 1987	20	159.74 ± 11	197.54 ± 9	100
Nov 17, 1987	47	187.90 ± 7	235.83 ± 8	73
Dec 16, 1987	34	127.43 ± 12	180.79 ± 14	53
Jan 20, 1987	44	126.04 ± 10	157.08 ± 10	5

dry weight) from November to December 1986 was not significant (Scheffe $p \geq 0.05$), and the percent survival for each collection date was 49% and 2%, respectively. The high mortality in 1986 may also have been affected by the low air temperatures (Fig. 12) and the greater amount of snow cover (Fig. 13) from October to January.

In 1987, significant differences in total lipid (μg lipid/mg dry weight) occurred among the 4 sampling periods (Duncan $p \leq 0.05$) (Table 3). The total lipid content in overwintering females was greater in November than in October, and there was a decrease in total lipids from November to January 1987. The percent survival at each collection date was 100% in October, 73% in November, 53% in December and 5% in January. The remainder of the experimental population was monitored on January 30, February 03 and February 25, but no living females were recovered.

Figure 12: The winter air temperature for 1986 (——) and 1987 (---) at Glenlea, Manitoba. Ambient air temperatures were obtained by recording a thermograph; additional data was from Environment Canada.

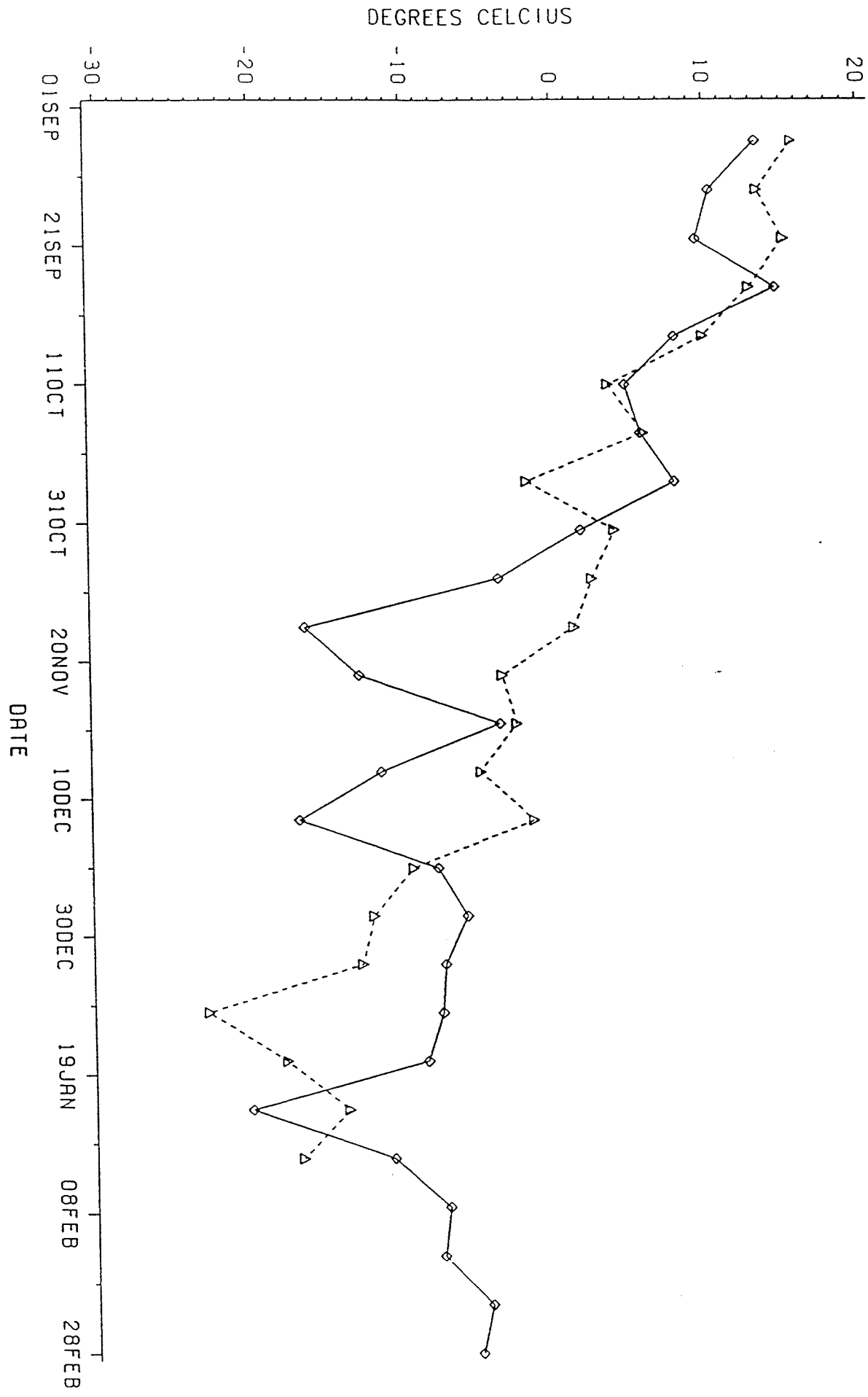
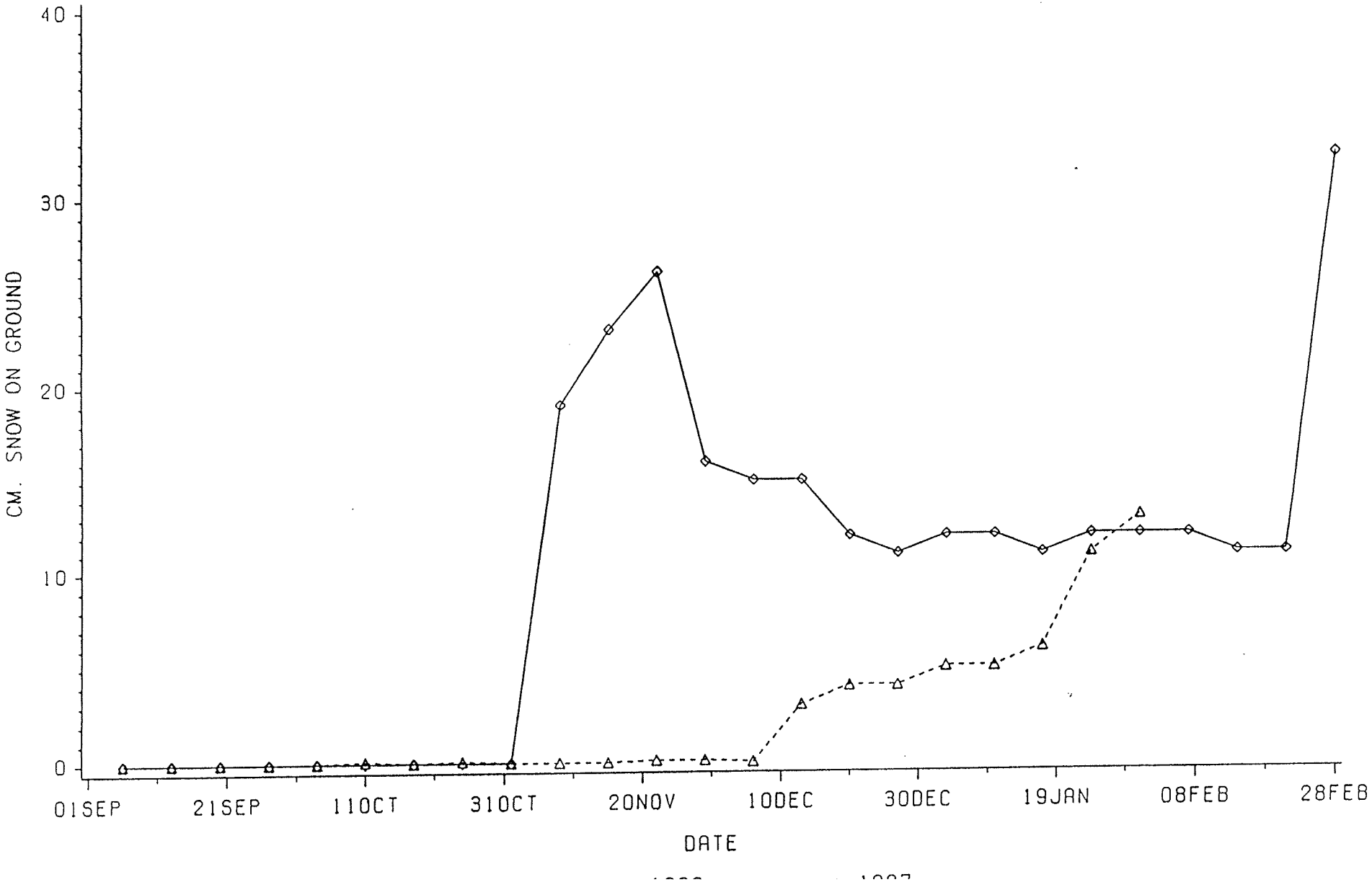

DEGREES CELCIUS
-30
-20
-10
0
10
20
01SEP
21SEP
11OCT
31OCT
20NOV
10DEC
30DEC
19JAN
08FEB
28FEB
DATE

Figure 13: The snow cover in 1986 (——) and 1987 (---). Data obtained from Environment Canada.


40
30
20
10
0
CM. SNOW ON GROUND
01SEP
21SEP
11OCT
31OCT
20NOV
10DEC
30DEC
19JAN
08FEB
28FEB
DATE

CHAPTER V

Discussion

Insects, like most multicelled organisms, are capable of storing reserves of metabolizable energy (Downer 1981). The accumulation of such reserves enables an insect to survive prolonged periods of metabolic activity without feeding (eg. embryogenesis, pupation, migration and diapause). The analyses of total lipid extracts from a variety of insect species clearly indicate that the dominant lipid class in the fat body is triglyceride (Fast 1964; Gilbert 1967; Downer and Matthews 1976; Beenakkers 1983; Downer 1981; 1985). The general characteristics which contribute to the success of lipids as a reserve of metabolic energy are: firstly, lipid reserves or triglycerides, yield almost two times more metabolic water than carbohydrate reserves, or glycogen. This source of non-imbibed water is particularly important to maintain homeostasis during the non-feeding stages of the insect. Secondly, lipids provide the most efficient and convenient means of energy storage. The advantages of triglyceride over glycogen as a metabolic fuel include a higher caloric content per unit weight of substrate and the capacity for

storage in an anhydrous form. Thus, the use of triglycerides as a primary metabolic substrate permits the accumulation of a large energy reservoir that can be metabolized and utilized in response to various bioenergetic demands (Downer and Matthews 1976; Downer 1981).

In this study, the total lipid content of adult Cx. tarsalis females was analysed to determine the environmental factors affecting lipid accumulation and depletion. Van Handel's (1985) colorimetric assay was used to establish the total lipid content of individual female mosquitoes. The correlation between optical density and the amount of lipid standard was high (r^2=0.99).

The average lipid content of Ae. aegypti when starved to death, fed 10% sucrose for 3 days and fed 10% sucrose for 7 days were shown to be 113, 495 and 550 μg lipid/female. Van Handel (1985), using these conditions, reported values of 18, 190 and 235 μg lipid/female, respectively. The reason for these differences was not established but may have been affected by different strains of laboratory reared Ae. aegypti, different rearing conditions and possible differences in the assay procedure (ie. soybean oil standard).

In the current study, the amount of lipid per Cx. tarsalis female at adult emergence, when fed 10% sucrose for 3 and 7 days, and when starved to death was 178, 453, 726

and 104 μg lipid/female, respectively. These data indicate that lipid content increased from adult emergence to 7 days with sucrose feeding and decreased when females were starved to death. The starved to death condition provided a baseline lipid level that included all non-nutritional and structural lipids. The proportional increase in total lipid per female from adult emergence to 3 and 7 days when fed 10% sucrose to the increase in lipids, using soybean oil standard at 25, 50, 100 and 150 μg per sample is considered sufficient evidence that the regression line was representative of lipid accumulation in adult Cx. tarsalis females.

It has been shown that lipid metabolism is modified by temperature and photoperiod, and in some insects this results in the accumulation of a suitable energy store to ensure overwintering survival (L'Helias 1970). Harwood and Halfhill (1964) found that at short photoperiod and low temperature, there was a general predominance of lipids in Cx. tarsalis. The major fatty acids detected in Cx. tarsalis females were palmitic, palmitoleic and oleic acids (Schaefer and Miura 1972). The accumulation of palmitoleic and oleic acids appeared to be necessary for overwintering survival in Cx. tarsalis females. Respective values of 43% and 26% were reported at low temperature and short photoperiod, whereas values of 31% and 20% were observed in

females reared at a moderate temperature and long photoperiod. Schaefer and Miura (1972) propose that nectar-feeding was the primary source of the newly synthesized fatty acids. Similar results were observed in A. sollicitans (Van Handel 1965; 1966; Magnarelli 1978), A. taeniorhynchus (Nayar and Sauerman 1971), A. triseriatus (Say) (Magnarelli 1986), and C. pipiens pipiens (Magnarelli 1978). Many researchers have also attempted to demonstrate a relationship between environmental temperature and the degree of fatty acid saturation within an insect (L'Helias 1970; Downer 1981). The melting point of fatty acids increases with the degree of saturation; hence, it is suggested that there is a greater proportion of unsaturated to saturated fatty acids in insects reared at lower temperature. Harwood and Takata (1965) confirmed that unsaturated fatty acids, except 18:3, increased as the rearing temperature of Cx. tarsalis was decreased. Photoperiod is also believed to affect the degree of lipid unsaturation in this species. Similar results were observed in H. cecropia (Beenakkers and Gilbert 1968), B. mori (Sridhara and Bhat 1965), M. autumnalis (Pitts and Hopkins 1965), and C. capitata (Municio et al. 1971). Although the accumulation of lipid reserves and the decreased level of saturation of fatty acids in overwintering insects is well-documented, there is no quantitiative data on total lipid

content (μg total lipid/female) in previous literature to provide an ecological comparison of total lipids in Cx. tarsalis females.

Several experiments were conducted under laboratory conditions to determine the relationship between temperature, development and lipid content. These results show that at low temperatures, development time was significantly increased whereas at higher temperatures, development time was decreased. The low temperature threshold for Cx. tarsalis was 14°C and the upper temperature threshold was 34°C or above. At both 32 and 34°C, 5 days were required for 50% pupation with 59.7% and 41.3% survival, respectively. The decrease in the percentage survival at 34°C suggests that perhaps 32°C was the optimum upper temperature threshold for development in Cx. tarsalis.

The total lipid content per female for each temperature treatment indicated that larger quantities of lipid were present in newly emerged females when larvae and pupae were maintained at 14 and 17°C. Significantly smaller quantities of lipid were present in females, when immatures were maintained at 25 to 34°C. This suggests that at lower temperatures, increased nutrient assimilation or reduced nutrient utilization occurred in Cx. tarsalis females. As a result, the adults contained significantly larger quantities

of lipid at emergence.

Six rearings were conducted outdoors at the University of Manitoba Campus in 1986 to determine the period of the summer or fall season in which lipid accumulation occurred. These results suggest that mean air temperatures significantly affected lipid levels (mg wet weight/female) on all dates by indirectly affecting the quantity of physiological water available to regulate homeostasis. The lipid levels, represented by dry weight, indicate that lipid content is not significantly different, except on July 7 and August 18. In the literature, lipid content is represented by both mg wet weight and dry weight. These data indicate that the mg dry weight of females is a more accurate representation of total lipids than wet weight.

The total lipid content on a dry weight basis (μg lipid/mg dry weight) indicated that lipid levels were significantly greater on July 7 and 23, and significantly lower on August 18. These results suggest that total lipid content in field-reared populations was not closely related to air temperature and development time. Therefore, the possible role of photoperiod is proposed. A decrease in photoperiod of 1 hours 29 minutes occurred between July 23 to August 18, 1986; hence, the relationship between total lipid content and photoperiod may be relevant. This hypothesis, however, needs to be tested under controlled conditions.

The field population of Cx. tarsalis females was also sampled at weekly intervals during 1986 to determine if the fall generation of females had a higher lipid content than summer generations. Unfortunately, females that are attracted to CO_2 and light represent a cohort of unknown age and physiological condition (ie. nulliparous vs parous), and lipid correlations with environmental factors cannot be made. However, the variation in total lipid content in these samples was likely related to nectar feeding, or access to other carbohydrate sources. Mosquitoes have been observed feeding on a wide variety of nectar sources and fructose, such as rotting fruit and vegetables (Van Handel 1972; 1984; Bidlingmayer and Hem 1973; Reisen et al. 1986c).

The effect of photoperiod (16L:8D and 12L:12D) and temperature (21 and 26.5°C) on lipid accumulation was investigated in 1987 in controlled environment conditions. These results indicated that females accumulated significantly larger quantities of total lipid at 16L:8D than at 12L:12D at both 21 and 26.5°C These results thus indicate that total lipid content was modifiable by long photoperiod; however, this appears of limited advantage to the overwintering female. Harwood and Halfhill (1964) found that a California-derived strain of Cx. tarsalis responded to short (8-hour) daily photoperiod at 22°C by increased size and compactness of the fat body, and by minimal ovary

length. Lower temperatures were shown to reinforce the response of the fat body. The data obtained in this study may reflect unsuitable stimuli (temperature and/or photoperiod) for the accumulation of total lipids in northern Cx. tarsalis populations, particularly with respect to temperature. This hypothesis, however, requires additional testing under controlled conditions.

The effect of sucrose feeding on total lipid content was determined by comparing females given water only and females given 10% sucrose in outdoor conditions. The concentration of lipid per unit dry weight (μg/mg) for females given 10% sucrose was significantly greater from 24 to 120 hours following emergence than females given water only. These data indicate the importance of sucrose, or nectar feeding in adult females, particularly to ensure overwintering lipid reserves (ie. lipid reserves do not appear to be carried over from the immature stages).

The depletion of total lipids in an experimental overwintering population was investigated in 1986 and 1987 to determine the relationship between overwintering survival and total lipid content. The total lipids per female (μg /female) were 158 and 146 μg for November and December 1986, respectively (Table 3). In 1987, the total lipids per female were 159, 187, 127 and 126 μg for October, November, December and January samples, respectively. The comparison

of these lipid quantities with total lipids for Cx. tarsalis at adult emergence (179 μg/female) suggests that large quantities of lipid were not present in overwintering females. Another explanation for the seemingly low overwintering lipid levels may be the calculation of total lipid for Cx. tarsalis (Table 2). The egg rafts used to determine total lipid in Cx. tarsalis adults were obtained from a colony that has been colonized for approximately 150 generations (obtained in 1973). Therefore, these quantities of lipid may not be comparable to those of natural populations. The total lipid content on a dry weight basis for natural populations of Cx. tarsalis females given water only in outdoor conditions, were 72, 58, 55, 57 and 61 μg lipid/mg dry weight at 24, 48, 72, 96 and 120 hours following emergence, respectively (Figure 11). The total lipids on a dry weight basis for females given 10% sucrose were 104, 130, 119, 120 and 144 μg lipid/mg dry weight at 24, 48, 72, 96 and 120 hours following emergence, respectively. It is clear from these data that lipid levels doubled when females were fed 10% sucrose. Overwintering females, therefore, had ample reserves of lipid when they died.

The low survival of females in 1986 was attributed to an excess accumulation of mold in the overwintering cages. Low temperatures and low snow cover may also have

contributed to adult mortality. In 1987, experimental females were placed under field conditions on October 22, without a water source. November samples had significantly higher lipid levels than October samples, indicating the possible conversion of carbohydrates into triglycerides. From November to January, a significant reduction in total lipid content was observed at each sampling date with a concurrent decrease in the percent survival. In January 1987, the mean quantity of lipid/female was 126 μg lipid/female or 157.08 μg lipid/mg dry weight. This suggests that overwintering females were not dying of starvation, since this is greater than the starved to death lipid level for colony Cx. tarsalis (104.0 μg/female), and about 2 times greater than the level of lipid at emergence in natural populations. Consequently, there must be other factors affecting overwintering survival.

The acclimation period to 10°C 0L:24D in 1986 and 1987 was 2 and 4 weeks, respectively. The longer acclimation period in 1987 may have resulted in an accumulation of increased lipid levels, as well as other reserves. As a result, these factors may have been responsible for increased overwintering survival. Increased levels of glycerol have been observed in diapausing H. cecropia (Gilbert 1967), Sarcophaga bullata Parker (Adedokun and Denlinger 1984), Sarcophaga crassipalpi Macquart, Ostrinia

nubilalis (Hubn.) (Nordin et al. 1984), Eurosta solidaginis (Fitch) (Baust et al. 1979; Baust and Lee 1981; 1982; Storey et al. 1981a; 1981b; Rojas et al. 1983), Tetanops myopaeformis (Roder) (Whitfield and Grace 1985), and Pectinophora gossypiella (Saunders) (Askisson et al. 1963). The increased level of glycerol in overwintering insects is believed to act as an "antifreeze" by increasing the osmotic pressure of the tissue fluids (Patton 1963). The accumulation of cryoprotectants was not investigated in this study; however, this may be another important factor for overwintering survival in Cx. tarsalis. Hudson (1977) found that Culex females survived better at 0°C, or at slightly lower temperatures. Warmer air temperatures until mid-December and less snow cover in 1987 may have been contributed to the improved survival of overwintering females in 1987, as compared to 1986.

CHAPTER VI

Conclusion

In this study, Van Handel's (1985) calorimetric assay using soybean oil standard provided an accurate and reliable representation of total lipid content in Cx. tarsalis females. The baseline lipid level for starved females was 104.0 μg total lipid per female. The total lipid content increased with sucrose feeding, with values as high as 453 and 726 μg lipid/female being obtained at 3 and 7 days, respectively.

The low temperature threshold for Cx. tarsalis development was 14°C and the upper threshold was 34°C or above. At low temperatures (ie. 14 and 17°C), an increase in development time and larger quantities of lipids were observed at emergence. At higher temperatures (ie. 25 to 34°C), a decrease in development time and decreased quantities of lipid were observed at emergence.

A series of experiments conducted under natural conditions showed that lipid levels in newly emerged females were lower in August than in July. Therefore, nutrient availability such as nectar feeding, or other carbohydrate sources, must have been different during these two periods. Nectar feeding has been shown to be the primary source of

lipid reserves for overwintering (Schaefer and Miura 1972).

Culex tarsalis females overwinter as inseminated, nulliparous females. Since females accumulated significantly larger quantities of total lipid at 16L:8D than at 12L:12D at both 21 and 26.5°C, it is concluded that photoperiod did affect lipid levels. However, it appears of limited advantage to the overwintering female, since this occurs at a time (July) when nectar sources are abundant.

This research indicated that there was a close relationship between overwintering survival and the lipid stores in Cx. tarsalis. The low survival of females in 1986 was attributed to an excess accumulation of mold in the overwintering cages. In 1987, overwintering females were sampled until January 20. The mean lipid content the January sample was 157 μg lipid/mg dry weight, indicating than females did not die of starvation.

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APPENDIX 1. LIPID ANALYSIS

PROCEDURE:

A. Vanillin-phosphoric acid reagent

Dissolve 600 mg. vanillin in 100 ml. hot water. Add 400 ml. 85% phosphoric acid and store in a dark place.

B. Lipid standard

Mix 100 mg. per 100 ml. soybean oil in chloroform.

C. Method of analysis

Freeze-dry and weigh individual female mosquitoes. Crush female in about 0.5 ml. chloroform-methanol. Transfer supernatant to a clean test tube, and place tubes in a heating block to evaporate the solvent. Add 0.2 ml. sulfuric acid and heat for 10 minutes. Cool. Pour vanillin reagent to 5 ml. mark on the test tube. Mix and allow the reddish color to develop for 5 to 30 minutes. Read the absorbance in Spectronic 501 at 525 nm. against a reagent blank. If the optical density is higher than 1.00, dilute the sample (usually a 1:5 dilution).

blank= 0.2 ml. sulfuric acid and 4.8 ml. vanillin-phosphoric acid reagent.