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Growth and Development of *Culex tarsalis* Mosquito Larvae and the Feeding Ecology of a Potential Biocontrol Organism, *Dugesia tigrina*.

by

Malcolm H. McKee

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The undersigned certify that they have read, and recommend to the Faculty of Graduate studies for acceptance, a thesis entitled, "Growth and Development of Culex tarsalis Mosquito Larvae and the Feeding Ecology of a Potential Biocontrol Organism, Dugesia tigrina" submitted by Malcolm H. McKee in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Concern over the impact of insecticides in the environment has led to a resurgence in integrated pest management (IPM) and biological control programs which require extensive knowledge of the ecology of mosquito larvae and their predators. This study develops a conceptual stage-structured model for larval growth and development rates. Experiments were performed to determine how the effects of food concentration and temperature on larval rates would be best represented in the model. Results showed the model could be simplified from 7 developmental stages to 5 by grouping the first three instars (I, II, III = early larval stage). Growth and development in this early larval stage was independent of food concentration and only weakly influenced by temperature. In the IV instar, sexually dimorphic growth and developments rates occur that are positively influenced by temperature and food concentration. While females showed slower development rates they gain significantly more weight per day than males.

The potential for the Turbellarian *Dugesia tigrina* for use in integrated pest management programs was investigated by studying the functional response of the predator. *Dugesia* has a Type II functional response, with small (II) instar mosquito larvae more vulnerable to predation than larger IV instars. A significant non-consumptive predator-induced prey mortality resulted; however, the ecological significance of this to the predator-prey interaction cannot as yet be determined. *Daphnia* are more vulnerable to this non-consumptive mortality than mosquito larvae. The significantly different prey mortality between the two size classes of mosquito larvae suggest *Dugesia* predation would have its strongest influence on the early larval stage.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

The ascendancy of synthetic organic insecticides for the management of pest insects began with the commercial introduction of DDT for agricultural use in 1946. From this time chemical insect management can be divided into three distinct phases: The Era of Optimism, 1946-1962; Era of Doubt, 1962-1976; and the Era of Integrated Pest Management, 1976-present (Metcalf 1980). These phases document a transition from viewing chemical insecticides as the panacea for all insect infestations and out-breaks (to the extent that proposals were put forward to develop conservation programs to prevent mosquitoes, cockroaches and lice from becoming extinct (Horn 1988)) to their recognition as a contributing, and often causal agent of severe environmental and insect management problems. A number of negative impacts are now associated with the use of insecticides including: the destruction of pollinators (Horn 1988), selection for insecticide resistant genomes (Luck *et al.* 1977, Wirth *et al.* 1987, Bisset *et al.* 1990), and food chain contamination (Blus *et al.* 1971, Schreiber 1980). In addition, the elimination of natural predators and competitors often resulted in rebound (Heinrichs *et al.* 1982, Madsen & Madsen 1982) and secondary pest outbreaks (Metcalf 1980, Horn 1988). Rebound outbreaks result when broad spectrum insecticides eliminate the invertebrate community in the habitat subsequently releasing recolonizing populations of the pest insect from regulation by predators and competitors. Secondary pest out-breaks

occur when the elimination of the invertebrate community results in rapid recolonization by a different pest insect that in the previous community was not considered a pest. As a result, disillusionment with chemical control has led to a resurgence of interest in integrated pest management and biological control (Chapman 1985, Service 1985, Horn 1988).

It is important to be clear on the distinction between population control and regulation. Regulation of a population is the maintaining of a population between upper and lower threshold densities, that if crossed will result in extinction of the population (Nicholson 1954a, Milne 1957). Control is the depression of the upper population peaks below densities perceived as causing economic loss (Milne 1954), and is now commonly referred to as the economic injury level or EIL (Horn 1988). Thus, biological control means the limitation of a pest to or below an upper threshold level equal to the EIL through the use of biological agents either as the sole controlling agent or in conjunction with insecticides in an Integrated Pest Management program (IPM).

A typical approach to biological control programs has been to release various potential control organisms in the hope that one or more will be effective (Murdoch *et al.* 1985). Godfray and Waage (1991) have noted some practitioners go so far as to advocate the introduction of all possible biological control candidates with no *a priori* selection. This broad spectrum approach has had limited success, with the large number of disappointing or inconclusive results casting doubt on the effectiveness of biological

control (Service 1985, Godfray and Waage 1991). The lack of basic biological, and ecological knowledge of both predators and prey has hindered *a priori* selection of candidates for biological control. To meet the practical need for predictive, scientific approaches to the selection of biological control candidates, detailed ecological and theoretical studies of both potential control organisms and pest species are required (Service 1983).

The high economic and health costs arising from the blood-feeding habits of mosquitoes has made them a target of massive insecticide applications (Metcalf 1980) and as a result, many of the negative ecological impacts associated with excessive application of insecticides listed earlier were first demonstrated in mosquito populations. Research into the biological control mosquitoes has suffered from premature promotion of potential biological control agents (Chapman 1985) based on sparse information on the potential control organism with little consideration of ecological interactions at the population level (Service 1985). To assess the biological control potential of a predator, pathogen, or parasitoid (insects that are parasitic as immatures, that kill their hosts in the latter stages of the parasitoids development or when emerging as adults) for biological control, an understanding of the underlying mechanisms driving the dynamics of both the pest insect and the control agent is required. Thus, this study examines the developmental biology of the mosquito *Culex tarsalis* Coquillett as well as the foraging behaviour of the turbellarian predator *Dugesia tigrina* Girard, a potential mosquito biological control agent.

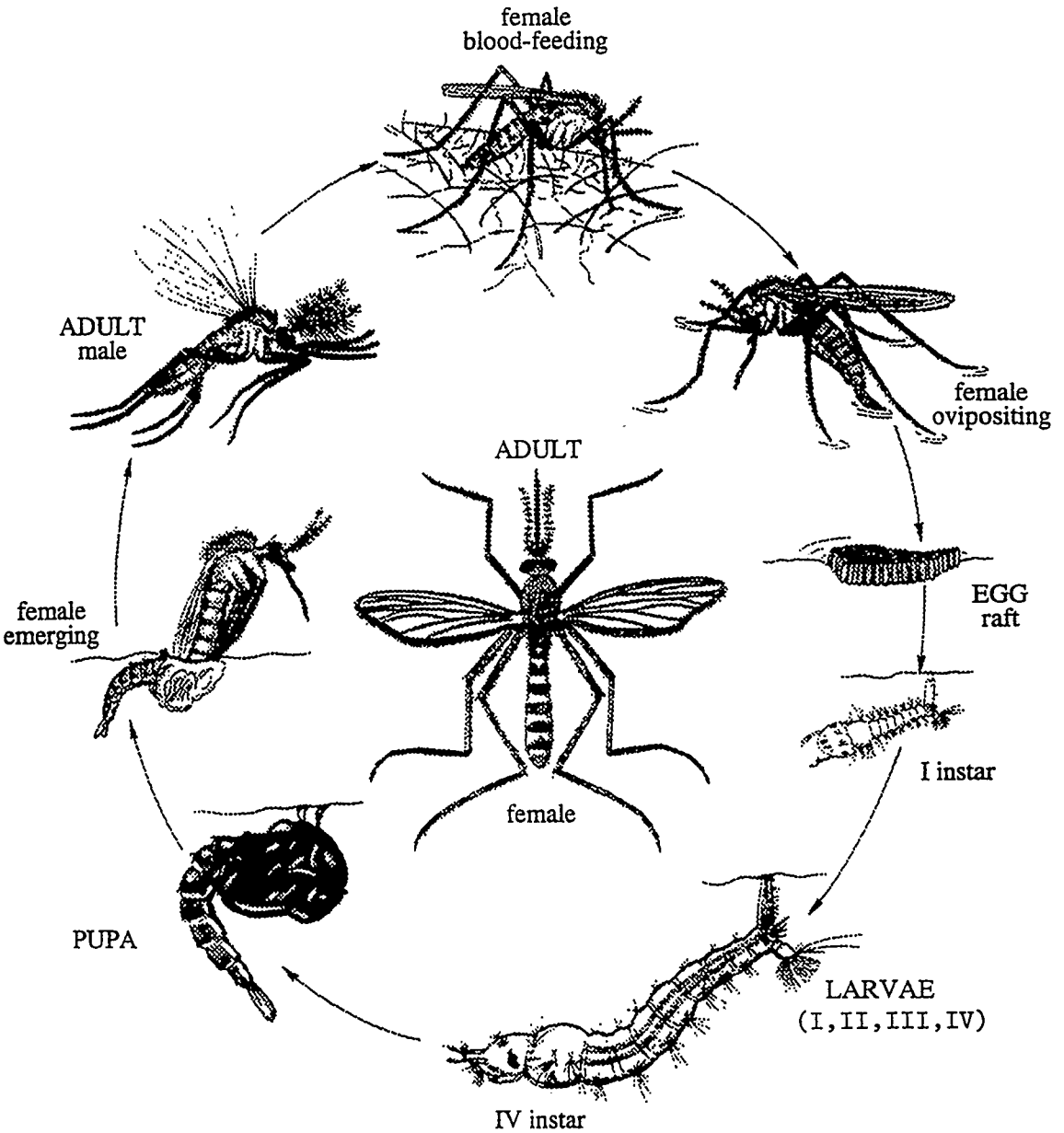
1.1 THE STUDY ANIMALS

1.1.1 THE PEST INSECT - *CULEX TARSALIS*

Female *C. tarsalis* transmit the arbo-viruses western equine encephalitis (Sekla 1976, 1982, Sekla *et al.* 1980, Artsob 1983) and St. Louis encephalitis and are thought to be the primary vector for both diseases throughout western North America (Reeves and Hammon 1962, Reeves 1965) They have also have demonstrated an increasing resistance to insecticides (Reeves 1972, Bohart and Washino 1978).

The life-cycle of mosquitoes consists of seven stages: the egg, four successively larger larval instars (I, II, III, IV), the pupa, and the adult (Fig. 1.0). The genus *Culex* deposit their eggs on the water in clusters (rafts) of 100 - 300 eggs (Edmunds 1935, Buth *et al.* 1990). All larval instars are aquatic, filtering food particles (predominantly micro-organisms) from the water column and/or grazing on fungal or microbial communities colonizing biotic and abiotic substrates (Merritt *et al* 1992). Respiration by instar I larvae is primarily cuticular, but instar II - IV larvae (with the exception of *Anopheline* sp.) respire through paired respiratory spiracles located in abdominal segment VII in a respiratory siphon that pierces the surface film to obtain atmospheric oxygen. Pupae are positively buoyant and when at rest, are found suspended at the surface. However, when disturbed, they exhibit a rapid flight response through contractions of their paddle-like tail. Pupae also utilize atmospheric oxygen obtained through two small respiratory

Figure 1.0. The seven basic stages in the mosquito life-cycle (egg, larva, pupa and adult) and some of the key moments in the life-history (emergence, blood-feeding and oviposition). (Modified from the Carolina Biological Supply Co. bioreview sheet 4588).


female
blood-feeding
ADULT
male
female
ovipositing
ADULT
female
emerging
EGG
raft
I instar
female
PUPA
LARVAE
(I,II,III,IV)
IV instar

trumpets projecting from the anterolateral portion of the thorax. The pupae is a non-feeding stage during which tissue is hystolized or reorganized into adult tissue.

At emergence, the pupal case ruptures mid-dorsally to allow the adult to emerge. Should the emerging adult contact the surface film (pleuston) with any body part other than the tarsus and tarsal claws, entrapment and death usually result (White 1980). Mating occurs within a day or two after emergence. Females mate only once while males continue to swarm throughout lifespan (Reisen *et al.* 1983, 1985). Both males and females utilize nectar for energy but females require a blood meal after mating to obtain sufficient protein for egg development (Wood *et al.* 1979, Briegel 1990a). Females may go through several gonotrophic cycles each requiring a separate blood meal (Koller and Raikhel 1991). Buth *et al.* (1990) estimated that in Manitoba *C. tarsalis* have 2.6 to 2.9 generations per year with five ovarian cycles a year, likely the maximum for any in Canada due to climatic limitations (Wood *et al.* 1979).

In Canada, in order to overwinter, mosquitoes must enter diapause. In the genus *Culex*, only non-blood fed, inseminated females with arrested ovarian development successfully overwinter (Reisen *et al.* 1983). As larvae in late summer (August and September) are exposed to a shortening day-length, the number of emerging females exhibiting reproductive diapause increases rapidly to approximately 70 % by the beginning of August, to near 80 % by the end of September (Buth *et al* 1990). These females seek out protected sites such as barns, attics, talus slopes and rodent burrows as

overwintering sites (Shemanchuck 1965). Rodent burrows are ideal overwintering sites owing to the buffering of temperature fluctuations by the soil layer. At sites not buffered from temperature fluctuations during the winter (e.g. chinook winds) diapausing females can become prematurely active resulting in diminished storage reserves, potentially decreasing the individuals survivorship and fecundity (Shemanchuk 1965).

Females terminate diapause and take flight in the spring as temperature increases. Newly active females immediately seek blood meals and begin ovipositing before the end of May (Shemanchuk 1965). A broad range of aquatic habitats are utilized for ovipositing and pre-imaginal development (Hagstrum and Gunstream 1971, Fanara and Mulla 1974, Buth *et al.* 1990). Standing water possessing a high organic content is preferred. Semi-permanent habitats provide the best trade-off between persistence, allowing time for complete development and transience, minimizing the time available for the development of large predator populations (White 1980).

1.1.2 THE PREDATOR - *DUGESIA TIGRINA*

Legner and Medved (1979), Legner and Sjorgren (1984) and Legner (1985) investigating potential biological control organisms for mosquitoes have recommended the study of invertebrate predators, especially flatworms (Platyhelminthes: Turbelleria). The reasons for using Turbellarians as potential control organisms have been primarily based on practical management considerations. A number of species can be easily reared in the laboratory (Tsai and Legner 1977, Legner 1979) and unlike many predacious insects, some turbellarians can be confined at high densities without cannibalism (Legner 1979). Turbellarians have a high tolerance to pesticides and growth regulators (Levy and Miller 1978) enhancing their potential for IPM programs. In addition they show an efficacy for mosquito larvae and have little impact on the abundance of non-target insect predators (Legner 1977). Two species *D. tigrina* Girard and *D. dorotocephala* Woodworth, have received the most attention as potential mosquito control agents (Legner 1977, Tsai and Legner 1977, George 1978, Levy and Miller 1978, Meyer and Learned 1981, Arshad and Mulla 1983, George *et al.* 1983).

In Alberta, *D. tigrina* occurs in central and southern areas of the province in permanent and semi-permanent ponds and lake margins (Folsom and Clifford 1978, Clifford 1991). It is a suctorial generalist predator feeding on a variety of aquatic invertebrates (Reynoldson and Davies 1970, Pickavance 1971a, b). There are two physiological varieties of *D. tigrina*, one of which reproduces asexually by fission

(Pennak 1978), and the other which alternates between sexual reproduction at cool temperatures and asexual reproduction at warm temperatures (Chandler 1966 in Folsom and Clifford 1978, Pickavance 1968). During periods of low food availability *D. tigrina* reabsorb body tissue and degrow (Reynoldson 1964). *D. tigrina* form aggregations in the field as well as under homogenous laboratory conditions (Reynierse 1967, Reynierse and Ellis 1967, Reynierse, Gleason and Ottemann 1969, de Silva 1976, 1978) and have been observed group feeding on individual mosquito larvae (Mead 1978, Kolasa 1984).

1.2 OVERALL RESEARCH OBJECTIVES

The objectives of this study are: (1) to develop a conceptual model of adult female *Culex tarsalis* emergence rates incorporating larval dynamics, and (2) to assess the potential of *Dugesia tigrina* as a biological control agent of mosquito larval populations. The basis of a mosquito population model incorporating the stage structure of the population and operating in continuous time will be outlined. This conceptual model will be used to provide a framework to investigate the dynamics of growth and development rates of *C. tarsalis* as they are effected by extrinsic variables such as temperature, food concentration and larval density, and ultimately the effect of larval history on adult female emergence, survivorship and fecundity. Experiments will be conducted to determine the effects of these extrinsic factors on larval mortality, and rates of growth and development on a stage-specific basis.

To predict the impact of a predator on its prey population, a detailed understanding of the predator's foraging behaviour is required. An understanding of the predator-prey relationship between *D. tigrina* and mosquito larvae will lead to incorporation of this interaction into a mosquito population model to assist in the generation of hypotheses that can be subsequently tested experimentally. Thus, laboratory experiments examining the functional response of *D. tigrina* feeding on mosquito larvae and an alternative prey will be conducted. The effects of prey size on the functional response will also be investigated.

SECTION I

CHAPTER TWO

2.0 MOSQUITO POPULATIONS AND MODELLING

Population models can be used as analytical tools for investigating population dynamics. They may be used strategically to organize and direct experimental approaches and tactically to investigate the mechanisms determining a populations dynamics (Gurney *et al.* 1983). Concerted attempts at mathematically modelling mosquito population dynamics are lacking (Dye 1984) and those that do exist generally - have two major weaknesses: (1) they focus primarily on adult densities and characteristics, neglecting larval characteristics and/or (2) they are discrete generational models which are inappropriate for the overlapping generations of mosquitoes. The majority of mosquito population models have been developed for disease vector research and sterile male release programs (Weidhaas 1974, Haile and Weidhaas 1977, Chubaki 1979, Fine *et al.* 1979, Birley *et al.* 1983) and generally have both major weaknesses. However, the few models that do incorporate larval dynamics have shown that adult densities are strongly influenced by larval density-dependent mortality (Miller *et al.* 1974, Dye 1984). There is also empirical evidence showing adult vital rates, such as fecundity and survivorship, are strongly influenced by the conditions experienced in the larval stages, including competitive interactions among larvae (White 1980, Reisen *et al.* 1984, Haramis 1985, Service 1985, Clements 1992). Gilpin and McClelland (1979) suggest density-dependent mortality on mosquito adults or eggs results in density-

dependent feedback in the larval stage from reduced mortality due to a decline in larval food exploitation. Rajagopalin *et al.* (1977) found that overcompensating density-dependent processes acting on larval populations of *Culex quinquefasciatus* Say, resulted in increased numbers of emerging adults when the density of I instar larvae was reduced. An ideal control agent may therefore be one that inflicts mortality on the latter larval stages allowing the negative effects of larval competition to operate in the earlier larval stages. Thus, individuals not killed by the control agent would still have experienced the negative effects of larval competition at the earlier instars and as a result have lowered fecundity. Control agents that cause large early instar larval mortality may release surviving larvae from larval competition, resulting in a higher probability of successful emergence and greater fitness for these individuals (Service 1985). For example, Lounibos (1983) found the phantom chaoborinae predator *Corethrella appendiculata* Coq. displayed a marked preference for I and II instar rather than III and IV instar larvae of the mosquito *Aedes triseriatus* Say. If *A. triseriatus* emergence rates are strongly influenced by larval competition, high predation rates by the predator may actually increase the numbers and individual fitness of emerging adult mosquitoes by releasing them from the negative effects of intraspecific competition early in their development.

Incorporating larval stage specific density-dependence with stage specific mortality rates inflicted by a control agent into a mosquito model would provide theoretical predictions that could be tested empirically. Hence, analysis of density-dependent effects on larval development and growth rates is necessary to understand mosquito population

dynamics. Moreover, a more realistic model is required that addresses larval density-dependence in a continuous time framework so that the effects of overlapping generations and age- or stage-structure of the population are considered.

2.1 CONCEPTUALIZATION OF THE MODEL

Two conceptually approaches are used by population ecologists when modelling predator-prey interactions: analytical and simulation. Analytical models, mostly based on Lotka-Volterra or Nicholson-Bailey models (Taylor 1974, Hassell 1978), make very broad predictions on system stability and equilibrium levels utilizing as few biological variables as possible. These models identify general patterns of predator-prey interactions, but are unlikely to apply to species-specific predator-prey interactions (May and Hassell 1988).

Simulation models are detai-intensive models which incorporate prey biology, predator biology and often include the food source of the prey as well as abiotic variables (eg. Guitierrez *et al.* 1975, Guitierrez *et al.* 1984a, b, Guitierrez *et al.* 1984, 1988a, b, c). Simulation models require determination of an enormous number of variables, and while valuable for predicting site specific predator-prey interactions, suffer from a lack of generality.

The strengths of simulation models are the weaknesses of analytical models and

vice-versa (Godfray and Waage 1991). Thus, there is a need for models of intermediate complexity which incorporate more biological complexity than analytical models, but less complexity than the detail intensive simulation models.

One modelling approach, classified by Godfray and Waage (1991) as intermediately complex, is that proposed by Nisbet and Gurney (1983), Gurney *et al.* (1983), Gurney and Nisbet (1985) and Murdoch *et al.* (1987) termed continuous-time-stage structured models. In these models the life-cycle of the prey is divided into identifiable stages (ie. age, weight, instars, etc.) each of which is represented by a balance equation of inputs and outputs. The natural division of insect life-cycles into discrete physiological stages (i.e. egg, larva, pupa, adult) makes insects especially amenable to this approach. The incorporation of age (or stage) structure significantly enhances the predictability of the model, as age structure is known to be of crucial importance to the stability properties of many populations, but is almost equally widely absent from population models (Gurney *et al.* 1983). Stage selection must meet the basic assumption that all individuals in a given stage at a specific time are functionally identical with the same per capita vital rates (eg. mortality risk, fecundity, etc.). However, it is important to identify the minimal number of stages required to capture the dynamics of a population. The elimination of even a single stage greatly enhances empirical determination of the model's components (Gurney *et al.* 1983).

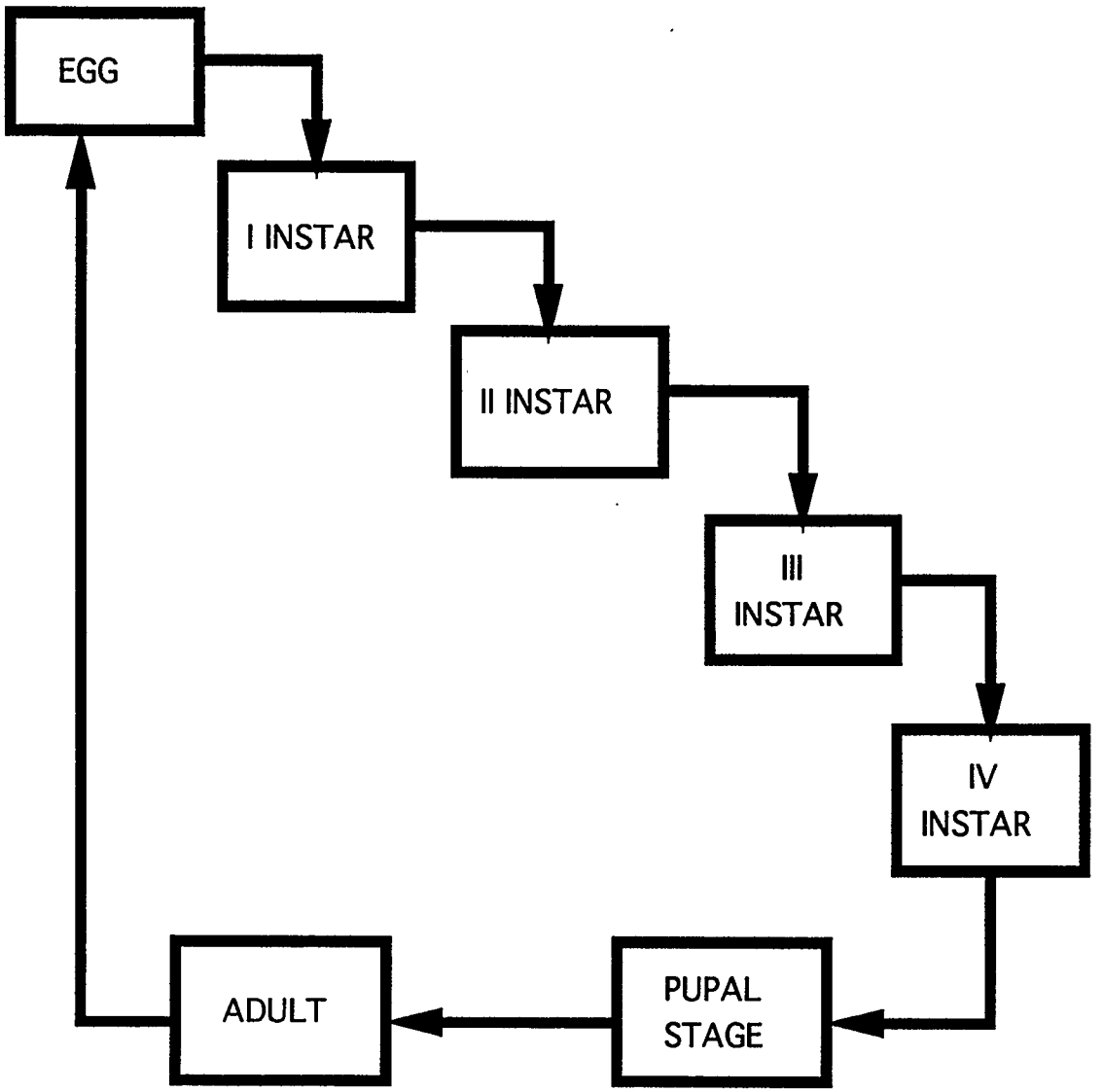
2.1.1 IDENTIFICATION OF INITIAL STAGE STRUCTURE

The seven developmental stages of egg (E), four larval instars (I, II, III, IV), pupa and adult will serve as the starting point for stage structure determination (Fig. 2.0). In this section each of the seven stages of the model will be examined, outlining the complexity needed to be incorporated into each stage of the model.

2.1.1.1 Egg

Egg production is the only source of recruitment into the population as adult immigration and emigration are assumed to be non-existent. Recruitment into the egg stage can operate in one of two ways. Egg input can be treated as being either dependent, or independent of the emergence rate of the adults. If the model is used to investigate the effects of certain conditions (ie. varying food availability, predation rates, etc.) on the number of females emerging from a cohort, then a single introduction of eggs would be appropriate. However, if the long term dynamics of the mosquito population is under investigation, egg input to the model should include a recruitment function related to the rate of adult female emergence. Egg density does not affect egg viability or development rates (White 1980), although larval density has been found to inhibit hatching in tree-hole mosquitoes (Livdahl and Edgerly 1987, Edgerly and Marvier 1992). This phenomenon has not been observed in non tree-hole species such as *C. tarsalis* and thus, will not be incorporated into the model.

Figure 2.0. The seven initial stages of the conceptional model for *Culex tarsalis*. Individuals within each of these stages may be considered to be functionally identical. Movement between the stage is uni-directional with ecdysis or mortality the only means of exiting a stage.


EGG
I INSTAR
II INSTAR
III INSTAR
IV INSTAR
PUPAL STAGE
ADULT

2.1.1.2 Larvae

2.1.1.2.1 Survival

The survival rate of mosquito larvae in both laboratory and field experiments varies as a function of density (Barbosa *et al.* 1972, South *et al.* 1972, Wijegaratne *et al.* 1974, Reisen 1975, Reisen 1979, White 1980, Madder *et al.* 1983). These density-dependent larval survival rates account for the resilience of mosquito populations following reductions in density (Weidhaas *et al.* 1971, Weidhaas 1974). Survival also varies greatly with temperature (Clements 1963, Southwood *et al.* 1972, Madder *et al.* 1983) and nutrition (Hagstrom and Workman 1971). However, no studies have assessed these effects on an instar-specific basis in *C. tarsalis*.

Ikeshoji and Mulla (1970a,b) and Moore and Fisher (1969) suggest that metabolites released by older cohorts are at least partly responsible for the reduced survival rates of younger cohorts reared in mixed cohort laboratory cultures. Dye (1984) found larvae of some strains of *Aedes aegypti* produced detectable quantities of a growth retardant whilst others did not. Increased mortality in *A. aegypti* not producing specific chemical growth retardants was due to the concentration of waste products from continuous rearing in the laboratory (Dye 1984). White (1980), however, failed to detect the presence of growth retardants in *C. tarsalis* populations and as *C. tarsalis* rarely oviposits in small container habitats, metabolite concentration would not likely occur in the field.

2.1.1.2.2 Development Time

Larval stage specific development times vary as a function of density (Hagstrum and Workman 1971, Barbosa *et al.* 1972, Reisen 1975, Madder *et al.* 1983) and are also influenced by temperature and nutrition (Hagstrum and Workman 1971, Wijegaratne *et al.* 1974, Madder *et al.* 1983). The effects of temperature, nutrition or density on development rates have not been investigated on an instar specific basis and will therefore be addressed in this study.

2.1.1.3 Pupal Stage

Pupal mortality in the field is very low (Reisen *et al.* 1989) with survivorship exceeding 90% (Rae 1990). In the laboratory Gilpin and McClelland (1979) found pupal mortality to be independent of larval nutritional history, with individuals which successfully pupate emerging as adults. They suggested mortality in the pupal stage was due to developmental errors (genetic mortality) and suggested the pupal stage could be treated in models as a temperature regulated transfer stage between the larval and adult stages.

2.1.1.4 Emerging Adults

Studies on the sex ratio of emerging adults have produced conflicting results. Barbosa *et al.* (1972) and Reisen (1975) found a trend towards a higher proportion of females as larval densities increased. In contrast, Frank *et al.* (1985) found the sex ratio skewed towards males in high density multiple cohort studies. White (1980) found that in *C. tarsalis* the sex ratio showed no correlation with density and did not differ significantly from a 1:1 ratio.

A strong positive correlation has been found between adult female body weight and fecundity (Clements 1992). Adult female body size has been positively correlated with enhanced ovarian development (Hosoi 1954a, b, Van den Heuvel 1963, Steinwascher 1984, Packer and Corbet 1989a), blood meal volume (Briegel 1990a), egg batch size (Briegel 1985a, 1990a), adult survivorship and number of gonotrophic cycles (Packer and Corbet 1989a, 1998b), and the ability to contribute lipids to egg contents from reserves (Volozina 1967, Briegel 1990b). Unlike higher Diptera, adult mosquitoes do not develop fat body cells differing in structure from that of the larvae (Clements 1992). Thus, it is thought fat body cells in adults are those carried over from the pupal stage. Adult feeding serves to meet immediate metabolic demands (nectar-for both sexes) or to provide protein for egg production (blood - females) with very little energy utilized to create storage reserves (Clements 1992). Thus, adult body weight and hence fecundity is primarily determined by conditions experienced by the larvae.

2.2 A GENERAL STAGE STRUCTURED MOSQUITO MODEL

The proposed model follows the structure shown in Figure 2.0. The seven compartments each represent a well defined physiological stage. Density-dependent feedback where present operates on survivorship and stage duration. The progress of an individual through a stage is followed using a development index (DI) (Gurney and Nisbet 1985), that represents some state of development (eg., weight or age) of the individuals in a stage at a given time that can be used as a modelling trigger for transition to the next stage. Development within a stage can be thought of as travelling along a conveyor belt moving at a constant or a variable speed (Crowley *et al.* 1987). Maturation from a stage occurs when some fixed point on the DI is attained, and the individual drops from one conveyor belt to another.

For the purpose of explaining the model a DI based on age (time) will be used. If the age upon entry into stage i is a_i and the age upon maturation into stage $i + 1$ is a_{i+1}, then the sub-population of stage i at time t is the number of individuals with weights ranging from $a_i \leq a < a_{i+1}$. Thus, each sub-population has the following balance equation

$$\frac{dN_i}{dt} = R_i - M_i - D_i \qquad (2.0)$$

where i is the stage being described (E = egg, I - IV = respective instars, P = pupal,

A = adult); $R_i(t)$ is the recruitment rate into stage class i from stage i-1 at time t; $M_i(t)$ is the rate of maturation out of stage i into stage i+1 at the same time; and D_i is the background density-independent mortality rate occurring for stage i at time t.

The balance equation for the emergent adult stage is similar but has no maturation component nor within-stage mortality as the model is only concerned with the number entering the stage:

$$\frac{dN_A}{dt}=R_A \qquad (2.1)$$

The stage specific density-independent mortality (D_i) incorporates what Gilpin and McClelland (1979) refer to as density-independent genetic load. This includes moulting failures between instars and pupation that are unrelated to larval density or food level and therefore are treated as stage specific background mortality. Consequently, D_i takes the simple form:

$$D_i=\delta_i(t)N_i(t) \qquad (2.2)$$

where δ_i is the instar specific background mortality; and N_i is the density of individuals in stage class i at time t.

Two other variables are required in order to define the recruitment and maturation

rates. These are τ_i, defined as:

$$\tau = a_{i+1} - a_i \tag{2.3}$$

representing the duration of the stage (age) class *i;* and $P_i(t)$, described as:

$$P_i(t) = \frac{S_{(t-a_{i+1}, a_{i+1})}}{S_{(t-a_{i+1}, a_i)}} \tag{2.4}$$

which represents the proportion of individuals recruited into age class i at time t-τ (denominator) which survive (S) to be recruited into stage class $i+1$ at time t (numerator). From this, the rate of maturation out of stage class i at time t is the rate of maturation into that class at time t-τ_i multiplied by the through stage class survival $P_i(t)$.

$$M_i(t) = R_i(t-\tau)P_i(t) \tag{2.5}$$

It follows that the rate of recruitment into stage i at time t is exactly equal to the rate of maturation out of stage i-1 at the same time.

$$R_i(t) = M_{(i+1)}(t) \tag{2.6}$$

From these equations, and the assumption that all members of a given stage class have the same per capita vital rates, the rates of change of the sub-populations of the various

groups of functionally identical individuals can be described as:

$$\frac{dN_i(t)}{dt} = R_i(t) - R_i(t-\tau)P_i(t) - \delta_i(t)N_i(t) \qquad (2.7)$$

To this point the procedures have followed Gurney *et al.* (1983) and do not incorporate density-dependent feedbacks. Density-dependence or *per capita* food availability can be incorporated into the model in two ways. Exploitative food competition can be modelled as operating on both the proportion of individuals entering a stage that survive to exit the stage (P_i) and on the duration of the larval stages (τ of I, II, III, IV).

Further complexity may be added to the model with the incorporation of a variable DI which is density or *per capita* food availability in the larval stages. This operates by varying τ_i as a function of the growth rate and density interaction. Utilizing weight as the DI as suggested by Gilpin and McClelland (1979), the larvae can be visualized as moving along the conveyor belt at a rate now varying according to their rate of weight gain which is density or food dependent. When the larvae attain a specific weight at they drop to the next conveyor belt. The model representation of this is given by:

$$\tau_i(t) = \int_{t-\tau_i}^{t} g_i(x)dx \qquad (2.8)$$

where g(x) is a growth function that varies with density. The model now incorporates density-dependent or per capita food effects in both survivorship and on the instar duration.

When fully developed the conceptual model outlined above can be manipulated to make predictions on the effects of various extrinsic factors on larval population dynamics and adult emergence rates which may then be tested empirically.

2.3 EXPERIMENTS ARISING FROM THE MODEL

In developing the conceptual model it becomes clear that several important pieces of information on *C. tarsalis* larval development are required. Experiments designed to begin paramaterization of the model are outlined in Chapter 3.

The model as it presently stands fails to incorporate temperature effects on larval development and survivorship. The simplest and most flexible means of including temperature effects is the utilization of degree days in a growth function. For most insects it has been found that time to emergence expressed as a reciprocal value approximates a positively linear relationship at temperatures between the lower developmental threshold and the upper developmental threshold. (Andrewartha and Birch 1954, Rockstein 1974). The appropriateness of the linear temperature model to *C. tarsalis* was investigated to determine whether the addition of a linear degree day

component will adequately address temperature effects. The effect of temperature on survivorship, mass at ecdysis (between stage moults), stage specific durations and final adult mass were also examined. In addition, temperature effects on head capsule width was tested to determine the appropriateness of this measurement as suitable means of identifying larval instars in mixed cohort experiments at various temperature regimes.

To determine stage-specific back-ground mortality (D_i), individuals were reared under non-stressful food conditions. Experiments investigating the most appropriate DI for stage transitions as well as whether stage durations (τ_i) are best represented by a fixed or variable DI were performed. To paramaterize the effects of density on through stage survivorship (P_i) as well as growth (g_i) and development rates (τ_i) requires both individual (food per head effects) and density manipulations for each stage. Experiments on larvae reared as individuals at various food concentrations were performed to isolate food per head effects and to establish the groundwork for future density manipulation experiments. For this purpose experiments were performed on larvae reared as individuals so stage specific rates could be identified without error, utilizing the presence of exuviae to distinguish the transition to the next stage. The hypothesis that food concentration has no effect on stage-specific duration, growth rates (mass), and survivorship as well as overall development time and mass of adults is tested. The robustness of larval head capsule width under a range of food concentrations, as a correlate for the identification of specific instars in density experiments was tested.

While density effects acting directly on mortality are more easily determined, they may be of less significance than increased mortality resulting from increased stage duration. Increased exposure time to the very high mortality factors of predation and catastrophic disturbance (negative stochastic environmental disturbance) that larvae are exposed to in the aquatic stages would significantly affect adult densities. Should sensitivity to extrinsic factors prove to be stage-specific, the stage that a potential control organism inflicts larval mortality may determine whether the mosquito population is positively or negatively affected. In Chapter 4 the predation biology of *D. tigrina* is assessed to determine its one aspect of its potential as a component in the control of mosquito populations. Experiments to determine the mortality rate experienced by mosquito larvae and an alternative prey source when exposed to individual *D. tigrina* are detailed. To investigate stage specific predation mortality for the two behaviourally distinct prey species (mosquito larvae and zooplankton) two stages or size classes of each prey type were utilized. The effect of prey density on the prey mortality rate and the mechanisms involved are discussed.

In addition, Chapter 4 identifies the relative importance of consumptive and non-consumptive prey mortality on total predator induced size and species specific prey mortality. Prey specific vulnerability to non-consumptive mortality and the functional responses (consumptive mortality) of *D. tigrina* predation are also determined. The significance of non-consumptive mortality to predator-prey models and in non-laboratory conditions is considered.

CHAPTER THREE

3.0 SIGNIFICANCE OF LARVAL ENVIRONMENT

Two of the principal components determining the rate of increase of a population are: time to first reproduction and fecundity of the individuals. For mosquitoes, environmental conditions experienced in the larval stages set larval growth and development rates, which in turn determine the time to first reproduction and adult fecundity. While both larval and adult nutrition affect the reproductive capacity of female mosquitoes, their reproductive potential is set by the end of the larval stage (Chapter 2). Hence, larval nutrition determines the potential fecundity of females but exploitation of this potential as realized fecundity is determined by both larval and adult nutrition. Potential fecundity is the potential reproductive capacity of an organism under optimal conditions, while realized fecundity is the actual reproductive performance of an organism measured as the number of viable offspring produced (Lincoln *et al.* 1982). Characterization of larval growth and the extrinsic factors affecting it are therefore, important in understanding population dynamics of mosquitoes.

In mosquitoes there is a strong positive correlation between female size and ovarial production (Steinwascher 1984, Packer and Corbet 1989a,b, Clements 1992). The number of eggs in an egg batch is often used as a correlate for fecundity as it is simpler to determine than ovarial production, although it does underestimate potential

fecundity. Several factors shown to affect egg batch size are: maternal body size (Steinwascher 1984, Haramis 1985, Briegel 1990a,b), maternal nutritional state (Macdonald 1956, Reisen 1975, Briegel 1990a,b), egg size (Steinwascher 1984, Hawley 1985), physiological age of the female (Gubler and Bhattacharya 1971, Hien 1976, Hawley 1985, Suleman 1990), and volume and source of the blood meal (Woke *et al.* 1956, Volozina 1967, Hien 1976, Briegel 1985, Briegel 1990a,b). Maternal body size positively influences egg batch size as larger females ingest larger blood meals (Briegel 1990a,b).

The total reproductive output of a female mosquito is a function of the number of eggs per batch and the number of gonotrophic cycles completed. Hence, body size further influences lifetime reproductive output as survivorship is positively related to body size (Haramis 1985). Consequently, larval history sets potential fecundity by determining ovarial production and influences realized fecundity by its feedback on adult characteristics of blood meal size, protein supplementation, time to first reproduction and longevity.

The principle extrinsic factors affecting growth and development rates and therefore the potential reproductive contribution to the next generation are temperature, food availability and larval density. The effects of temperature and food availability on *C. tarsaslis* larval growth and development are investigated in this chapter. Weight and development time to the reproductive stage are used as correlates for potential reproductive output.

3.0.1 TEMPERATURE

The effect of temperature on development rate, growth and reproductive potential has received a great deal of attention (Haufe and Burgess 1956, Stewart 1974, Slater and Pritchard 1979, Trpis 1972, Gilpin and McClelland 1979, Milby and Meyer 1986). The temperature experienced by mosquito larvae inversely affects adult characteristics such as size, dry weight and ovarial number (van den Heuvel 1963, Gilpin and McClelland 1979, Bock and Milby 1981). The general conclusion has been that larger females developing at slower rates, result when larvae are reared at lower temperatures (Clements 1992). Temperature effects on mosquito larval characteristics can be summarized into three main points: (1) growth and development occurs only within a temperature range defined by a lower developmental threshold and an upper lethal threshold, (2) within most of this temperature range the rate of development is positively correlated with temperature and (3) the temperature range varies among species.

The experimental procedures utilized in previous work investigating temperature effects have failed to isolate food availability as a potentially significant cofactor. Recent work identifying mosquito larval food in the field, indicate that bacteria, protista as well as fungal and some algal material are the major food sources (Walker and Merritt 1988, Walker *et al.* 1988, Merritt *et al.* 1990, Merritt *et al.* 1992a). In laboratory experiments, an artificial food medium (eg. tetramin, liver powder, dry dog food, etc.) is frequently utilized (Gerberg 1970). The medium is not the principal nutritional source

but acts as the substrate and/or nutrient source for the bacterial and fungal populations that colonize the medium and are the primary food resource. Hence, traditional approaches of investigating temperature effects by culturing larvae in trays held at specific temperatures and provided with equal amounts of artificial food (Brust 1967, Brust and Kalpage 1967, Gilpin and McClelland 1979, Chambers and Klowden 1990), fail to isolate potential differences in food availability and quality from the experiments. Since the growth of bacterial and fungal cultures are strongly influenced by temperature, cool temperature treatments will also be low food treatments while high temperature treatments will be high food treatments. In this study, temperature effects on growth, development characteristics and survivorship of *C. tarsalis* are investigated while minimizing possible food-temperature interactions.

3.0.2 FOOD AVAILABILITY

Food availability significantly affects larval characteristics such as time to emergence (Marcovich 1960, Hagstrum and Workman 1971, Moore and Whitacre 1972) and adult characteristics of survivorship (Nayar 1968), mass at emergence (Nayar 1969, Reisen 1975), average size of blood meal (Reisen 1975), and realized fecundity (Macdonald 1956, Reisen 1975), all of which are positively correlated with food availability. Starvation prevents growth but does not necessarily prevent larval development as ecdysis to the next instar has been shown to occur even when no food was provided during the previous instar (Brust 1968a). While adult and pupal mass are

positively correlated with food availability, food effects on post ecdysal mass of the previous developmental stages are unknown. To utilize a stage-structured approach for modelling *C. tarsalis* population dynamics, stage-specific effects of food on survivorship, mass and development times must be determined.

The amount of food available to an individual larva is dependent on both the concentration of food in the environment and the number of larvae exploiting the food. Thus, food availability may be manipulated in two ways: (1) varying the amount of food provided per volume of water, and (2) holding food constant and varying larval density. In experiments with more than one larvae per microcosm it is difficult to isolate food effects(available food per individual), from density effects. Larval density can also exert additional negative interactions by the release and accumulation of growth retardants (Ikeshogi 1977) or behavioural disturbance due to physical interactions (Dye 1984) and positive interactions such as enrichment of the food environment by larval cadavers and bacteria exploiting these cadavers (Gilpin and McClelland 1979). Thus, to isolate food effects from density effects, larvae were reared individually and exposed to a wide range of food concentrations.

3.1 METHODS and MATERIALS

3.1.1 COMMON PROTOCOLS

All experiments with larvae were conducted in 50 ml culture tubes containing 25 ml food medium. Buth *et al.* (1990) found photo-period had no effect on larval developmental characteristics but a short day-length resulted in adults that entered reproductive diapause. Thus, since this investigation involves reproductive potential, a light-dark regime of 16 h:8 h was used. Eggs were obtained from laboratory cultures maintained using the procedures outlined by (Gerberg 1970) with blood meals provided by arm feeding. Experiments were initiated with I instar larvae hatched within the last 4 h.

A standardized food medium in which the larvae were reared was produced from a specified amount of dry food mixture (see appendix A) added per litre of artificial pond water (see appendix B) cultured at 25°C for 24 h to establish bacteria. The mixture was gravity sieved through a 75 micrometer sieve to remove large or clumped particles above the upper limit of particle size ingested by IV instar larvae (Dadd 1971, Merritt *et al.* 1978, Merritt 1987). Since *C. tarsalis* is rarely found in treeholes, small containers or phytotelmata, stressful concentrations of metabolic waste products often experienced in laboratory cultures rarely occur in the field. The food medium in larval experiments was replaced every 24 h by transferring the larvae to a new tubes with fresh medium

minimizing the build-up of metabolic waste products and the influence of possible periods of short-term starvation due to food depletion. Three experimental food concentrations (0.05, 0.1, 0.5 g/L) were identified by preliminary experiments as representing a range between the upper and lower food thresholds capable of supporting complete development (to emergence) of an individual larva in 25 ml of media at 25° C. An additional food concentration of 0.01 g/L was used to investigate a treatment marginally incapable of supporting full development.

3.1.2. TEMPERATURE

Six experimental temperature treatments (10°, 15°, 20°, 25°, 30°, and 35°C) were established at a constant food concentration of 0.1 g/L. Each treatment had 25 replicates, with each replicate consisting of a single larva reared individually. Observations were made every 12 h, when larval mortality and time to ecdysis, identified by the presence of exuviae, were recorded. Dead individuals found to have ecdysed from stage i to stage $i+1$ since the last observation interval were recorded as unsuccessful moults and treated as stage i individuals. Feeding medium was replaced every 24 h to minimize the influence of temperature on microbial growth.

To investigate temperature effects on adult emergence characteristics, an experimental series (25 individuals) was run at each temperature and terminated when the individuals had either emerged as adults or died. Stage-specific development times

were recorded on an individual basis. Newly emerged adults were sexed, frozen at 30°C and air dried 5 days at 40°C prior to weighing using a Mettler MT5 microbalance. All experimental dry weights were determined in this manner.

Stage-specific post-ecdysal masses were determined for each developmental stage (II, III, IV, pupal) at 15°, 25° and 30°C. A series (25 replicates) was run at each of the three temperatures for each developmental stage, with individuals sacrificed upon ecdysis into the specific final stage. These larvae or pupae were plunged into water at 80°C, to produce an instant kill, and quickly removed. Head capsule width was recorded with sex determination of the pupae. Individuals were then dried and weighed as outlined above.

3.1.2.1 Statistics

Through-stage survivorship was determined by calculating the percentage of those individuals that entered stage i successfully ecdysing into stage $i+1$. Determination of temperature or developmental effects on through-stage survivorship was carried out utilizing a two-way ANOVA on arcsine square root transformed data (Zar 1984). Only data from 15° 25° and 30°C were analysed as only these treatments provided replicate survivorship values .

Total development rate is the inverse of the time to adult emergence. Hence,

temperature treatments of 10° and 35° C where no individuals survived to adult, were excluded from the analysis. Linear regression analysis was used to determine the effect of temperature and sex on total development rate. Sex was treated as a dummy variable (Zar 1984) with males designated as zero and females as one. The original regression equation:

$$rate = constant + B_1 sex + B_2 temp. + B_3 sex * temp. \qquad (3.0)$$

was used (temp. = temperature) with coefficients with non-significant F vales removed prior to repeating the regression analysis. Coefficients that were not significant but were present in a significant interaction coefficient remained in the model.

Since the goal was to investigate stage-specific development characteristics to aid construction of a stage-structured model, development times and stage durations were analyzed on a stage-specific basis using the temperature treatments in which larvae can complete development. The effect of temperature on stage-specific development rates was assessed as described for emergence rates. Identification of the stage or stages responsible for any sexual dimorphism in emergence rates was made by determining the earliest (youngest) stage at which males and females developed at the same rate. Analysis was then performed on the rate of development within each of the later developmental stages for temperature and sex effects.

To investigate which stages comprise the greatest proportion of time to

emergence, stage specific proportions were calculated. A three way ANOVA with treatment factors of temperature, stage and sex was used with an arcsine square root transformation.

Two analytical approaches were used to determine the factors affecting stage-specific post ecdysal weights. A two-way ANOVA with main effects of temperature and stage was performed on the complete data set (stages II - adult at three temperatures) followed by a three-way ANOVA with the additional main affect of sex and the interaction term applied to the pupal and adult weights.

To investigate the rate of weight gain per day on a stage-specific basis, the average mass obtained at stage i -1 was subtracted from the weight of each individual at stage i giving an estimate of the individual weight gain in the interval. Each individuals weight gain during this period was divided by the duration of this stage interval to obtain an estimate of weight gain per day. This method is the most conservative approach for the analysis since it has the effect of increasing the error estimates, minimizing the chances of producing a Type I error. Statistical procedures were as outlined for the ecdysal weight analysis. In addition, to provide a descriptive model of weight gain per day, regression models were fit to the data. The effect of sex and temperature on weight gain per day in the IV and pupal stages were analyzed using equation 3.0 with the coefficient sex omitted for stages which could not be sexed.

A two-way ANOVA with main effects of temperature and stage was used to determine whether head capsule widths are robust indicators of larval instar when exposed to a range of temperatures.

3.1.3 FOOD AVAILABILITY

Assuming no behavioural interference, food availability to mosquito larvae is dependent on the amount of food in the system, the number of individuals exploiting the food and their feeding rates. To assess the effects of food availability, independent of potential behavioural interference, experiments were performed on larvae reared individually. Four food treatments were tested (0.01, 0.05, 0.1, 0.5 g/L). For each developmental stage 25 individuals were reared at every food concentrations. Mortality and ecdysal times up to the stage of sacrifice were recorded with dry weights determined. Individuals reared to adult and pupal stages were sexed.

3.1.3.1. Statistics

Analyses were similar to those described for the temperature data. The effect of food concentration (0.01 - 0.5 g/L) on through-stage survivorship up to the pupal stage was analysed with a two-way ANOVA. Analysis was not performed on the pupal stage as there were no replicate values. To provide a descriptive model of development rates as a function of food concentration and sex, regression analyses were done using a linear

model:

$$Rate = constant + B_1 food + B_2 sex + B_3 food*sex \qquad (3.1)$$

or the simple non-linear regression model:

$$Rate = \frac{a*food\ concentration}{(b+food\ concentration)} \qquad (3.2)$$

Residuals were analyzed to determine the suitability of the linear or curvi-linear models. Development rates were modelled incorporating the 0.01 g/L food concentration on the data set with both sexes combined. The data were then re-analysed on a sex specific basis (hence data from the 0.01 g/L food concentration were not included) to determine whether there was a difference between sexes. The parameter estimates of *a* and *b* obtained for each of the sexes were compared using the *t* statistic.

The effect of food concentration on ecdysal weights, weight gain per day and head capsule width were analysed as outlined for the temperature experiments.

3.2 RESULTS

3.2.1 TEMPERATURE EXPERIMENTS

3.2.1.1 Experimental Population Stage Composition and Stage Survivorship

At the extremes of the temperature range tested (10° and 35°C) complete development from egg to adult did not occur (Table 3.0, Figs. 3.0 and 3.1). At 10°C larval lifespan ranged from 3 to 37 days with the lone individual which successfully ecdysed into II instar dying within 1 day of ecdysis at an age of 6.5 days. Through-stage survivorship, or the probability upon entering stage i of successfully ecdysing into stage i+1, was zero for all stages other than the I instar where the probability was 8% (Table 3.0).

At 35 °C larval lifespan ranged from 0.5 - 7.5 days with four individuals reaching IV instar. Mortality was more evenly distributed with a through-stage survivorship for I instar of 52% and 33% and 25% survivorship for instars II and III, respectively. Thus, a high fatal temperature increased development rates but resulted in mortality after a relatively short exposure period. In contrast, larvae exposed to a low fatal temperature can persist under such conditions for an extended period of time but experience little development.

Table 3.0. Stage specific through-stage survivorship of *C. tarsalis* for each experimental temperature. Values in brackets are the number of replicate series of 25 larvae and the standard error of the estimate respectively.

	TEMPERATURE					
STAGE	10°C (%)	15°C (%)	20°C (%)	25°C (%)	30°C (%)	35°C (%)
I	8 (1,-)	91.8 (5,4.2)	88.0 (1,-)	94.5 (8,1.1)	83.2 (5,3.9)	52.0 (1,-)
II	0 (1,-)	100 (4,0)	100 (1,-)	95.8 (7,2.3)	83.25 (4,6.9)	33.0 (1,-)
III	--	95.3 (3,2.6)	95.0 (1,-)	95.2 (5,2.6)	100 (3,0)	25.0 (1,-)
IV	--	100 (2,0)	100 (1,-)	92.0 (3,4.2)	93.5 (2,6.5)	0
Pupal	--	89.0 (1,-)	100 (1,-)	89.5 (2,0.5)	100 (1,-)	--
I-ADULT	0	68.0	84.0	84.0	92.0	0

Figure 3.0. Stage structure of *C. tarsalis* experimental populations at 10°, 15°and 20°C as a function of time. Stage composition was calculated as the percentage of the surviving population in each developmental stage at that time interval (I instar = ●, II instar = ▼, III instar ■, IV instar = ▲, Pupal stage = ○, Adult stage = △).

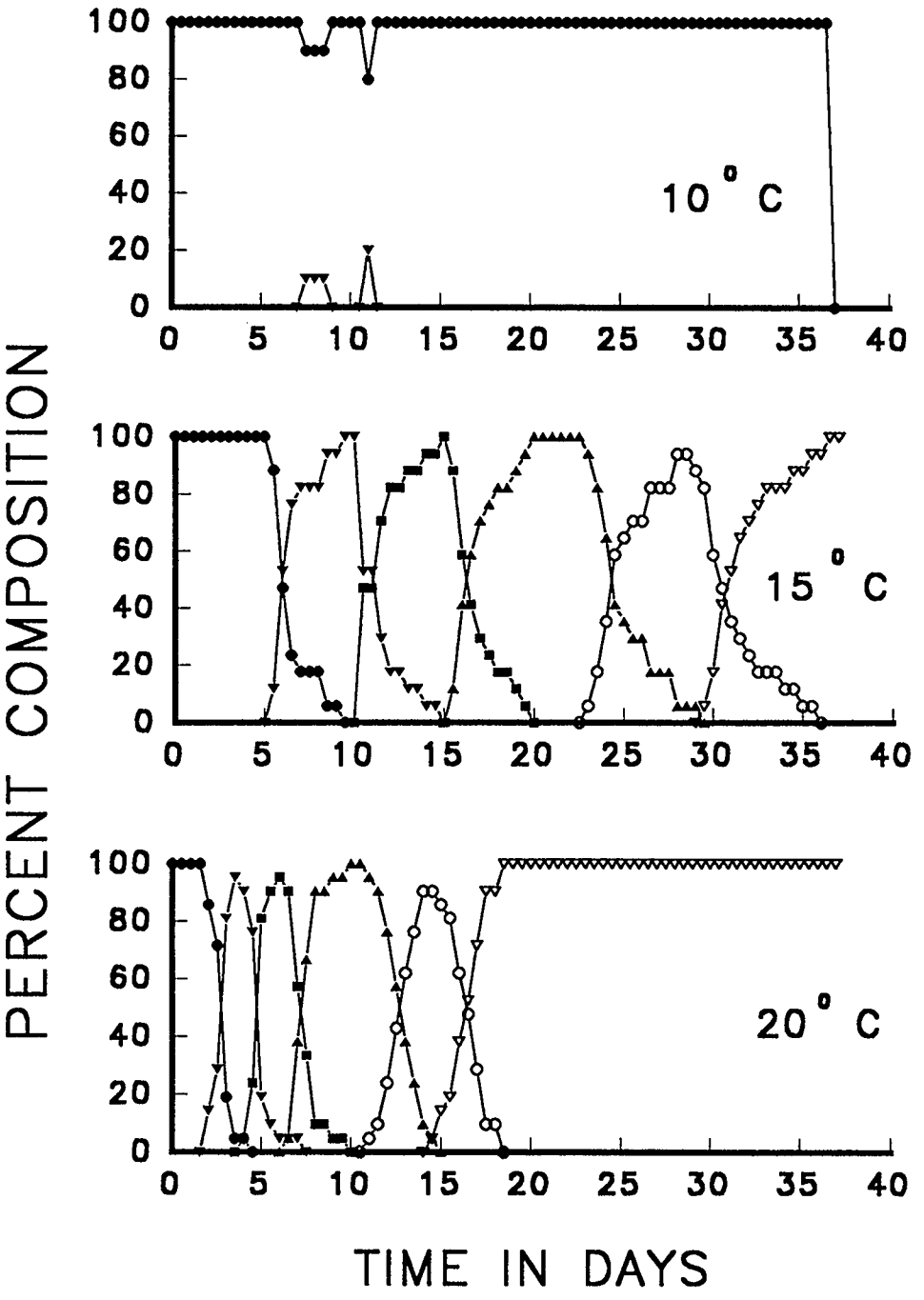
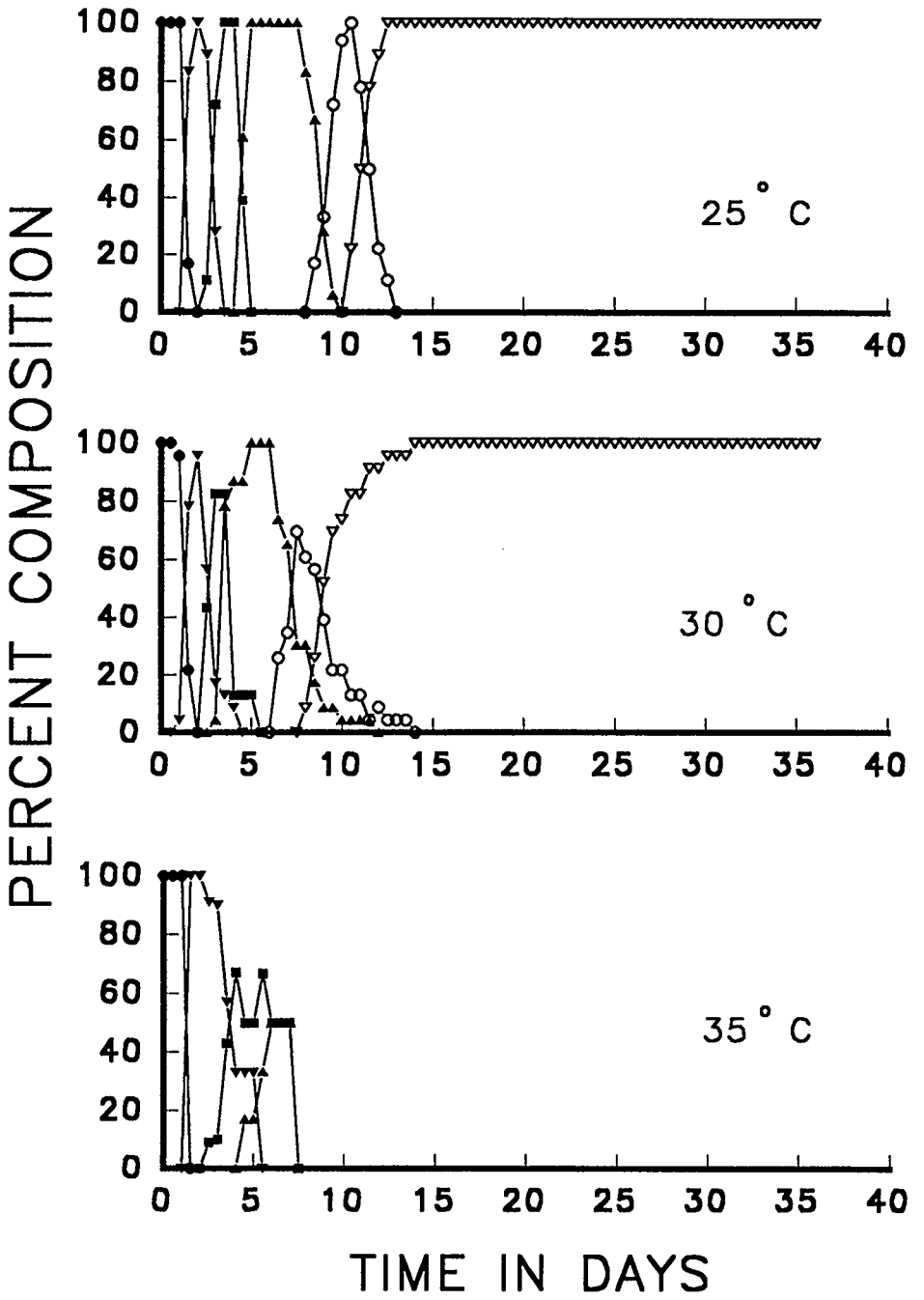

PERCENT COMPOSITION
100
80
60
40
20
0
10° C
15° C
20° C
0 5 10 15 20 25 30 35 40
TIME IN DAYS

Figure 3.1. Stage structure of *C. tarsalis* experimental populations at 25°, 30°and 35°C as a function of time. Stage composition was calculated as the percentage of the surviving population in each developmental stage at that time interval (I instar = ●, II instar = ▼, III instar ■, IV instar = ▲, Pupal stage = ○, Adult stage = △).


PERCENT COMPOSITION
100
80
60
40
20
0
25 ° C
30 ° C
35 ° C
0 5 10 15 20 25 30 35 40
TIME IN DAYS

For temperatures at which complete development from egg to adult occurred, through-stage survivorship was not significantly affected by temperature ($F_{2,40}=0.62$, $P>0.5$), but was significantly by stage ($F_{8,40}=2.75$, $P<0.05$). A significant temperature*stage interaction ($F_{8,40}=2.61$, $P<0.05$) occurs, primarily due to significantly lower stage-survivorship at 30°C for stage I (contrast, $F_2=4.79$, $P<0.05$) and stage II (contrast, $F_2=10.51$, $P<0.01$) compared to the other temperatures (Table 3.0). The lowest stage-specific survival probability occurred in the transition from I to II instar with mortality occurring either at I instar or immediately after ecdysis into instar II. Through-stage survivorship for other instars exceeded 89%.

3.2.1.2 Development Times and Stage Durations

Over a wide and biologically significant range of temperatures development rates of most insects increase linearly with temperature to an optimal temperature. Above this optimal temperature the development rate declines until the high fatal temperature is reached (Taylor 1981). Extrapolation from the linear portion of the development curve to the temperature axis indicates the theoretical developmental zero (DZ) or the threshold temperature at which no development can occur. For *C. tarsalis* the development rate increases with temperature (Fig. 3.2). When a linear regression equation is fitted to the temperatures at which complete development is possible (Table 3.1) a DZ of 7.4°C for males and 7.6°C for females is obtained. However, the experiments showed that temperatures in excess of 10° C are required for full development. When

Figure 3.2. Mean *C. tarsalis* development rates (1/days, ±SE) to emergence as a function of temperature for both sexes (male-○, female-●). Lines generated from regression model in Table 3.1B fit to raw data. Standard errors not shown if incorporated within the symbol.

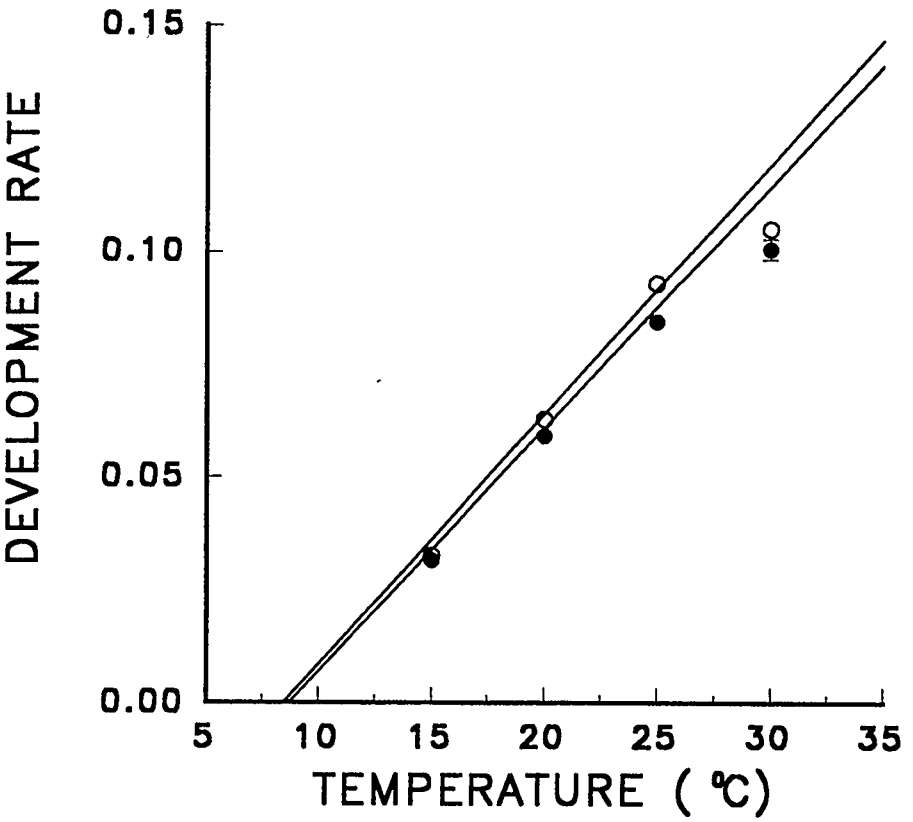

DEVELOPMENT RATE
0.15
0.10
0.05
0.00
5
10
15
20
25
30
35
TEMPERATURE (℃)

Table 3.1. Regression analysis of *C. tarsalis* development rate (1/days) to adult for both males and females as a function of temperature (rate = constant + B_1sex + B_2temperature + B_3sex*temperature). Sex was analyzed using dummy variables; male = 0, and female = 1. Two regressions were performed, (A) the temperature range from 15°-30°C and the temperature range from 15° - 25°C.

A) TEMPERATURES (15 - 30°C) MODEL r^2 = 0.925

Source	Sum-of-squares	df	MS	F	P
Regression	0.0578	3	0.0193	377.817	<0.001
Residual	0.00470	92	0.000051		

Variables	Coefficient	SE	t	P
Constant	-0.0468	0.00605	-7.721	<0.001
Sex	0.0137	0.00736	1.865	>0.05
Temperature	0.00547	0.000265	21.0	<0.001
Sex*temperature	-0.000930	0.000316	-2.948	<0.0.01

B) TEMPERATURES (15° - 25°C) MODEL r^2 = 0.976

Source	Sum-of-squares	df	MS	F	P
Regression	0.0391	3	0.013	944.319	<0.001
Residual	0.000952	69	0.000014		

Variables	Coefficient	SE	t	P
Constant	-0.0580	0.00354	-16.0	<0.001
Sex	0.0110	0.00472	2.345	<0.05
Temperature	0.00604	0.000161	38.0	<0.001
Sex*temperature	-0.000772	0.000217	-3.551	<0.0.01

the linear regression model is applied without data from 30°C, DZ temperatures of 9.6°C for males and 8.9°C for females are obtained (Fig. 3.2, Table 3.1), which are closer to the empirical results. At temperatures in excess of 15°C males have a higher development rate than females and these sexually dimorphic development rates become more pronounced as temperature increases (sex*temperature interaction, $t=-2.948$, $P<0.001$, or $t=-3.551$, $P<0.001$) (Table 3.1, Fig. 3.2).

Stage-specific development times and durations decrease as temperature increases (Fig. 3.3). The ability to track individual development makes it possible to identify the stage or stages responsible for the sexual dimorphism in emergence times. The latest development stage with no significant difference in sex specific development times is III instar (Table 3.2). Upon ecdysis into IV instar males begin to develop faster than females (Table 3.2, Fig. 3.4). However, the significantly faster rate of development of males in IV instar (sex*temperature $t=-3.159$, $P<0.01$) shows the majority of the difference in final emergence times occur in the IV stage (Table 3.3, Fig. 3.4). Pupal development plays no role in determining the sexually dimorphic emergence rates (Table 3.4, Fig. 3.4).

When the proportion of total development time to emergence for each stage is plotted against temperature, it can be seen that the proportion of total time spent in stages I, II and III declines as temperature increases. This corresponds with an increase in the proportion of time spent in IV instar (temperature*stage $F_{12,355}=14.15$, $P<0.0001$) (Fig.

Figure 3.3. Mean (±SE) stage-specific development times for 10°C-△, 15°C-□, 20°C-▲, 25°C-■, 30°C-▼, and 35°C-◇. Solid lines represent temperatures at which complete development from egg to emergence does not occur. Standard errors not shown if incorporated within the symbol.

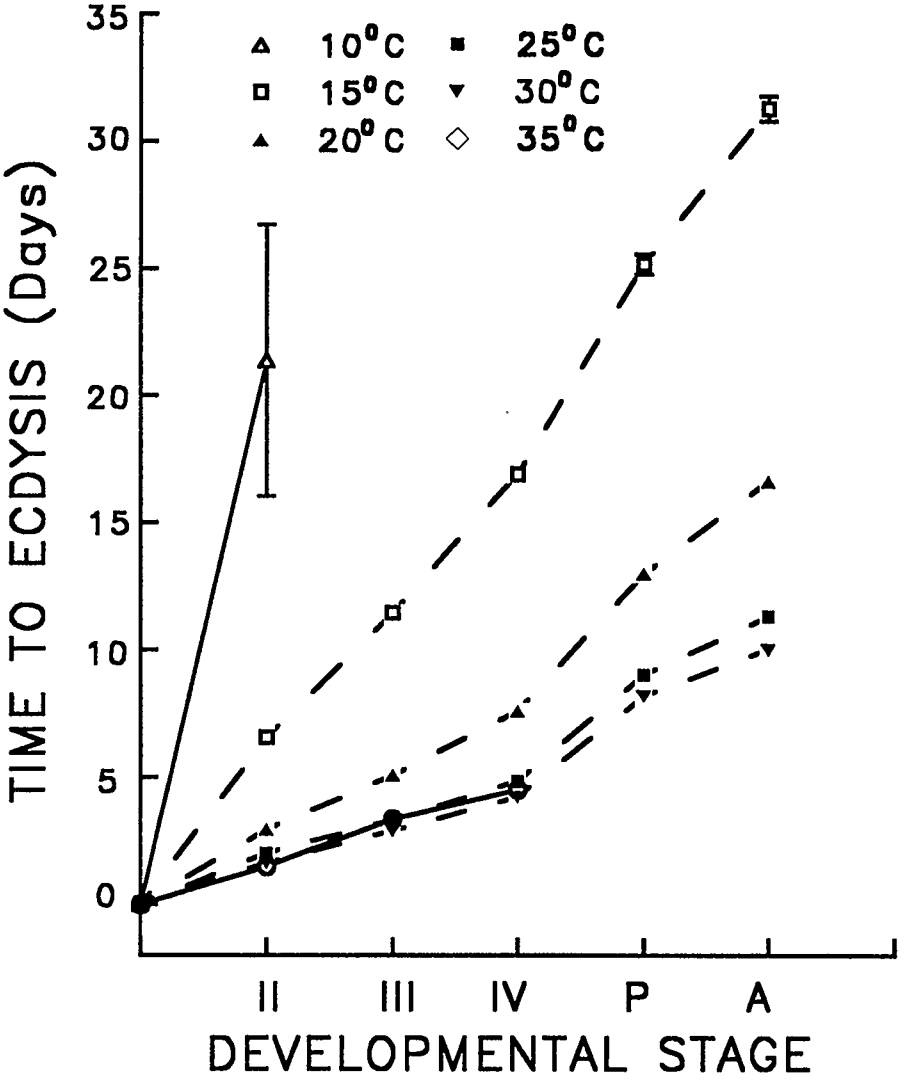

35
30
25
20
15
10
5
0
TIME TO ECDYSIS (Days)
10°C
15°C
20°C
25°C
30°C
35°C
II
III
IV
P
A
DEVELOPMENTAL STAGE

Figure 3.4. Stage specific mean (±SE) development rates for pupal duration (◆), IV instar duration (■), and time to IV instar (early larval stage-●) of *C. tarsalis*. Open symbols represent males, closed symbols females. Standard errors not shown if incorporated within the symbol. Lines generated from regression models in Tables 3.2, 3.3 and 3.4 fit to raw data.

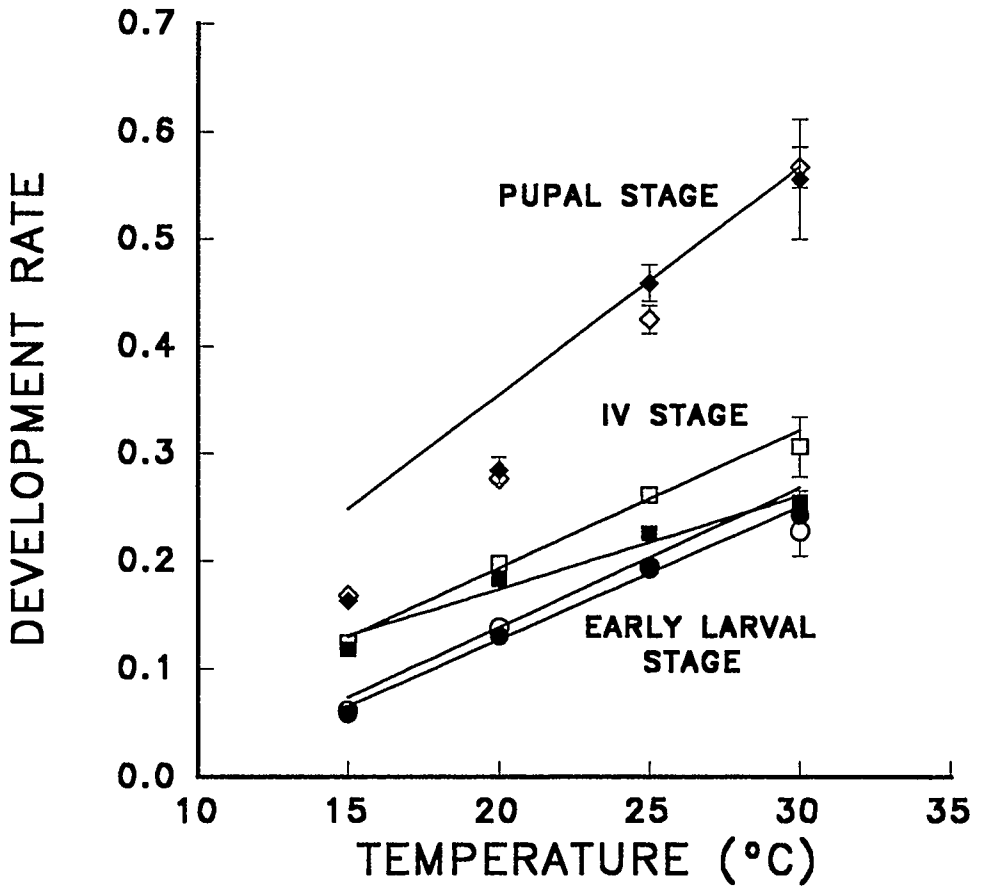

DEVELOPMENT RATE
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
PUPAL STAGE
IV STAGE
EARLY LARVAL STAGE
10
15
20
25
30
35
TEMPERATURE (°C)

Table 3.2. Linear regression analysis for the development rate to instars III and IV for both sexes of *C. tarsalis*. The original regression model was rate = constant + B_1sex + B_2temperature + B_3sex*temperature and was fit to the raw data. Sex was analyzed using dummy variables; male = 0, and female = 1.

TO III INSTAR: MODEL $r^2 = 0.858$

Source	Sum-of squares	df	MS	F	P
Regression	0.830	1	0.830	566.74	<0.001
Residual	0.1376	94	0.00146		

Variable	Coefficient	SE	t	P
Constant	-0.166	0.0182	-9.156	<0.001
Temperature	0.018	0.00076	24.0	<0.001

TO IV INSTAR (Early Larval Stage): MODEL $r^2 = 0.908$

Source	Sum-of squares	df	MS	F	P
Regression	0.389	2	0.1947	458.797	<0.0001
Residual	0.0395	93	0.000424		

Variable	Coefficient	SE	t	P
Constant	-0.113	0.00987	-11.0	<0.0001
Sex	-0.0124	0.00439	2.834	<0.005
Temperature	0.0125	0.000414	30.0	<0.0001

Table 3.3. Linear regression analysis for rate of development during IV instar for both sexes of *C. tarsalis*. The origonal regression model was; rate = constant + B_1sex + B_2temperature + B_3sex*temperature with sexes analyzed using dummy variables; male = 0, and female = 1. The regression was applied to the raw data.

MODEL $r^2 = 0.7575$

Source	Sum-of squares	df	MS	F	P
Regression	0.255	3	0.0851	95.384	<0.001
Residual	0.0820	92	0.00892		

Variable	Coefficient	SE	t	P
Constant	-0.0624	0.0253	-2.1468	<0.025
Sex	0.0638	0.0308	2.0730	<0.05
Temperature	0.0128	0.00111	12.0	<0.001
Sex*temperature	-0.00417	0.00132	-3.159	<0.01

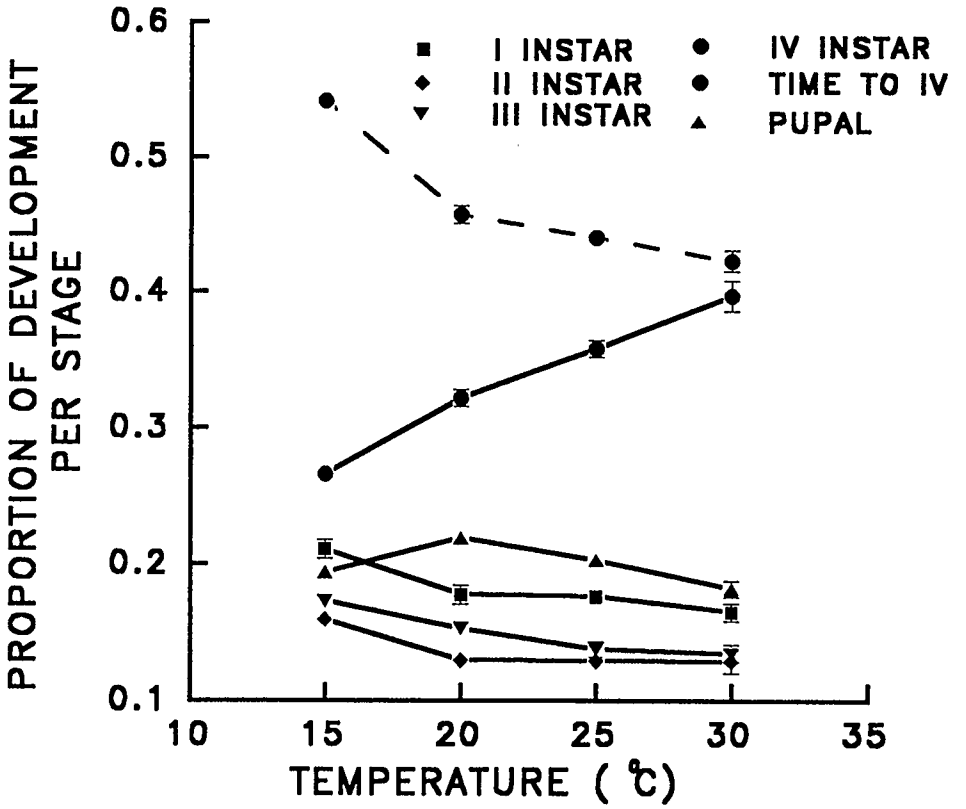
Table 3.4. Linear regression analysis for the rate of development during the pupal stage for both sexes of *C. tarsalis*. The raw data were fit to the original regression model of rate = constant + B_1sex + B_2temperature + B_3sex*temperature with sex analyzed using dummy variables; male = 0, and female = 1.

MODEL $r^2 = 0.855$

Source	Sum-of squares	df	MS	F	P
Regression	1.903	1	1.903	553.954	<0.001
Residual	0.323	94	0.00344		

Variable	Coefficient	SE	t	P
Constant	-0.252	0.0278	-9.066	<0.001
Temperature	0.027	0.00116	24.0	<0.001

Figure 3.5. Total time to emergence for *C. tarsalis* subdivided into stage-specific periods and plotted as a function of temperature. (I instar ■, II instar ◆, III instar ▼, IV instar ●, time to IV instar or early larval stage --●--, pupal stage ▲).


0.6
0.5
0.4
0.3
0.2
0.1
PROPORTION OF DEVELOPMENT PER STAGE
I INSTAR
II INSTAR
III INSTAR
IV INSTAR
TIME TO IV
PUPAL
10
15
20
25
30
35
TEMPERATURE (℃)

3.5). The proportion of total development time to IV instar (early larval stage) decreases with temperature, so that IV stage duration increasingly accounts for a higher proportion of emergence time (Fig 3.5). The II and IV instars display a significant temperature*stage*sex interaction ($F_{12,355}=2.73$, $P>0.001$) with females in IV instar spending proportionately more time (contrast $F_1=7.67$, $P<0.01$) and those in II proportionately less time ($F_1=14.46$, $P<0.001$) than males with these differences increasing with temperature.

3.2.1.3 Post Ecdysal Weight

Stage-specific post ecdysal weights increase with each stage up to and including the pupal stage followed by a decrease in weight upon emergence to the adult stage (Fig. 3.6). A significant temperature*stage interaction occurs, with temperature having a greater effect on weight for the latter three stages (temperature*stage $F_{8,320}=101.64$, $P<0.0001$)(Figs. 3.6, 3.7). Ecdysal weights are independent of temperature until IV instar (contrast $F_4=8.38$, $P<0.01$) when ecdysal weights differ in each temperature treatment. Pupal mass increases nearly five fold from that of IV instar at 15°C but increase only two-fold at 25°C and 30°C (Figs. 3.6, 3.7). A three-way ANOVA shows pupal and adult stages are significantly affected by temperature ($F_{2,95}=385.84$, $P<0.0001$) stage ($F_{1,95}=177.68$ $P<0.0001$) and sex ($F_{1,95}=35.09$, $P<0.001$) with only a very weak stage*sex interaction ($F_{1,95}=4.27$, $P<0.05$). Females have significantly higher emergent and pupal masses than males in all temperature treatments.

Figure 3.6. Mean (±SE) stage-specific post-ecdysal weights at 15°C (●), 25°C (■) and 30° C (◆) for *C. tarsalis*. Open symbols represent males and closed symbols represent females. Standard errors not shown if incorporated within the symbol.

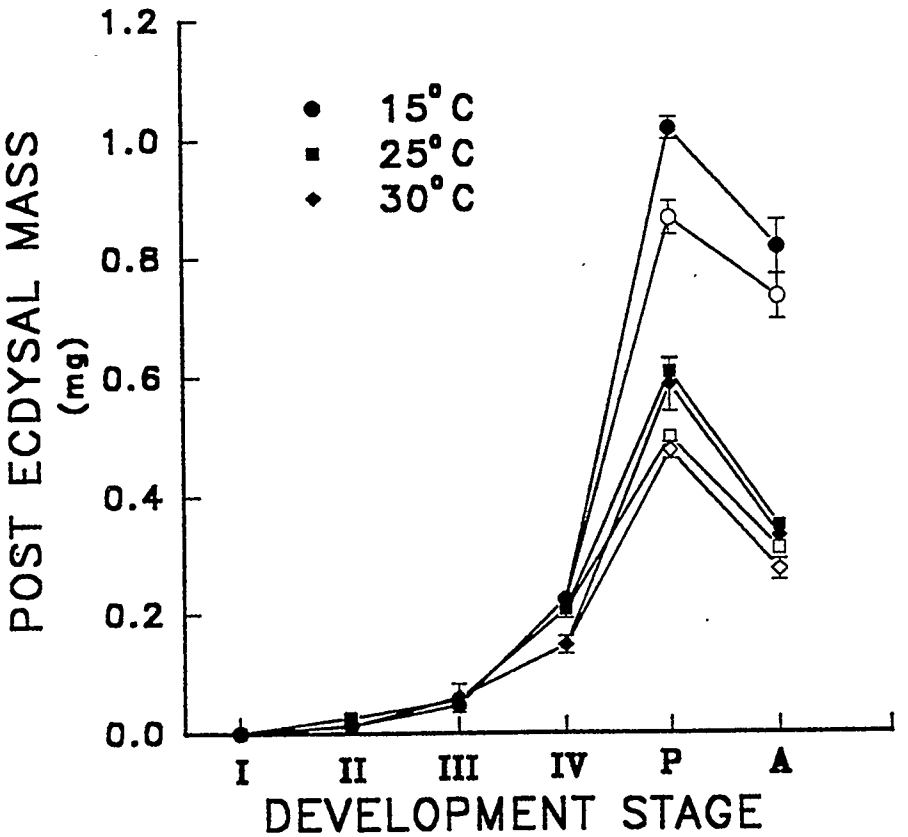
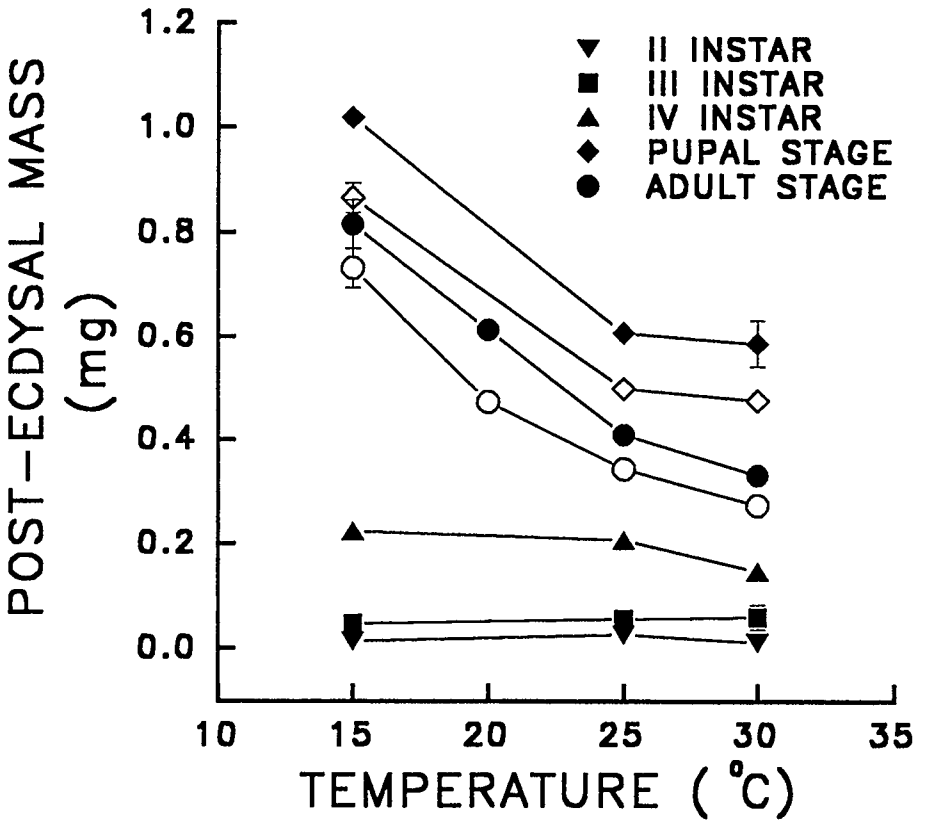

POST ECDYSAL MASS
(mg)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
15°C
25°C
30°C
I
II
III
IV
P
A
DEVELOPMENT STAGE

Figure 3.7. Mean *C. tarsalis* (±SE) stage-specific post-ecdysal weights (II instar ▼, III instar ■, IV instar ▲, pupal stage ◆, and adult ●) as a function of temperature. Open symbols represent males and closed symbols represent females. Standard errors not shown if incorporated within the symbol.


POST-ECDYSAL MASS (mg)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
II INSTAR
III INSTAR
IV INSTAR
PUPAL STAGE
ADULT STAGE
10
15
20
25
30
35
TEMPERATURE (°C)

Weight gain per day increases with temperature and is highest in the IV stage. The pupal stage has a net decrease in weight (Fig. 3.8). The increase in weight gain per day at 25°C for stages III and IV and the marked increase in weight loss per day in the pupal stage at 30°C accounts for the significant temperature*stage interaction ($F_{8,321}=1.50$, $P<0.0001$). In addition to the temperature*stage interaction occurring in the IV and pupal stages ($F_{2,106}=52.38$, $P<0.0001$) a three-way ANOVA shows a stage*sex interaction ($F_{1,106}=12.24$, $P<0.001$) also occurs with females during IV stage gaining mass at a greater rate per day than males. The difference in weight loss per day at 15°C between the sexes in the pupal stage does not produce a significant temperature*stage*sex interaction ($F_{2,95}=0.46$, $P>0.5$). The linear regression model is a significant fit for the early larval stage and the IV instar. However, the model does not account for a great deal of the variance around the means (Table 3.5).

3.2.1.4 Head Capsule Width

Head capsule width responded inversely to temperature with widths at IV stage displaying the most temperature variation (stage*temperature $F_{4,176}=2.78$ $P<0.05$, Table 3.6). Head capsule widths differ greatly between stages ($F_{2,176}=1948.71$, $P<0.0001$) so that identification of development stage was possible using head capsule measurements (Table 3.6).

Figure 3.8. Mean weight gain per day (mg/day) as a function of temperature during the early larval stage (●), IV instar (▲), and the pupal stage (♦) for *C. tarsalis*. Open symbols represent males and closed symbols represent females. Lines generated from regression models in Table 3.5 fit to the raw data. Standard errors not shown if incorporated within the symbol.

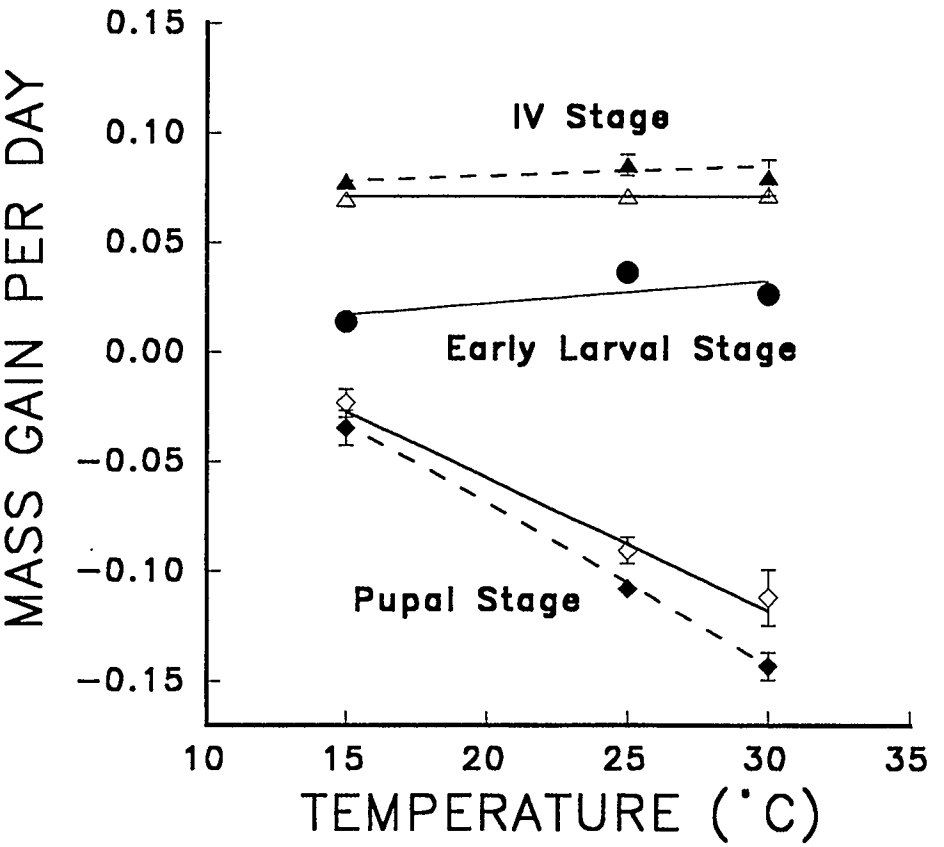

0.15
0.10
0.05
0.00
−0.05
−0.10
−0.15
MASS GAIN PER DAY
IV Stage
Early Larval Stage
Pupal Stage
10
15
20
25
30
35
TEMPERATURE (°C)

Table 3.5. Linear regressions for *C. tarsalis* weight gain per day (mg/day) for the (A) early larval stage, (B) IV instar and (C) Pupal stage. The raw data was fit to the original model; rate = constant + B_1sex + B_2temperature + B_3sex*temperature. Sex was analyzed using dummy variables; male = 0, and female = 1.

A) EARLY LARVAL STAGE: MODEL $r^2 = 0.366$

Source	Sum-of squares	df	MS	F	P
Regression	0.00271	1	0.00271	32.852	<0.001
Residual	0.00470	57	0.0000824		

Variable	Coefficient	SE	t	P
Constant	0.000464	0.00461	0.101	>0.05
Temperature	0.00111	0.000193	5.732	<0.001

B) IV INSTAR: MODEL $r^2 = 0.136$

Source	Sum-of squares	df	MS	F	P
Regression	0.00114	1	0.00114	7.546	<0.01
Residual	0.00728	48	0.000152		

Variable	Coefficient	SE	t	P
Constant	0.0712	0.00237	30.0	<0.001
Sex	0.00960	0.00350	2.747	<0.01

C) PUPAL STAGE: MODEL $r^2 = 0.805$

Source	Sum-of squares	df	MS	F	P
Regression	0.120	2	0.060	111.752	<0.001
Residual	0.029	54	0.000538		

Variable	Coefficient	SE	t	P
Constant	0.085	0.0123	6.866	<0.001
Sex	-0.0177	0.00643	-2.750	<0.01
Temperature	-0.00696	0.000502	-14.0	<0.001

Table 3.6. *C. tarsalis* stage-specific mean head capsule widths for larvae reared at 15°, 25° and 30°C in a constant food medium of 0.1 g/L.

		HEAD CAPSULE WIDTH (mm)		
TEMPERATURE (°C)	STAGE	N	MEAN	SE
15	II	22	0.521	0.003
	III	25	0.837	0.003
	IV	20	1.268	0.006
25	II	24	0.518	0.002
	III	20	0.784	0.009
	IV	22	1.175	0.005
30	II	23	0.447	0.005
	III	12	0.747	0.025
	IV	17	1.155	0.045

3.2.2 FOOD CONCENTRATION EXPERIMENTS

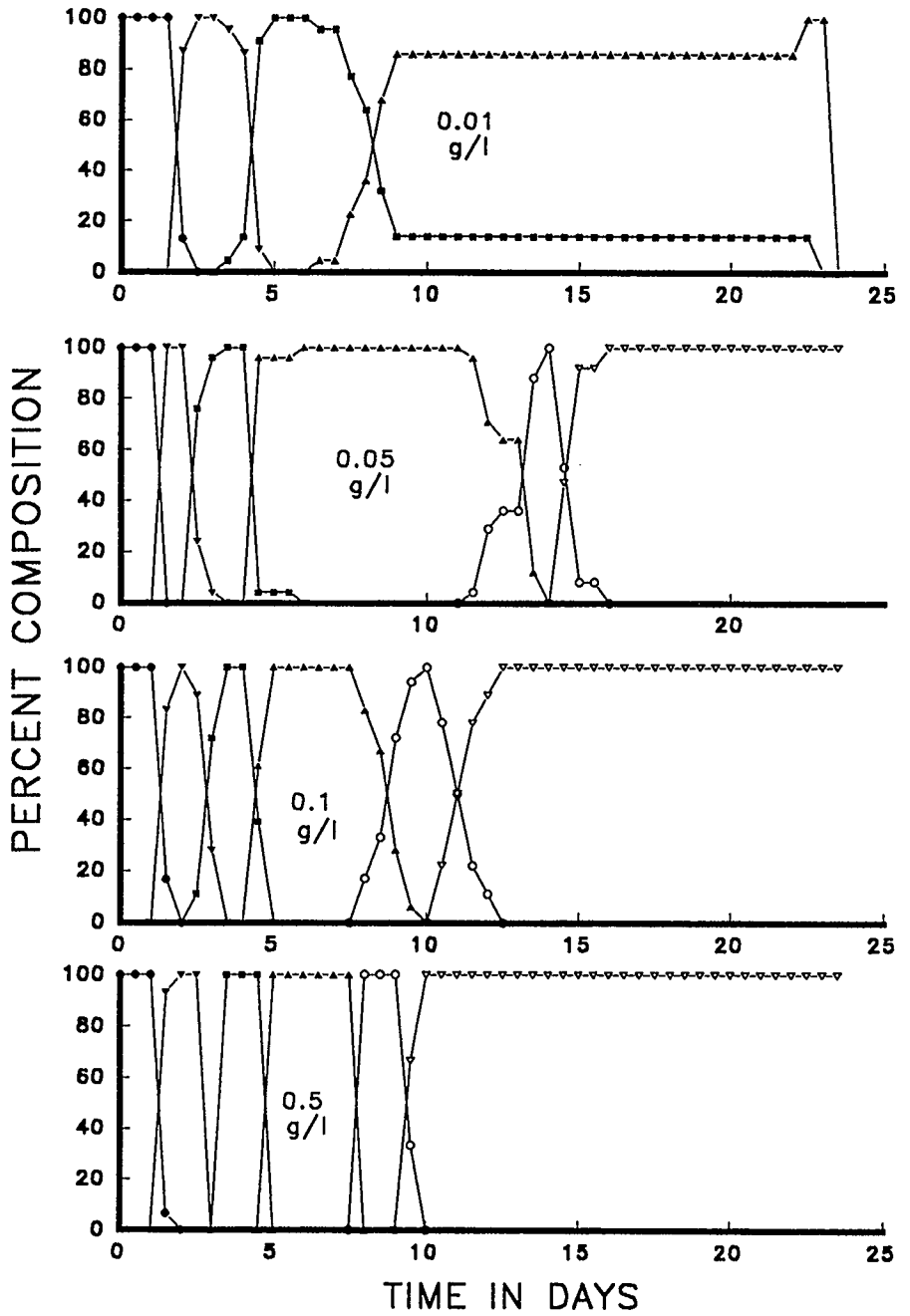
3.2.2.1 Experimental Population Stage Composition and Stage Survivorship

At the lowest food concentration (0.01 g/L) development did not occur beyond IV instar (Table 3.7, Fig. 3.9). Survivorship at this food concentration was high for the first three instars (88.9% - I instar, 89.6% - II instar, and 100% - III instar) (Table 3.7). In IV instar through-stage survivorship drops to 57.3% with no individuals surviving the pupal stage (Table 3.7). In the three higher food concentrations, complete development to emergence occurred, with the rate of transition between development stages increasing positively with increases in food (Figs. 3.9, 3.10). Stage specific survivorship varied significantly with an interaction between development stage and food concentration (stage*food, $F_{9,40}=2.26$, $P<0.05$). The source of this interaction becomes clear when the effects of food concentration within each stage as well as the effect of stage within each food treatment are isolated. Food treatment produces significant effects in stage I (contrast $F_{3,40}=5.14$, $P<0.01$) and a very weak effect in stage IV (contrast $F_{3,40}=2.79$, $P<0.05$). Food treatments of 0.01 g/L (contrast $F_{3,40}=4.78$, $P<0.01$) and 0.5 g/L (contrast $F_{3,40}=5.57$, $P>0.01$) have significantly different survivorship between stages. Thus, the low survivorship in stage I in the 0.5 g/L food treatment (64.0%) and stage IV in the 0.01 g/L food treatment (57.3%) are the sources for the significant stage*food interaction. Survivorship in food concentrations of 0.05 g/L and 0.1 g/L is >90% for all stages with no significant differences between stages (contrast 0.05 g/L, $F_{3,40}=0.08$, $P>0.05$ and 0.1 g/L, $F_{3,40}=0.29$, $P>0.05$) (Table 3.7).

Table 3.7. Stage specific through-stage survivorship for *C. tarsalis* at food concentrations of 0.01, 0.05, 0.1, 0.5 g/L. Values in brackets are the number of replicate series comprised of 25 larvae and the standard error of the estimate respectively.

	FOOD CONCENTRATION (g/L)			
STAGE	0.01 (%)	0.05 (%)	0.1 (%)	0.5 (%)
I	88.9 (6,6.5)	96.0 (5,1.8)	94.4 (8,1.1)	64.0 (5,9.2)
II	89.6 (6,6.5)	96.9 (4,1.9)	95.8 (7,2.3)	94.8 (4,3.1)
III	100.0 (3,0.0)	95.4 (3,4.6)	95.2 (5,2.6)	96.67 (3,3.3)
IV	57.3 (2,2.5)	94.3 (2,0.3)	92.0 (3,4.2)	95.0 (2,5.0)
Pupal	--	64.0 (1,-)	89.5 (2,0.5)	100.0 (1,-)
I - ADULT	--	64.0	73.9	50.0

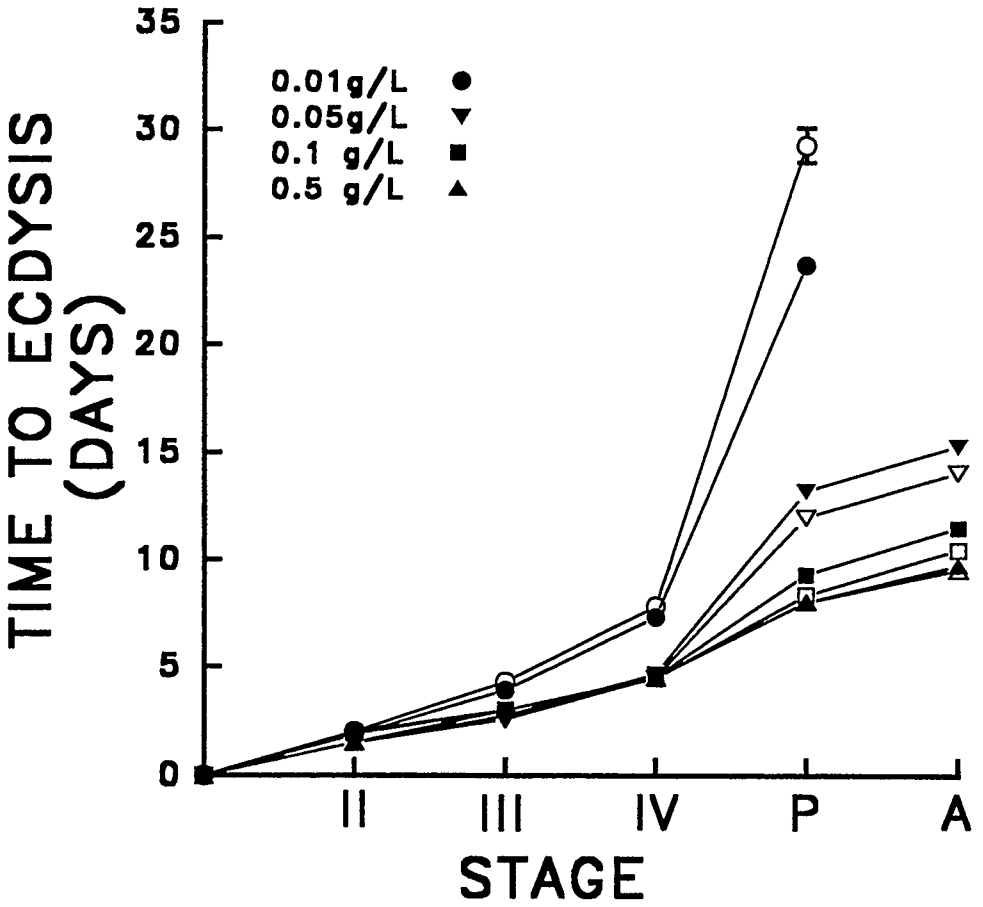
Figure 3.9. Stage structure of *C. tarsalis* experimental populations at food treatments of 0.01, 0.05, 0.1, 0.5 g/L. Stage composition calculated as the percentage of the surviving population in each developmental stage (I instar = ●, II instar = ▼, III instar■, IV instar = ▲, Pupal stage = ○, Adult stage = △).


PERCENT COMPOSITION
0.01 g/l
0.05 g/l
0.1 g/l
0.5 g/l
TIME IN DAYS

3.2.2.2 Development Times and Stage Durations

When time to ecdysis is plotted for each developmental stage against food concentration, only at the lowest food treatment (0.01 g/L) does development time prior to the pupal stage differ (Fig. 3.10). Since complete development cannot occur at this concentration data from this treatment were not included in the any ANOVAs. Development rates to adult are significantly affected by the interaction of food concentration and sex ($F_{2,39}=7.396$, $P<0.001$). At low food concentrations males develop at a faster rate than females (contrast 0.05 g/L, $F_{1,39}=8.983$, $P<01$, 0.1 g/L, $F_{1,39}=49.9$, $P<0.001$), however, at the highest food concentration (0.5 g/L) female development rates increase, so there is no significant difference between sexes (contrast $F_{1,39}=1.448$, $P>0.05$) (Fig. 3.11). A similar significant food * sex interaction ($F_{2,39}=5.568$, $P<0.01$) occurs for development rates to the pupal stage. Males develop to the pupal stage faster than females at food concentrations of 0.05 g/L (contrast $F_{1,39}=27.665$,$P<0.001$) and 0.1 g/L ($F_{1,39}=18.397$,$P<0.001$) with no significant difference between sexes occurring at 0.5 g/L ($F_{1,39}=0.0$, $P=1.0$) (Fig. 3.10). However, development time to ecdysis into IV instar is not affected by sex ($F_{1,39}=0.436$, $P>0.05$) or food concentration ($F_{1,39}=0.494$, $P>0.05$) (Fig. 3.10). Hence, the significant food * sex interaction effect that occurs for development rates to the pupal and adult stages, results from sex specific variation in food concentration effects on development within the IV instar. A two way ANOVA on IV instar duration supports this conclusion ($F_{2,39}=4.985$, $P<0.01$), with males spending significantly less time in IV instar at the

Figure 3.10. Mean (±SE) *C. tarsalis* stage-specific development times for food concentrations of 0.01 g/L(●), 0.05 g/L(▼), 0.1 g/L(■), and 0.5 g/L(▲). Open symbols represent males and closed symbols females with error bars contained within the symbols not shown.


TIME TO ECDYSIS
(DAYS)
35
30
25
20
15
10
5
0
0.01g/L
0.05g/L
0.1 g/L
0.5 g/L
II
III
IV
P
A
STAGE

lower food concentrations (contrast 0.05 g/L, $F_{1,39}=26.399$, $P<0.001$, and 0.1 g/L, $F_{1,39}=7.728$, $P<0.01$) and both sexes having identical IV instar durations at the highest food concentration (contrast $F_{1,39}=0.0$, $P=1.0$). In the pupal stage only the high food treatment (0.5 g/L) is significantly lower than the other treatments with no difference between sexes.

The simple non-linear model proved to be the most appropriate fit for development rates as a function of food concentration. The coefficients *a* and *b* determine the asymptote and the rate of approach to the asymptote. When *b* is held constant increasing *a* decreases the asymptote. When *a* is held constant and *b* increased the rate of approach to the asymptote decreases.

Development rates for each of the stages were found to increase at a decelerating rate to an asymptote as food concentration increased (Tables 3.8, 3.9, 3.10, 3.11, Figs. 3.11, 3.12, 3.13, 3.14). The regressional fits to this model show the same patterns as the analysis of variance. Development rates of the sexes converge as food concentration increases, as shown by the sex specific values of *a* and *b*. Males have a higher maximum development rate than females (*a*, $t_{43}=-2.08$, $P<0.05$) (Table 3.8, Fig. 3.11), however, both sexes reach their respective asymptotes at the same rate (*b*, $t_{43}=0.001$, $P>0.05$). Female pupal development rates rapidly plateau in comparison to males (*b*, $t_{43}=3.4$, $P<0.05$) but do not differ significantly in their maximum development rates (*a*, $t_{43}=1.9$, $P>0.05$)(Fig. 3.12). In IV instar females have a significantly lower maximum

Table 3.8. Non-linear curve fit using y = a*food/(b+food) for *C. tarsalis* emergence rate (data at 0.01 g/L not included). A) Model summary table and parameters B) Model summary table for sex specific development rates. C) Sex specific parameter estimates with two-sample t-tests for comparison between the sexes.

A) **SEXES COMBINED:** $r^2 = 0.996$

Source	Sums-of-squares	df	Mean-square
Regression	0.348	2	0.174
Residual	0.00136	43	0.000032
Total	0.349	45	

Parameter	Estimate	SE
a	0.113	0.00194
b	0.029	0.00217

B)

MALE: $r^2 = 0.996$

Source	Sums-of-squares	df	Mean-square
Regression	0.1707	2	0.085
Residual	0.0006	19	3.3×10^{-5}
Total	0.1713	21	

FEMALE: $r^2 = 0.999$

Source	Sums-of-squares	df	Mean-square
Regression	0.1776	2	0.089
Residual	1.5×10^{-4}	22	7.0×10^{-6}
Total	0.1777	24	

C)

Parameter	Sex	Estimate	SE	t	P
a	Male	0.118	0.004	-2.08	<0.05
	Female	0.11	0.001		
b	Male	0.030	0.004	0.001	>0.05
	Female	0.031	0.001		

Table 3.9. Non-linear curve fit using y = a*food/(b+food) for *C. tarsalis* pupal development rates (1/days). A) Model summary table and parameter estimates B) Model summary table for sex specific development rates. C) Sex specific parameter estimates with two-sample t-tests for comparison between the sexes.

A) **SEXES COMBINED:** $r^2 = 0.959$

Source	Sums-of-squares	df	Mean-square
Regression	12.306	2	6.153
Residual	0.531	44	0.121
Total	12.838	46	

Parameter	Estimate	SE
a	0.582	0.0351
b	0.012	0.00598

B)

MALE: $r^2 = 0.983$

Source	Sums-of-squares	df	Mean-square
Regression	5.240	2	2.620
Residual	0.0885	20	0.00442
Total	5.329	22	

FEMALE: $r^2 = 0.958$

Source	Sums-of-squares	df	Mean-square
Regression	7.435	2	3.717
Residual	0.324	22	0.0141
Total	7.759	24	

C)

Parameter	Sex	Estimate	SE	t	P
a	Male	0.666	0.0428	1.9	>0.05
	Female	0.550	0.0414		
b	Male	0.0372	0.00895	3.4	<0.005
	Female	0.000737	0.00581		

Table 3.10. Non-linear curve fit using y = a*food/(b+food) for *C. tarsalis* development rate (1/days) during IV instar. A) Model summary table and parameter estimates (0.01 g/L food concentration included). B) Model summary table for sex specific development rates. C) Sex specific parameter estimates with two-sample t-tests for comparison between the sexes.

A) **SEXES COMBINED:** $r^2 = 0.976$

Source	Sums-of-squares	df	Mean-square
Regression	2.405	2	1.203
Residual	0.059	60	0.001
Total	2.464	62	

Parameter	Estimate	SE
a	0.335	0.012
b	0.061	0.006

B) **MALE:** $r^2 = 0.974$

Source	Sums-of-squares	df	Mean-square
Regression	1.296	2	0.648
Residual	0.035	23	0.002
Total	1.331	23	

FEMALE: $r^2 = 0.992$

Source	Sums-of-squares	df	Mean-square
Regression	1.262	2	0.631
Residual	0.010	22	0.0001
Total	1.271	27	

C)

Parameter	Sex	Estimate	SE	t	P
a	Male	0.343	0.025	0.232	>0.05
	Female	0.337	0.010		
b	Male	0.052	0.011	2.417	<0.05
	Female	0.081	0.007		

Table 3.11. Non-linear curve fit using y = a*food/(b+food) for *C. tarsalis* development rate in the early larval stage. A) Both sexes combined with 0.01 g/L food concentration included. Model summary table and parameter estimates. B) Model summary table for sex specific development rates (0.01 g/L deleted) C) Sex specific parameter estimates with two-sample t-tests for comparison between the sexes.

A) **SEXES COMBINED:** $r^2 = 0.994$

Source	Sums-of-squares	df	Mean-square
Regression	3.224	2	1.612
Residual	0.0189	80	0.000236
Total	3.243	82	

Parameter	Estimate	SE
a	0.241	0.00283
b	0.00877	0.000538

B)

MALE: $r^2 = 0.998$

Source	Sums-of-squares	df	Mean-square
Regression	1.753	2	0.877
Residual	0.00298	64	0.000088
Total	1.756	36	

FEMALE: $r^2 = 0.997$

Source	Sums-of-squares	df	Mean-square
Regression	1.877	2	0.938
Residual	0.00583	37	0.000157
Total	1.883	39	

C)

Parameter	Sex	Estimate	SE	t	P
a	Male	0.219	0.0351	0.206	>0.05
	Female	0.221	0.00386		
b	Male	-0.000522	0.00108	0.644	>0.05
	Female	0.000525	0.00120		

Figure 3.11. Non-linear curve fit for *C. tarsalis* male and female emergence rates (1/days) as a function of food concentration using the regression equation in Table 3.8.

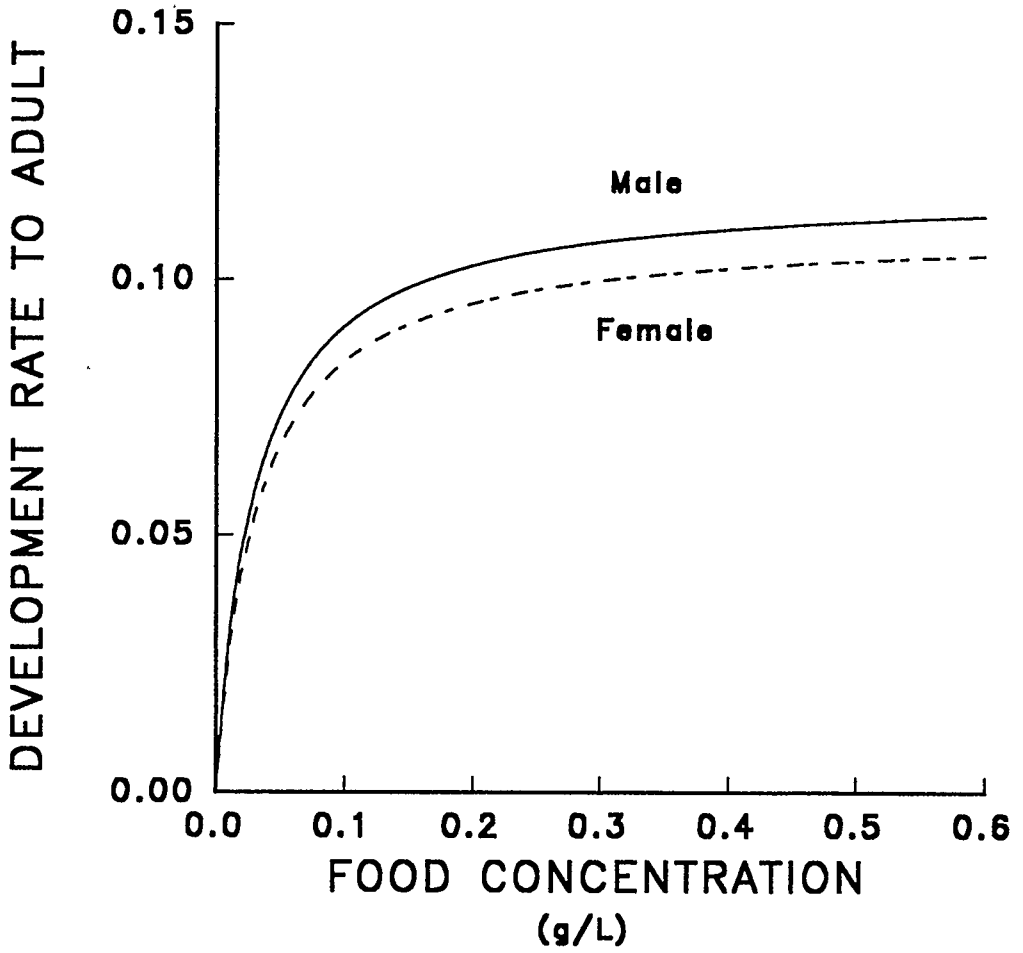

DEVELOPMENT RATE TO ADULT
0.15
0.10
0.05
0.00
Male
Female
0.0
0.1
0.2
0.3
0.4
0.5
0.6
FOOD CONCENTRATION
(g/L)

Figure 3.12. Non-linear curve fit for *C. tarsalis* male and female development rates (1/days) during the pupal stage as a function of food concentration using the regression equation in Table 3.9.

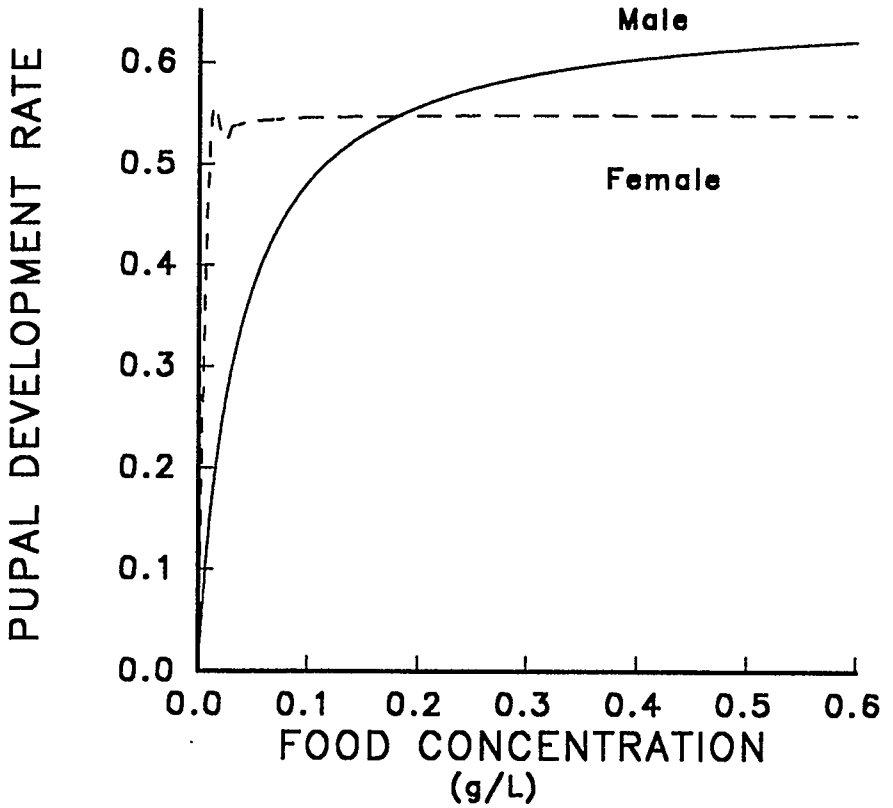

Male
Female
PUPAL DEVELOPMENT RATE
0.0
0.1
0.2
0.3
0.4
0.5
0.6
FOOD CONCENTRATION
(g/L)

Figure 3.13. Non-linear curve fit for *C. tarsalis* male and female development rates (1/days) during the IV instar as a function of food concentration using the regression equation in Table 3.10.

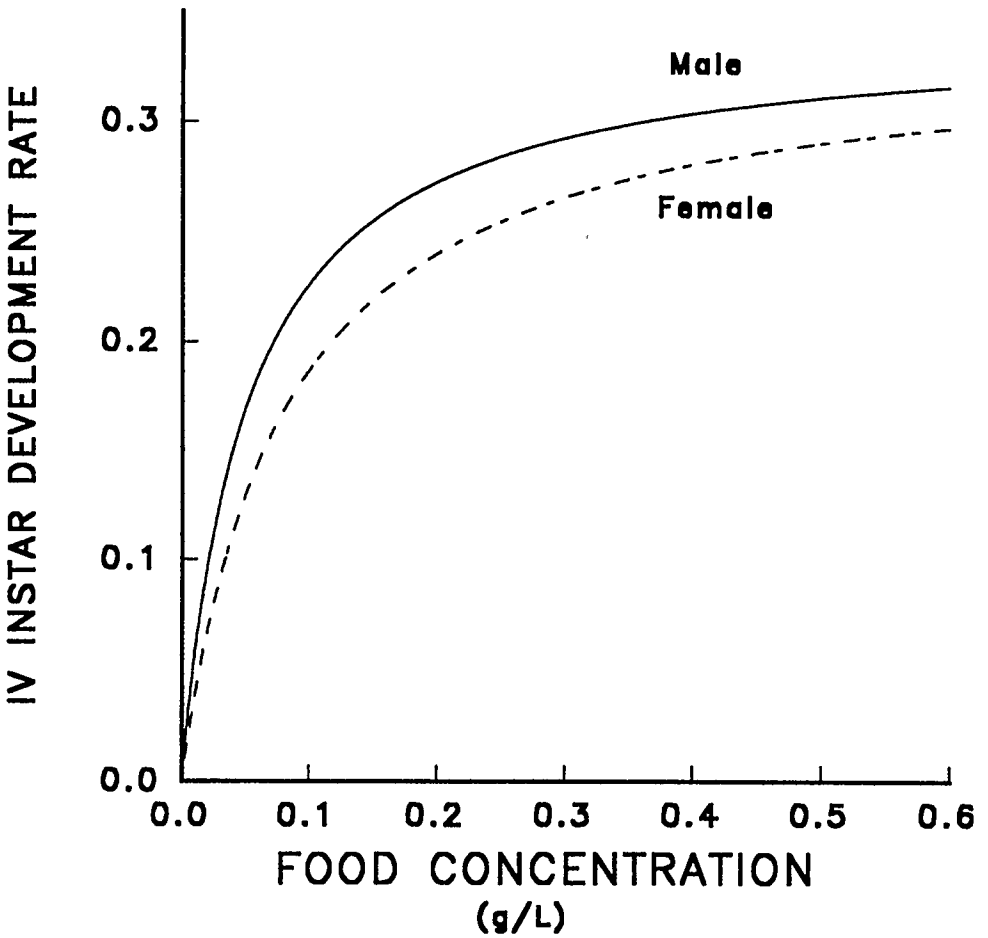
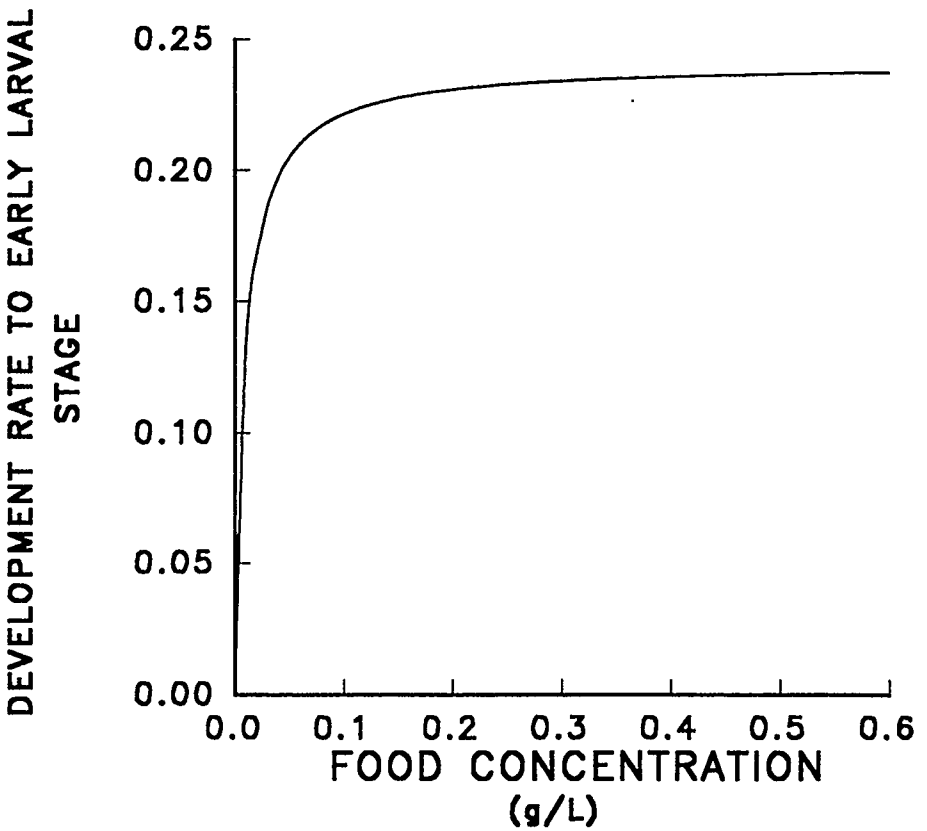

Male
Female
IV INSTAR DEVELOPMENT RATE
0.0
0.1
0.2
0.3
0.0
0.1
0.2
0.3
0.4
0.5
0.6
FOOD CONCENTRATION
(g/L)

Figure 3.14. Non-linear curve fit for *C. tarsalis* male and female development rates (1/days) in the early larval stage as a function of food concentration using the regression equation in Table 3.11.

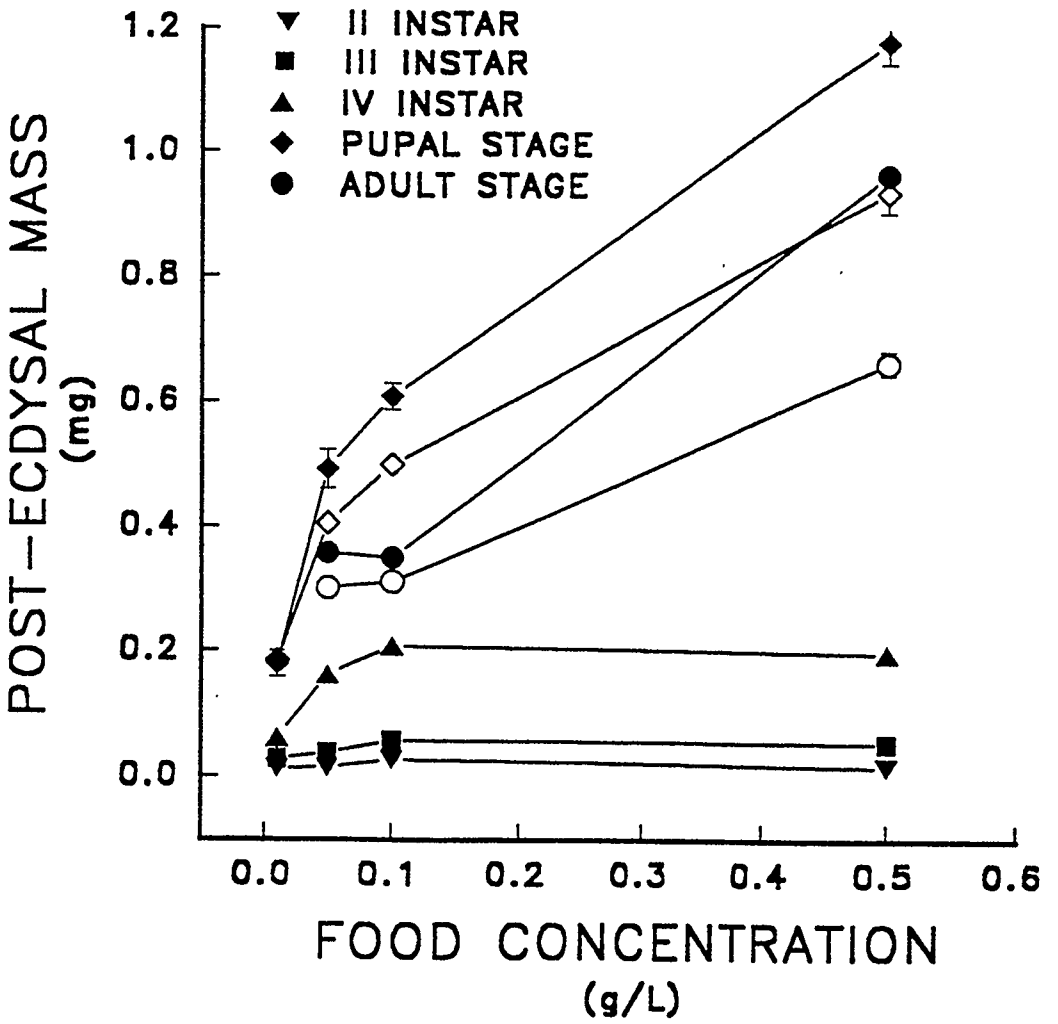

DEVELOPMENT RATE TO EARLY LARVAL STAGE
0.25
0.20
0.15
0.10
0.05
0.00
0.0
0.1
0.2
0.3
0.4
0.5
0.6
FOOD CONCENTRATION
(g/L)

development rate (*b*, t_{51}=-2.417, $P<0.05$) and require a higher food concentration to achieve it (*a*, t_{51}=-0.232, $P<0.05$) (Table 3.10, Fig. 3.13). Analysis of variance showed development times to ecdysis into IV instar (during early larval stage) were not significantly affected by food concentration or sex when data from the lowest food concentration (0.01 g/L) were deleted. When data from this treatment are included, the curvi-linear regression model best fits the data with no significant differences between sexes (Table 3.11, Fig. 3.14).

3.2.2.3 Post-Ecdysal Weights

Stage-specific post-ecdysal weights plotted against food concentration (Fig. 3.15) show a pattern similar to development rates. Individuals reared at food concentrations of 0.01 g/L have lower body weights than the other treatments (Fig. 3.15). The decrease in mass from the pupal stage to adult emergence is due to the loss of the pupal exuviae and the inability of pupae to feed. Both weight at emergence and weight at pupation are significantly affected by a food*sex interaction (adult, $F_{2,40}$=49.75, $P<0.001$; pupal, $F_{2,47}$=6.521, $P<0.01$) (Figs. 3.15) due to increased sexual dimorphism in body weight as food concentration increases. For example, adult females are on average 0.30 mg larger than males in the 0.5 g/L food treatment but only 0.056 mg larger than males in the 0.05 g/L food treatment. A similar relationship occurs for pupal weights, with average female weights exceeding male weights by 0.24 mg in the 0.5 g/L food treatment and only 0.087 mg the 0.05 g/L food concentration. Food concentration has no

Figure 3.15. Mean (±SE) stage-specific post ecdysal weights (II instar ▼, III instar ■, IV instar ▲, pupal stage ◆, adult ●) for *C. tarsalis* as a function of food concentration. Standard errors not shown if incorporated within the symbol.


II INSTAR
III INSTAR
IV INSTAR
PUPAL STAGE
ADULT STAGE
POST-ECDYSAL MASS
(mg)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
FOOD CONCENTRATION
(g/L)

significant effect on weight at ecdysis into IV instar ($F_{2,70}$=2.943, P>0.05) (Figs. 3.15). Thus, the significant food effects on pupal or emergent weights result from weight gain in the IV stage.

The effect of food concentration on weight gain becomes clear when the weight gain per day is calculated for each individual stage (Fig. 3.16). Weight gain per day up to ecdysis into IV instar, increases at a decelerating rate to an asymptote as food concentration increases (Tables 3.12 for early larval stage, Fig. 3.16 for stage specific rates). The asymptote is rapidly reached, with little difference in weight gain per day between food treatments of 0.01 g/L and 0.5 g/L (Fig. 3.16). The variation in sex specific pupal and adult weights occurs during IV instar. In IV instar weight gain per day accelerates linearly with food concentration with sex specific rates diverging as food concentration increases (Table 3.13, Fig. 3.16). Weight gain per day for females in the highest food concentration (0.5 g/L) is approximately 84 times that at the lowest food treatment (0.01 g/L) and seven times that experienced in the lowest food treatment (0.05 g/L) at which complete development occurs. Weight gain per day for males in the highest food concentration is approximately 44 times that in the lowest treatment level (0.01 g/L) and six times that in the lowest food treatment at which emergence can occur (0.05 g/L). During the pupal stage weight loss is non-linear withfood concentration, with weight loss per day increasing at a decelerating rate to an asymptote (Table 3.14, Fig. 3.16).

Figure 3.16. Mean weight gain per day (mg/day) for *C. tarsalis* as a function of food concentration during I instar (●), II instar (▼), III instar (■), IV instar (◆), and pupal stage (▲). Open symbols represent males and closed symbols females. Lines generated from regression models in Table 3.13 and 3.14 fit to the raw data. Standard errors not visible are incorporated within the symbols.

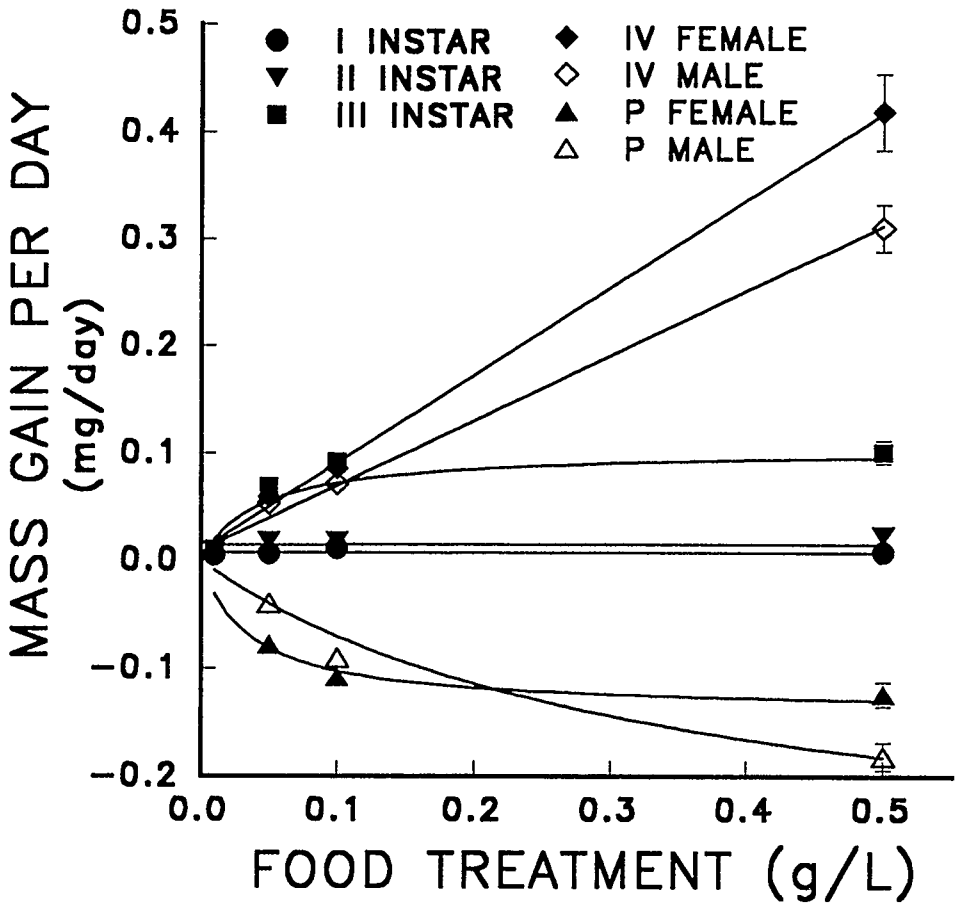

I INSTAR
II INSTAR
III INSTAR
IV FEMALE
IV MALE
P FEMALE
P MALE
MASS GAIN PER DAY (mg/day)
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
0.0
0.1
0.2
0.3
0.4
0.5
FOOD TREATMENT (g/L)

Table 3.12. Non-linear curve fit using y = a*food/(b+food) for *C. tarsalis* weight gain per day (mg/day) during development in the early larval stage. Sexes combined since sex determination not possible at this stage of development. The food concentration of 0.01 g/L was included. A) Summary table for regression analysis to the model. B) Parameter estimates for the model.

A) MODEL

$r^2 = 0.816$

Source	Sums-of-squares	df	Mean-square
Regression	0.4898	2	0.244
Residual	0.110	121	0.000914
Total	0.600	123	

B)

Parameter	Estimate	SE
a	0.0996	0.012
b	0.048	0.018

Table 3.13. Regression analysis of *C. tarsalis* weight gain per day (mg/day) during IV instar as a function of sex and food concentration (growth = constant + B_1sex + B_2food + B_3sex*food). Sex was analyzed using dummy variables; male = 0, female = 1.

MODEL $r^2 = 0.92$

Source	Sum-of-squares	df	MS	F	P
Regression	1.447	3	0.482	260.23	<0.001
Residual	0.126	68	0.00185		

PARAMETER ESTIMATES

Variable	Coefficient	SE	t	P
Constant	0.011	0.00806	1.341	>0.05
Sex	-0.00493	0.0139	-0.355	>0.05
Food	0.603	0.0366	16.0	<0.001
Sex*Food	0.225	0.0536	4.189	<0.001

Table 3.14. Non-linear curve fit using y = a*food/(b+food) for *C. tarsalis* weight loss per day during the pupal stage for both males and females. The food concentration of 0.01 g/L was included. A) Summary table for regression analysis to the model. B) Two-sample t-tests for comparison of the parameter estimates between the sexes.

A) MODEL

MALE: $r^2 = 0.860$

Source	Sums-of-squares	df	Mean-square
Regression	0.221	2	0.111
Residual	0.036	30	0.001
Total	0.257	32	

FEMALE: $r^2 = 0.832$

Source	Sums-of-squares	df	Mean-square
Regression	0.608	2	0.304
Residual	0.123	54	0.002
Total	0.731	56	

B)

Parameter	Sex	Estimate	SE	t	P
a	Male	-0.302	0.073	2.712	<0.01
	Female	-0.139	0.018		
b					
	Male	0.327	.073	3.007	<0.01
	Female	0.035	0.018		

3.2.2.4 Head Capsule Width

Head capsule widths varied with food conditions ($F_{3,222}=6.57$, $P<0.01$) and significantly differed with stage ($F_{2,222}=4689.59$, $P<0.0001$). However, sensitivity to food conditions proved to be stage specific (stage*food concentration, $F_{5,222}=6.38$ $P<0.0001$) with II instar head capsule widths not differing (contrast $F_{3,222}=2.30$, $P>0.05$) with food conditions while the III and IV instar head capsules did (contrast III $F_{3,222},=4$, $P<0.0084$ and IV $F_{3,222}=11.34$, $P<0.0001$) (Table 3.15).

3.3 DISCUSSION

3.3.1 Physiological Time

Physiological time is a better predictor of insect development than actual time (Gilpin and McClelland 1979) and is based on the premise that the more development to occur between two calendar dates the faster time has passed for the organism. Since the actual development time is dependent on temperature, development is often expressed as the total number of degree-days required above the DZ temperature to complete development (Daly *et al.* 1978). The number of degree-days required for full development is calculated using the equation:

Table 3.15. *C. tarsalis* stage-specific mean head capsule widths reared in food concentrations of 0.01, 0.05, 0.1 and 0.5 g/L at a constant temperature of 25°C.

		HEAD CAPSULE (mm)		
FOOD LEVEL	STAGE	N	MEAN	SE
0.01	II	21	0.493	0.005
	III	25	0.799	0.005
	IV	15	1.106	0.010
0.05	II	24	0.490	0.004
	III	22	0.762	0.005
	IV	22	1.167	0.015
0.10	II	24	0.517	0.002
	III	20	0.784	0.009
	IV	22	1.220	0.019
0.50	II	16	0.496	0.006
	III	12	0.803	0.010
	IV	11	1.185	0.035

$$\frac{1}{t} = \frac{1}{K}(T - c) \qquad (3.3)$$

where t is the duration of development, T the temperature, c the DZ and K the thermal constant. The mean K, calculated from temperatures on the linear portion of the development curve (to emergence), is the degree-days for the organism. Using the DZ resulting from the regression in Table 3.10b, produces the K estimates in Table 3.16. From these estimates mean values of 166.2 degree-days for males and 190.7 degree-days for females are obtained. These values are higher than those reported for females of the tropical species *Aedes aegypti* (114.2 degree-days)(Bar-Zeev 1958) but are lower than reported for sub-arctic species *A. communis* (males K=271, females K=318), *A. hexodontus* (females K=220) and *A. excruciens* (females K=440) (Haufe and Burgess 1956). This places *C. tarsalis* between the sub-arctic and the tropical species as expected from its geographic distribution.

3.3.2 MORTALITY AND THROUGH-STAGE SURVIVORSHIP

The two fatal temperatures of 10° and 35°C produced different patterns of stage-specific mortality. The low lethal temperature produced the longest larval lifespan, primarily spent in I instar. The high lethal temperature resulted in a very short lifespan

Table 3.16. Calculated temperature specific K values (degree-days) for both sexes of *C. tarsalis* using equation 3.3. The DZ (*c*) was calculated from the regression equation in Table 3.1B.

	Sex	Temperature (°C)			
		15°	20°	30°	35°
K	Males	166.2	166.4	165.98	197.2
	Females	193.0	188.2	190.8	262.3

with development progressing as far as IV instar. Similar patterns were reported by Bar-Zeev (1958) for *A. aegypti*, Trpis and Shemanchuk (1969) for *A. flavescens*, Trpis and Shemanchuk (1970) for *A. vexans*, Hanec and Brust (1967) for *Culiseta inornata*, and Tauthong and Brust (1977) for *A. campestris*. Due to possible interactions between food concentration and temperature in the experiments reported in the literature, food limitation cannot be eliminated as a causal mechanism. The use of pre-conditioned food treatments and replacement of the culture media on a daily basis in the present study, means food limitation is minimized. It is likely that temperature effects on physiological processes such as threshold temperatures required for enzyme synthesis and the kinetics of enzyme reactions are the limiting factors. This would affect functions as diverse as digestion (Gooding 1966a) or the production of developmental hormones such as ecdysone, which may explain why nearly all larval mortality at 10 °C occurred during or immediately after ecdysis.

The high fatal temperature, which increased ecdysal rates to IV instar also produced more evenly distributed mortality through the first four larval stages. The ability of larvae to develop as far as IV instar at high fatal temperatures is likely related to the temperature effects on metabolic activity and high ecdysone concentrations. This may serve to mediate temperature effects as ecdysone also induces synthesis of heat-shock proteins which increases cell temperature tolerance (Berger and Woodward 1983). While nearly all mortality at the low lethal temperature occurred in individuals attempting to ecdyse, this was not the case at the high fatal temperature. This also suggests the

involvement of ecdysone as immature stages exhibit greater thermotolerance during periods of high ecdysone titre (Benedict *et al.* 1991).

Larval mortality varied significantly with stage and food concentration. Even in the lowest food concentration (0.01 g/L) through-stage survivorship was high in the early instars (I - IV) and not until entering IV instar did larval mortality exceed 10.5%. This suggests that even at this low food concentration food requirements are met for instars I-III. In IV instar increased food requirements result in starvation for >40% of larvae with none able to survive the pupal stage. The high mortality of I instars in the 0.5 g/L food treatment is consistent with the literature, where high food concentrations produce significant mortality in the early instars (Gerberg 1970, Hayes *et al.* 1974, Gilpin and McClelland 1979) due to the formation of a surface film (pellicle) preventing penetration of the siphon. Pellicle formation was not noticed in this study, and since I instar larvae predominantly respire cutaneously this explanation cannot explain the observed mortality. It is possible high bacterial populations stress I instar larvae as the addition of bacteriacides such as tetracyclines reduces larval mortality (de St. Jeor and Nielson 1964, Hayes *et al.* 1974). How the bacteria populations adversely affect larvae is unknown but interference with cutaneous respiration, through depletion of dissolved oxygen concentrations, may be involved. With the exception of I instar larvae in the 0.5 g/L food treatment and pupae at 0.05 g/L food treatment had little effect on stage-specific survivorship which remained >90% for all treatments. Thus, only extreme temperature or food conditions significantly influence pre-imaginal mortality.

3.3.3 DEVELOPMENT RATES

The lack of a recognized standard larval food for experiments makes comparisons of larval development difficult. The procedure used in this study of pre-conditioning the media at a constant temperature combined with filtering and replacement, not only standardizes food concentrations but also allowed the use of higher food concentrations without the negative effects of pellicle formation and the accumulation of metabolites associated with other culturing procedures. Removing aliquots of the media for quantification of a selected nutrient such as organic carbon or protein content would provide a quantitative standard for comparative purposes and identification of temperature treatment effects on food concentration.

Despite the difficulties in comparing results of this study to those in the literature, some trends are clear. Mean development times were consistently lower than those reported in the literature (Hagstrum and Workman 1971, Reisen *et al.* 1984, and Buth *et al.* 1990) for *C. tarsalis* (Table 3.17). Differences in development times are more pronounced at the lower temperatures when compared to those reported by Buth *et al.* (1990). This may reflect limitation of bacterial growth at the lower temperatures producing poorer food concentrations in the latter experiments as well as further evidence that the incubation, filtering and replacement of media provides a more nutritious rearing medium.

Table 3.17. Comparison to literature values of *C. tarsalis* mean development times (days) to pupal and adult stages as a function of temperature .

Author(s)	Sexes	Temperature (°C)			
		15°	20°	25°	30°
McKee	Male	24.6	12.4	8.6	7.8
	Female	25.7	13.3	9.5	8.2
H and W[1]	Combined	---	14.6[a]	---	11.7[a]
Reisen[2]	Male	---	---	15.0[b]	11.0[c]
	Female	---	---	17.0[b]	14.0[c]
Buth[3]	Combined	34.6	18.2	12.8	---

[1] Hagstrum and Workman 1971
[2] Reisen *et al.* 1986
[3] Buth *et al.* 1990
[a] selected food treatment that produced shortest development time.
[b] actual experimental temperature = 26 °C
[c] actual experimental temperature = 31 °C

The increase in pupal development rates with temperature was expected as this is a non-feeding stage driven only by the metabolic rates of tissue reorganization. The pupal development rates (intercept=-0.252, slope=0.027) are similar to those for *A. aegypti* (intercept=-0.249, slope=0.039) calculated from Nielsen and Evans (1960) rather than those reported by Fouque *et al.* (1992) for *A. vexans* (intercept=-0.047, slope=0.016).

Mosquitoes exhibit sex specific development rates, with males developing at a greater rate than females (see Clements 1992 for review). However, no one has documented the interaction effect in this study, with development rates of the sexes diverging as temperature increases. Re-examination of Gilpin and McClelland's (1979) work on *A. aegypti* suggests the same interaction effect. While sexually dimorphic development rates result as early as III instar, the increased time spent by females in IV instar accounts for the majority of sexually different emergence rates. The pivotal role of the IV instar in determining emergence times is emphasized by the increasing proportion of total emergence time that IV instar accounts for with increasing temperature. This may be due to the rapid development through the previous instars not allowing the accumulation of some essential component required prior to ecdysis into the pupal stage. The non-feeding nature of the pupal stage means energy and tissue requirements for metamorphosis to adult must be met prior to ecdysis from IV instar. It is possible, that energy reserves not acquired in the early larval stages must be obtained in the IV instar.

Emergence and pupal development times were also significantly affected by food concentration and sex in a non-linear manner. While Gilpin *et al.* (1976) and Gilpin and McClelland (1979) found larval development rates for *A. aegypti* to be independent of food density, re-examination of their results shows this may be due to pupal collection only every 24h combined with the problems associated with food preparation and mass rearing. In this study the sexes developed at the same rate up to IV instar with the non-linear model best describing the nature of the food dependence. However, the extremely low value for coefficient *b* (Table 3.11) means the function asymptotes very rapidly, indicating that the maximum development rate is attained at low food concentrations. Hence, through-out the range of food concentrations at which complete development is possible, there is no variation between the sexes and only a marginal impact by food concentration. The IV instar was the stage responsible for the expression of sexually dimorphic emergence rates in both the temperature and food experiments. However, females respond to incremental increases in food concentration at a greater rate than males so that at the highest food concentration sexually dimorphic development rates do not occur. Thus, males and females have the same minimum duration for IV instar with males attaining this minimum time at lower food concentrations. Female pupae respond very little to food concentrations in excess of 0.01 g/L while males increase their pupal development rate, asymptoting near food concentrations of 0.5 g/L. Since pupae do not feed, these effects are due to the nutritional environment experienced in earlier stages. The reasons males and females respond differently are not known.

3.3.4 ECDYSAL WEIGHTS AND DAILY WEIGHT GAIN

Temperature and food concentration had only marginal influence on II, III, and IV instar post ecdysal weights and differences between sexes. However, pupal and adult weights were negatively influenced by temperature and positively influenced by food concentration. There appears to be a trade-off between development time and biomass accumulation. For example the higher pupal and adult weights at 15°C, are tempered by an increased development time (in IV instar), so that weight gain per day in IV instar is marginally lower than at the other temperatures. Thus, while temperature negatively influences weight it has little influence on weight gain per day. Increased rate of development at high temperatures results in less time for biomass accumulation with more energy required to meet metabolic demands. The inability of the pupae to feed means they cannot meet the temperature enhanced metabolic requirements and as a result have a net weight loss per day which is enhanced by the shedding of the exuviae.

Both pupal and adult development rates and ecdysal weights respond positively to increases in food concentrations. At high food concentrations larger individuals are produced in a shorter period of time. This is especially pronounced in females so that for each incremental increase in food concentration females experience a greater weight gain per day than males. This has been demonstrated in other species (Christophers 1960, Brust 1967), but until this study, the stage at which this occurred had not been identified. Possible explanations for this are sex specific food filtration rates, digestion

efficiencies or metabolic rates.

3.3.5 MODELLING IMPLICATIONS

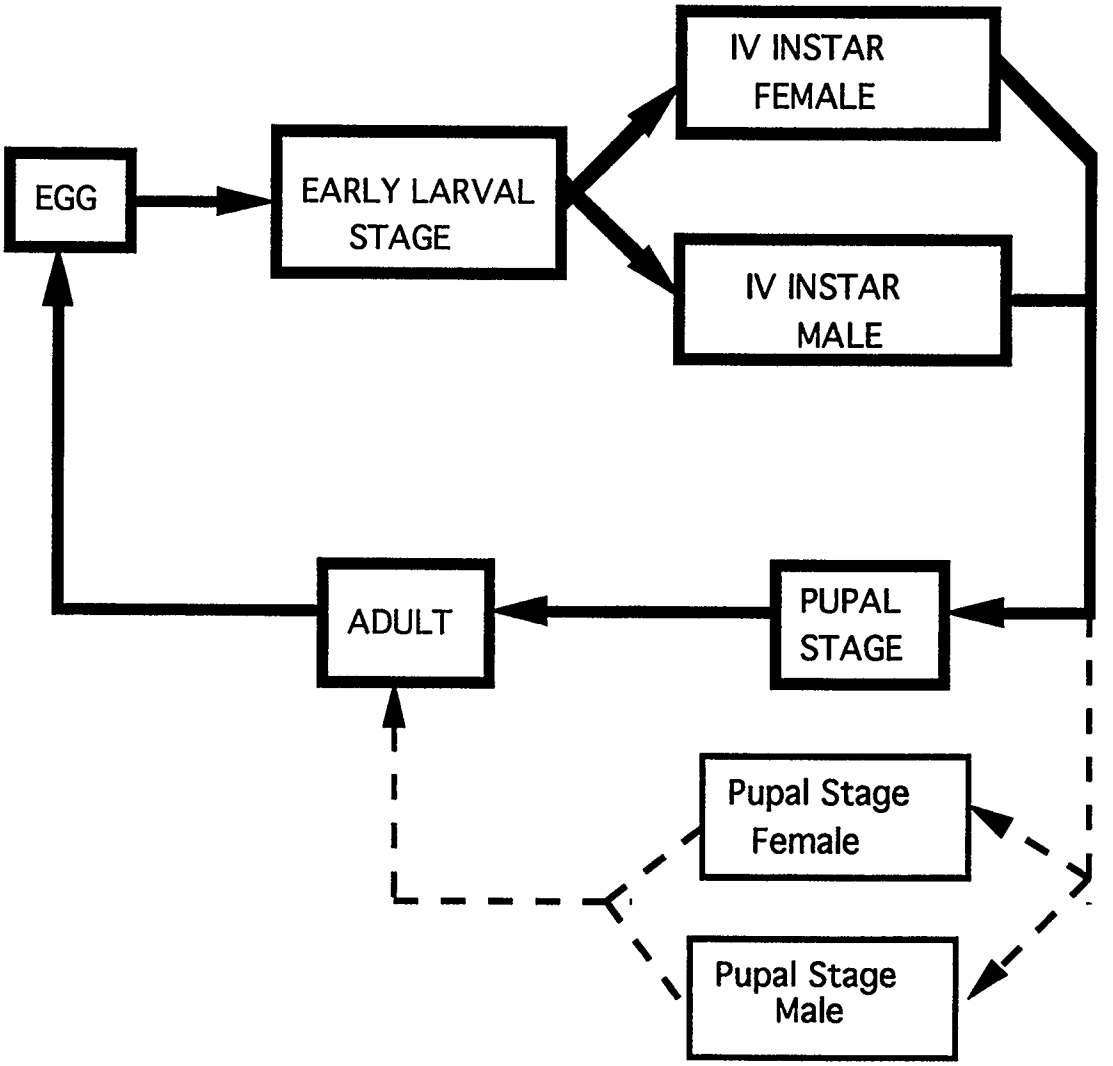
The development characteristics of the first three larval stages are primarily independent of food concentration and temperature. Thus, these stages can be grouped as the early larval stage, which has the benefit of decreasing the number of stages in the model from seven to five (Fig. 3.17). While development time for all of the stages increases linearly with temperature, differences in rates between sexes does not occur until ecdysis into III instar. However, sex specific differences at this stage are minor compared to the sexually dimorphic rates in IV instar. Thus, for the model, development in the early larval stage can therefore be considered to be linear with temperature and independent of sex. In addition the extremely low *b* coefficient in food dependent development rates in the early larval stage means development may be considered a fixed rate for most food concentrations. Ecdysal weights into IV instar were also independent of food concentration. Thus, either a fixed time or fixed weight may be used as the development index (DI) for this stage. Due to the positive relationship between temperature and development rates, a temperature dependent development time is probably the best DI.

The pivotal stage in the development of *C. tarsalis* is clearly the IV instar. Nearly all of the emergence characteristics influenced by temperature and food concentration

occur in this stage. Hence both temperature and food concentration must be modelled on a sex specific basis. This means IV instar must be subdivided into male amd female specific rates (Fig. 3.17). While temperature influences IV instar duration linearly, food concentration influences it non-linearly. Thus, neither time nor weight alone make a suitable DI for IV instar. This stage would best be modelled by requiring that both a minimum time and minimum weight be met in order to pupate. The asymptoting of the development curve at high food concentrations identifies a maximum rate or the minimum time that must be spent in each stage. The increased proportion of total emergence time spent in IV instar as temperature increases and food concentration decreases, supports the view that a minimum weight requirement must be met prior to pupation. A minimum weight is either not required in the earlier instars or is so easily met that it is rarely limiting. The requirement that a minimum time and weight be met prior to pupation may account for the great variation found in pupal masses. Large pupae result when individuals in high food conditions continue to gain weight for the minimum time requirement. Small individuals arise when poor food conditions result in the minimum weight being achieved in the minimum time. The DI for IV instar would be a sliding scale with females having higher minimum weight and minimum time requirements than males.

The pupal stage, as a non-feeding stage, is most strongly influenced by temperature but shows no temperature specific differentiation between sexes. The significantly different effect of food concentration on male and female pupal development

Figure 3.17. Flow diagram of final organization for the *C. tarsalis* stage-structured model. The dashed lines for the sex specific pupal stages denotes the possibility that future work may show a need for sex specific balance equations in this stage.


IV INSTAR
FEMALE
EGG
EARLY LARVAL
STAGE
IV INSTAR
MALE
ADULT
PUPAL
STAGE
Pupal Stage
Female
Pupal Stage
Male

rates needs to be further investigated.

Stage-specific mortality proved to be extremely high within a wide range of temperatures and food concentrations capable of allowing full development. The observed temperature or food dependent mortality within these ranges suggests that the majority of the mortality is due to genetic deficiencies that cause death irrespective of environmental conditions (Gilpin and McClelland 1979). Food requirements for survival in the early larval stage appear minimal. However, a starvation function needs to be generated for the IV and pupal stages under low food conditions.

Investigation of the effect larval density has on development and growth is now required to produce a functional model. This has not been investigated on a stage-specific basis due to the difficulty in accurately determining larval instar using larval saddle hairs. While head capsule width was significantly affected by temperature and food concentration within a stage, there is no significant overlap between stages. Thus, the head capsule widths provided here for each of the stages may be used for stage identification in time series density experiments for *C. tarsalis*.

3.3.6 ECOLOGICAL IMPLICATIONS

Larval mortality is only affected by extreme temperature and food conditions. This is supported by field work which shows larval mortality is primarily a result of

catastrophic events such as the temporary nature of many larval habitats or periods of high lethal temperature and predation (Lakhani and Service 1974, Riesen and Siddiqui 1979, Mogi *et al.* 1984, Reisen *et al.* 1989, Mogi and Okazawa 1990). However, while extrinsic factors of food concentration, temperature and possibly density may not directly inflict mortality, they may indirectly affect it.

Mosquitoes in the aquatic habitat face a trade-off. Since mortality due to predation and catastrophic events is high, rapid emergence is beneficial. However, individuals experiencing rapid development pay the subsequent costs of reduced fecundity. Yet reduced fecundity is better than the possibility of zero fecundity should mortality result in the larval stage. The evolution of a minimum pupation weight at which an individual will have some reproductive success would appear advantageous in this scenario. Stressful, but not fatal, extrinsic events that delay the attainment of this minimal weight or time threshold increase an individuals vulnerability to mortality in the aquatic habitat. It is possible that the sexual dimorphism in development and growth rates may be due to sex specific evolutionary strategies. The reproductive benefits of body size for females may exceed that for males. Hence, for females it is worth the increased mortality risk to remain longer in the aquatic habitat to reach a larger size with greater fecundity.

SECTION II

CHAPTER FOUR

4.0 BIOLOGICAL CONTROL THEORY

The aquatic stages of the mosquito lifecycle are the primary targets for chemical control programs due to the concentration of mosquitoes in identifiable habitats. As mentioned in Chapter 1 determining the potential of an organism for use in mosquito control programs has been previously based on applied management considerations (eg. culturing and application procedures), with little or no consideration of ecological theory in general or biological control theory in particular (Service 1983). This has resulted because mosquitoes are predominantly consumed by generalist (polyphagous) predators which were thought to be inappropriate control agents.

Classical biological control theory, developed primarily from predictions of the discrete time Nicholson-Bailey models, emphasises attributes of predator biology that result in low, stable equilibrium prey abundances (Hassell 1978): a) prey specific feeding preferences (monophagous), (b) synchronous development with the prey, (c) capacity to respond numerically to changes in prey density, (d) require few prey to complete its life-cycle, and (e) a high search ability (Beddington *et al.* 1978, Horn 1981). Few predators meet these criteria, which more appropriately describe specialists such as parasitoids.

In re-evaluating the characteristics of predators best suited for biological control, Murdoch and Bence (1987) suggested that a stable prey equilibrium is not the only means to achieve successful control of a prey species. Local extinction of the prey with a polyphagous feeding strategy by the predator will also achieve effective control (Washburn and Cornell 1981, Murdoch *et al.* 1985, Chesson and Murdoch 1986, Murdoch and Bence 1987). A polyphagous predator that is persistent in a habitat, resistant to starvation, and displays a high efficacy for the prey upon reinvasion or density increase, could control the prey by driving populations so low that local extinction occurs (Murdoch *et al.* 1985). Extended control results when the predator persists in the habitat by exploiting alternative prey species, enabling it to switch preference to the target prey upon any subsequent resurgence or re-colonization. For example, the invertebrate predator *Notonecta* (Hemiptera) and the fish *Gambusia affinis affinis* (Baird and Girard), both polyphagous predators have been shown to control mosquitoe larvae in this manner (Murdoch *et al.* 1985, Murdoch and Bence 1987).

Freshwater Turbellaria (Platyhelminthes), in particular members of the order Tricladida, are benthic predators in both lentic and lotic systems (Reynoldson 1966, Young and Reynoldson 1966, Davies 1969, Pickavance 1970, Reynoldson and Davies 1970, Folsom and Clifford 1978, Armitage and Young 1990, Blaustein 1990, Blaustein and Dumont 1990). Although turbellarians, have been proposed as potential mosquito larval control organisms (Legner and Sjorgren 1984, Legner in Chapman

1985, Legner and Medved 1979) there is little detailed research on their interaction with mosquito larvae. Two species, *Dugesia dorotocephala* and *Dugesia tigrina* (hereafter *Dugesia*) have proved to be effective in some field trials (George 1978, George *et al.* 1983, Legner 1985) but failed in others (Service 1983). Investigation of the mechanisms involved in producing success in some sites and failure in others have not been pursued.

A number of characteristics exhibited by *Dugesia* are beneficial in a mosquito control organism. They are polyphagous predators with a high resistance to starvation, and can survive and reproduce during periods when mosquitoes are absent, both requirements for the local extinction hypothesis. *Dugesia* also display attributes outlined in classical biological control theory such as the potential for a rapid numerical response and synchronous development with the mosquito lifecycle (George 1978). In addition, the resistance of *Dugesia* to high concentrations of mosquito insecticides further enhances their potential for integrated pest management (Wrona and Davies unpublished)

To determine whether triclads exert a stabilizing (tendency to approach an equilibrium) or destabilizing (move away from an equilibrium state) force on prey populations, basic predator responses affecting prey population stability (functional, aggregative, developmental and numerical responses) must be investigated. Generalist predators are typically sources of instability for coupled predator-prey systems owing

to an inability to respond in a non-time lagged, density-dependent manner to fluctuations in prey densities. Murdoch and Bence (1987) proposed that, the time delays displayed by freshwater predators in their developmental, and numerical responses relative to those of their prey would be de-stabilizing. Thus, only the functional response could act as a potential source of stabilizing mortality for freshwater predators. Hence, an understanding of the nature of the functional response is required to obtain a better mechanistic understanding of how, or to what extent *Dugesia* is capable of controlling larval mosquito population densities.

This chapter investigates the triclad *Dugesia*-zooplankton and mosquito predator-prey system to assess the potential for *Dugesia* in mosquito control programs. The initial steps involve obtaining a greater understanding of the predation biology of *Dugesia*, the vulnerability of prey species to predation and the nature of the functional response.

Observations on foraging behaviour show *Dugesia* induce prey mortality through direct and indirect means. Direct predator induced mortality occurs when *Dugesia* strikes a prey entangling and subduing it in mucus followed by the penetration of the pharynx and injection of digestive enzymes. In contrast, indirect predator induced mortality results when prey become entrapped in mucus trails and subsequently die through no other action by the triclad (Hyman 1951). Whether prey killed through indirect predator induced mortality are consumed at a later date is

unknown. Thus, there is the potential for non-consumptive mortality or wasteful killing (Johnson *et al.* 1975). A further complication for suctorial predators such as *Dugesia* is the inverse relationship between feeding efficiency and feeding duration (Cook and Cockerel 1978, Wrona and Calow 1988). This can often result in only partial ingestion of the prey. These complicating factors must be considered when determining the amount of energy ingested. The extent to which prey populations are influenced by these forms of predator induced mortality will also vary for behaviourally distinct prey species.

Identifying the mechanisms involved in the *Dugesia*-prey interaction is required prior to incorporation into a predator-prey model. Failure to separate consumptive mortality from non-consumptive mortality when utilizing predator-prey models coupled through the functional response, could result in over-estimation of the predators ingestion rate. Alternatively, excluding non-consumptive mortality could result in under-estimation of induced prey mortality. Thus, this chapter investigates the feeding biology of *Dugesia* on mosquito larvae (*A. aegypti*) and an alternative zooplankton prey (*Daphnia magna* Strauss). The effects of prey type, density and size on total prey mortality were examined. The suitability of simple experiments recording prey mortality as a measure of the functional response of the predator was also investigated. The relative importance of partial prey ingestion and/or non-consumptive prey mortality to total prey mortality was determined in conjunction with identification of the functional response. Experiments were also conducted to

determine whether prey killed through indirect predation were subsequently ingested and should be included as a component of consumptive predation.

4.1 METHODS AND MATERIALS

4.1.1 GENERAL PROCEDURES

Dugesia (10-12 mm in length) were fed *ad libitum* on *Tubifex tubifex* Müller for 1 day followed by a 4 day starvation period prior to the start of the experiments. Four prey classes of two prey species (types) (*Daphnia magna*, hereafter *Daphnia*, and *Aedes aegypti*, hereafter *Aedes*) and two sizes (*Daphnia*, small = 1.7±0.1 mm, large 2.9±0.06 mm; and *Aedes*, small-II instar (2.3±0.07 mm) and large-IV instar (7.0±0.66 mm, $\bar{X}$±SE)) were used. Trials were run for 4 days with individual *Dugesia* in glass bowls containing 175 ml filtered pond water at 20°C with a 12 h:12 h light:dark regime using nine prey densities (3, 6, 9, 12, 24, 48, 60, 72, 84 per bowl).

Prey mortality was determined daily with replacement to maintain initial prey densities. Only dead prey with mucus attached (eg. to antennae of *Daphnia*) were considered as *Dugesia* induced mortality. Controls (predator free trials) were run at three of the nine prey densities (9, 48, and 84 per bowl).

4.1.2 TOTAL PREY MORTALITY

The effect of prey density on total prey mortality was investigated using prey densities ranging from low to high (3, 6, 9, 12, 24, 48, 60, 72 and 84 prey per bowl) each with a minimum of three replicates. The effects of prey density and prey size on total prey mortality were analyzed separately for each prey species utilizing a repeated measures ANOVA on log10 transformed data to correct for heteroscedasity of variance (SAS Institute 1988). All statistical tests were performed at a $P = 0.05$ level of probability.

4.1.3 COMPONENTS OF TOTAL PREY MORTALITY

To define the functional response and quantify the role of partial prey ingestion and non-consumptive prey mortality on total predator induced prey mortality, experiments were performed at four densities (9, 24, 48 and 84 prey per bowl) for *Daphnia* and three prey densities (9, 48 and 84 prey per bowl) for *Aedes*. Due to difficulties in culturing the *Aedes* larvae, the prey density of 24 larvae per bowl was not run. Nine replicate trials were conducted for each treatment combination (i.e., all size and species combinations) under the same environmental conditions outlined above.

Total prey mortality (consumptive plus non-consumptive) was assessed daily.

Discrimination between consumptive and non-consumptive prey mortality was done at 10X magnification with a dissecting microscope. Prey killed by penetration of the pharynx exhibit discolouration around the puncture wound. *Daphnia* exhibiting no movement of the thoracic appendages and *Aedes* displaying no movement of the dorsal vessel and the trachea were classified as dead. Prey retaining at least 66% of body contents were categorized as partial prey ingestion.

The hypothesis that none of the components of prey mortality (consumptive, non-consultive and partial prey ingestion) were affected by density or prey class was tested with a repeated measures, two factor ANOVA on data log10 transformed to correct for heteroscedasity of variances. To determine the relationship between non-consumptive mortality and/or partial prey ingestion to prey density, data on unconsumed prey were analyzed using a one-way ANOVA for each prey class as a proportion (arcsine square root transformation) of prey density. Nonlinear curve fitting (SYSTAT 1990) to Holling's (1959) functional response models was used to estimate the attack coefficient (a′) and handling time (T_h) for each prey class. Variation among prey classes in a′ and T_h was estimated using standard errors calculated from the curve fit data, in a one-way ANOVA following Zar (1984).

4.1.4 INFLUENCE OF DEAD PREY

Two experiments were run to determine the fate of dead prey trapped in mucus. To investigate whether these prey are subsequently ingested by *Dugesia*, experiments providing only dead large *Daphnia* (24 per bowl, killed by freezing) were conducted. To determine if *Dugesia* showed a preference for live or dead prey , a combination of 12 living and 12 dead large *Daphnia* per bowl was provided. Experiments ran for 4 days with ingested prey replaced daily and all prey (dead and live) replaced after 48 h to minimize any potential biases from decomposition.

4.2 RESULTS

As *Dugesia* recently introduced to the experimental bowls showed increased movement and often killed prey without feeding, prey capture data from day 1 were not included in the statistical analyses. Mean daily prey mortality in the predator free controls ranged from 0 to a maximum of 1.1 prey per day for large *Daphnia* at the highest density treatment. None of the other control treatments exceeded a mean daily prey mortality of 0.44 (small *Daphnia*) per day. Thus, no correction factor for background mortality was required (Table 4.0).

Table 4.0. Daily mortality (mean ± SE, n=9) of *Aedes aegypti* and *Daphnia magna* in control treatments (no predators) at densities of 9, 48 and 84 individuals per bowl.

Density	*Aedes aegypti*		*Daphnia magna*	
	Large	Small	Large	Small
9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
48	0.0 ± 0.0	0.2 ± 0.14	0.1 ± 0.03	0.2 ± 0.14
84	0.11± 0.1	0.2 ± 0.14	1.1 ± 0.3	0.4 ± 0.44

4.2.1 TOTAL PREY MORTALITY

Total mortality for both size classes of *Aedes* increased at a decelerating rate to an asymptote with increasing prey density (Fig. 4.0). Mortality for small *Aedes* exceeded that of large larvae and increased with prey density at a greater rate with a significant density by size interaction (density*size, $F_{8,102} = 4.64$, $P < 0.0001$). Repeated measures ANOVA showed a significant day effect with greater mortality occurring on day 2 of the experiment for small *Aedes* at densities equal to and exceeding 48 larvae per bowl (day*size*density, $F_{16,204} = 4.12$, $P < 0.0001$). Based on non-linear curve fitting and examination of residuals and r^2 values, a Holling's type II functional response model proved to be the best and most parsimonious fit for both size classes of *Aedes* (Fig.4.0).

Mortality for both size classes of *Daphnia* was significantly affected by prey density ($F_{8,102} = 79.06$, $P < 0.0001$), with a decelerating rise to a temporary asymptote at intermediate densities, followed by an acceleration of mortality at higher densities (Fig. 4.1). Total mortality was not significantly affected by *Daphnia* size ($F_{1,102} = 0.009$, $P > 0.50$), although the sharp increase in mortality at prey densities of 84 prey per bowl is the likely reason for the significant density*size interaction ($F_{8,102} = 5.38$, $P < 0.0001$). Repeated measures ANOVA showed a significant day*density*size interaction ($F_{16,204} = 5.19$, $P < 0.0001$) arising from increased mortality occurring on days 3 and 4 at the higher prey densities for the large size class of *Daphnia*. None of the Holling's

Figure 4.0. Total prey mortality (± SE) in relation to prey density for II instar (○) and IV instar (●) *Aedes aegypti* exposed to a single *Dugesia tigrina* predator. Lines are non-linear curve fits to Holling's Type II functional response model ($f = a'x/[1+a'xb]$) where x = prey density, a' = attack coefficient and b = handling time (II instar MODEL, $a' = 0.98$, $b=0.06$, $r^2 = .96$, $P<0.05$; IV instar MODEL; $a'=0.6$, $b=0.36$, $r^2 = 0.85$, $P<0.05$).

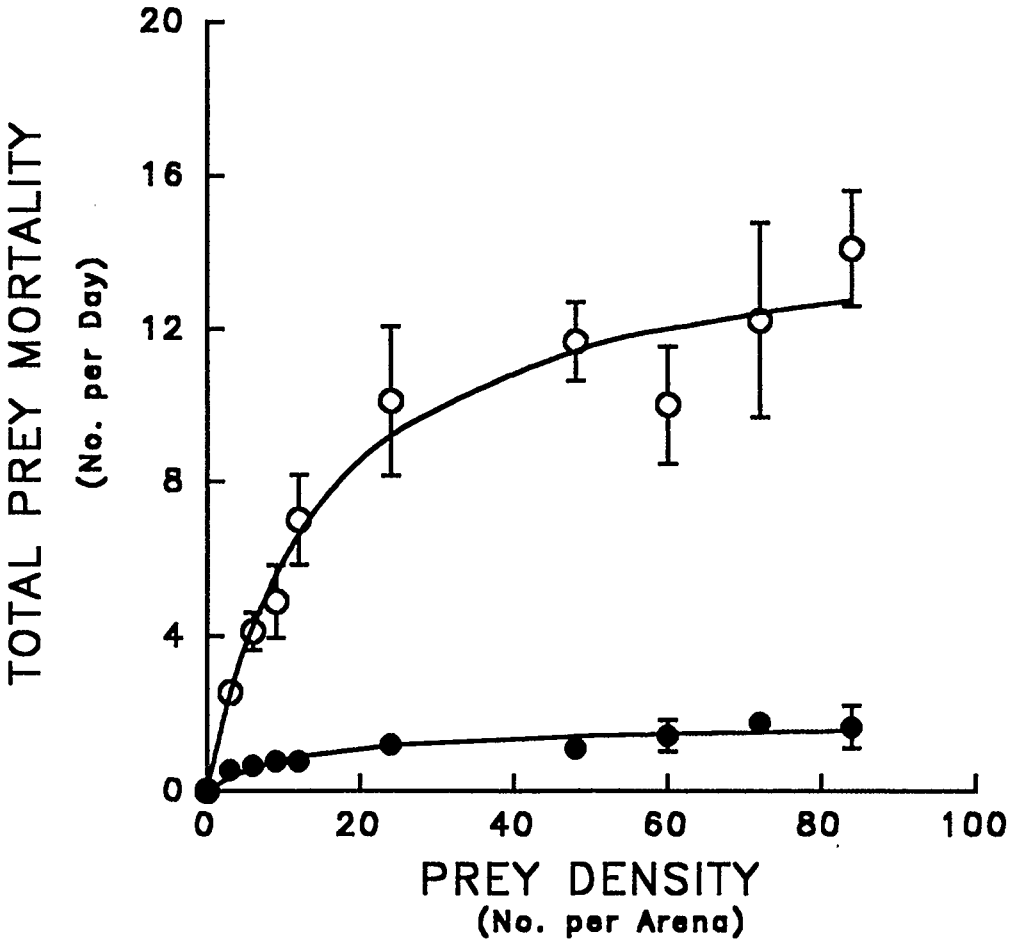
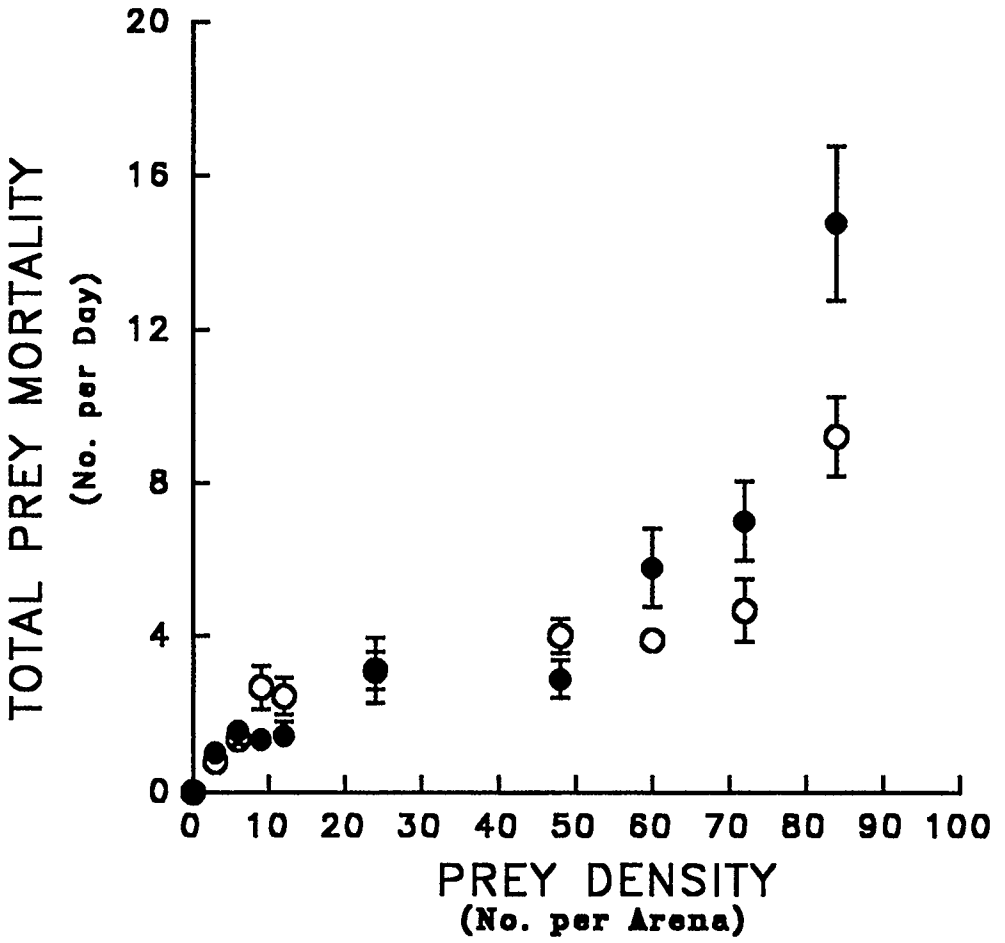

TOTAL PREY MORTALITY
(No. per Day)
20
16
12
8
4
0
0
20
40
60
80
100
PREY DENSITY
(No. per Arena)

Figure 4.1. Total prey mortality (± SE) in relation to prey density for small (○) and large (●) *Daphnia magna* exposed to a single *Dugesia tigrina* predator.


TOTAL PREY MORTALITY
(No. per Day)
20
16
12
8
4
0
0 10 20 30 40 50 60 70 80 90 100
PREY DENSITY
(No. per Arena)

functional response models gave a suitable fit to the *Daphnia* total mortality curves.

4.2.2 COMPONENTS OF TOTAL PREDATION

As non-consumptive mortality of large *Aedes* did not occur, this prey class was omitted from subsequent experiments. Partial prey ingestion was not observed for any of the prey species and therefore did not contribute to total prey mortality.

Consumptive mortality was significantly affected by prey density increasing at a decelerating rate with increasing prey density ($F_{2,78}=34.15$, $P<0.001$). Prey class also significantly affected consumptive mortality ($F_{3,78}=244.01$, $P<0.001$), with the number of prey ingested ranked in descending order from small *Aedes* and *Daphnia* to large *Daphnia* and *Aedes* (Fig. 4.2). Based on r^2 values and residual plots, a Holling's Type II functional response gave the best and most parsimonious fit for consumptive mortality for all prey classes (Fig. 4.2). Attack coefficients (a'), a measure of prey vulnerability (Spitze 1985), were significantly affected by prey class (ANOVA, $F_{3,19}=5.9$, $P<0.0025$) with a Tukey's multiple comparison test identifying two overlapping groups, with only small *Daphnia* and large *Aedes* significantly different (Table 4.1). Handling time (T_h) was also significantly affected by prey class (ANOVA, $F_{3,19}=11.34$, $P<0.0005$). Comparison of treatment means with a Tukey's means test identified three overlapping groups with the shortest handling time for small *Aedes*, intermediate handling times for

Figure 4.2. Total daily prey mortality ($\bar{X} \pm SE$) divided into consumptive (●) and non-consumptive (■) mortality for (A) small *Aedes aegypti*, (B) large *Aedes aegypti*, (C) small *Daphnia magna* and large *Daphnia magna*. Consumptive mortality curves were fitted utilizing Holling's type II functional response model (*Aedes aegypti* small $r^2 = 0.99$, $P < 0.002$; large $r^2 = 0.85$, $P < 0.0005$, *Daphnia magna* small $r^2 = 0.99$, $P < 0.002$; large $r^2 = 0.95$, $P < 0.005$).

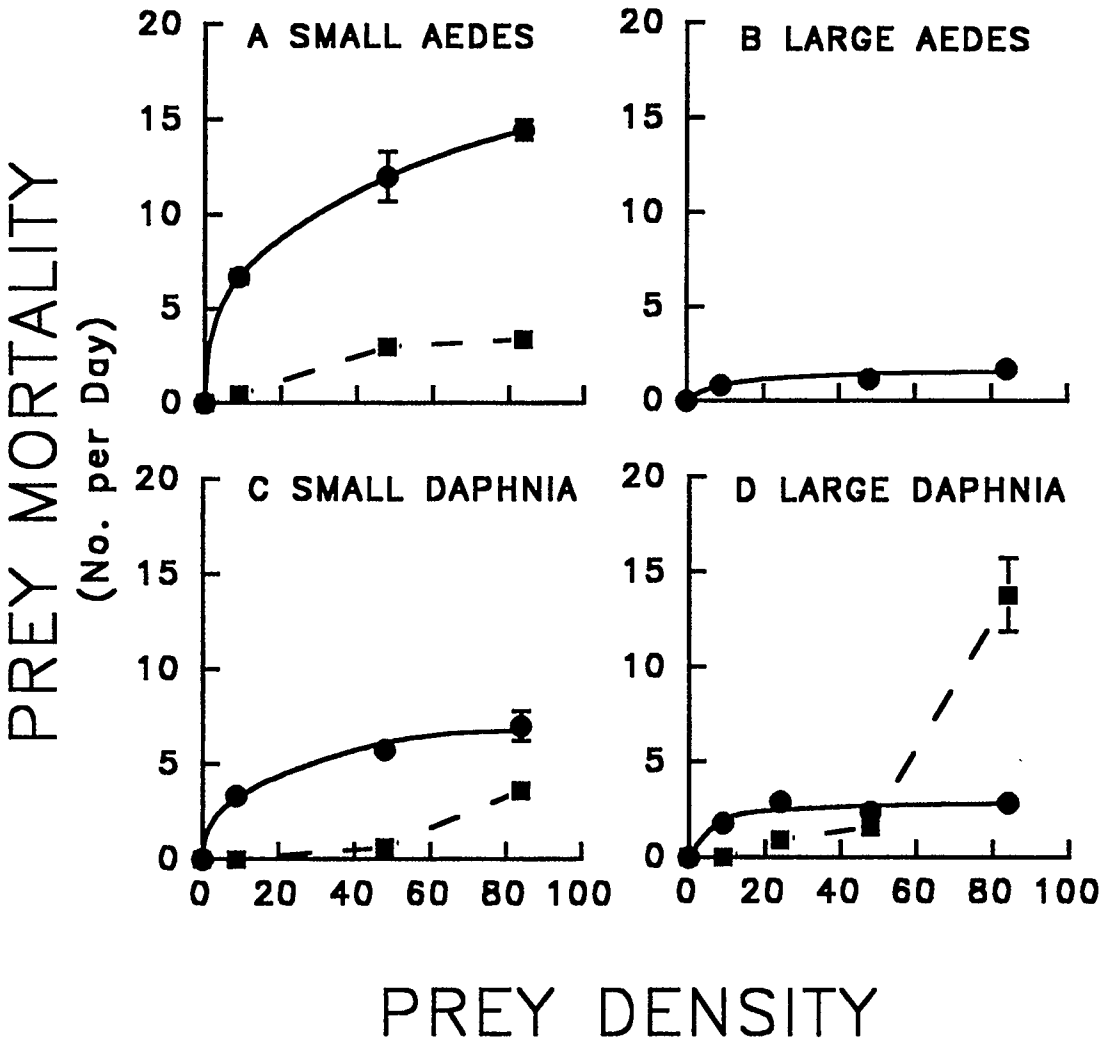

A SMALL AEDES
B LARGE AEDES
C SMALL DAPHNIA
D LARGE DAPHNIA
PREY MORTALITY
(No. per Day)
PREY DENSITY
0
5
10
15
20
0
20
40
60
80
100

Table 4.1. Effect of prey class on mean estimation of attack coefficient (a') (prey/day, ±SE) and handling time (prey/day, ±SE) (T_h) generated from non-linear curve fits of Holling's (1959) type II functional response model (Fitted value ±SE). Letters designate groups whose values are not significantly different (Tukey's multiple comparison test $\alpha = 0.05$).

	a′	Th
Small *Daphnia*	0.40 (0.13)[a]	0.11 (0.16) [ac]
Large *Daphnia*	0.61 (0.45)[ab]	0.36 (0.05) [ab]
Small *Aedes*	1.33 (0.21)[ab]	0.06 (0.003)[c]
Large *Aedes*	0.15 (0.05)[b]	0.47 (0.06) [b]

small and large *Daphnia*, and the largest handling time for large *Aedes* (Table 4.1).

Non-consumptive mortality of *Daphnia* increased with prey density (repeated measures ANOVA, $F_{6,78}=3.37$, $P<0.005$) with large *Daphnia* experiencing the greatest mortality at high prey densities (Fig. 4.2). The non-consumptive mortality curves for both size classes of *Daphnia* were qualitatively similar, although the rate of non-consumptive mortality for large *Daphnia* accelerated more rapidly at the higher prey densities (Fig. 4.2). Repeated measures ANOVA detected a significant interaction between time and prey type (Wilk's Lambda, $F_{6,154}=5.35$, $P>0.0001$). In contrast, small *Aedes* showed qualitative and quantitative differences with non-consumptive mortality increasing at a decelerating rate to an asymptote (Fig. 4.2).

To investigate the relationship between prey density and non-consumptive mortality, non-consumptive mortality was examined as a proportion of prey density. For small *Aedes* this proportion was not significantly affected by prey density (ANOVA on arcsine transformed, $F_{2,78}=2.79$, $P>0.05$), reaching a maximum of 19% at both 48 and 84 prey per bowl. In contrast, the proportion of non-consumptive mortality for *Daphnia* increased significantly with prey density for small (ANOVA on arcsine transformed, $F_{2,78}=63.39$, $P<0.0001$) and large prey (ANOVA on arcsine transformed, $F_{2,78}=202.6$, $P<0.0001$), accounting for up to 33% and 83% of total mortality respectively at high prey densities (Table 4.2). Thus, over the densities tested, non-consumptive mortality is a constant proportion of total prey density for *Aedes* and an increasing proportion for

Table 4.2. Effect of increasing prey density on the percent contribution of consumptive (C) and non-consumptive (NC) mortality to total *Dugesia* induced prey mortality. Non-consumptive mortality also shown as a proportion (Prop) of prey density (* = P < 0.0001).

Density	Small *Aedes*			Small *Daphnia*			Large *Aedes*		
	C	NC	Prop	C *	NC	Prop	C *	NC	Prop
9	95	5	0.05	100	0	--	100	0	--
24	--	--	--	--	--	--	76	24	0.04
48	81	19	0.06	91	9	0.01	60	40	0.03
84	81	19	0.04	66	33	0.04	17	83	0.16

both size classes of *Daphnia* (Table 4.2).

4.2.3 INFLUENCE OF DEAD PREY

With only dead *Daphnia* available, mean daily ingestion by starved *Dugesia* was low (0.22 ± 0.22 prey per day) but when given a choice of living or dead prey daily ingestion of dead prey decreased to 0.11 ± 0.33 per day with 2.4 ± 0.24 live *Daphnia* ingested per day. Thus, *Dugesia* shows a strong preference for living prey (Manly's alpha coefficient of preference (Krebs 1989), $\alpha = 0.96$ for living and 0.04 for dead prey).

4.3 DISCUSSION

The predation rate of *Dugesia* was significantly different between the two prey species. While total mortality for both size classes of *Aedes* followed a Holling's Type II functional response, with mortality increasing at a decelerating rate to an asymptote at higher prey densities (Fig. 4.0), total mortality of *Daphnia* increased at a decelerating rate to a temporary asymptote at intermediate prey densities, followed by an accelerating mortality rate at higher prey densities (Fig. 4.1). Both size classes of *Aedes* displayed qualitatively similar predator induced mortality curves but small *Aedes* mortality asymptotes at a much higher daily mortality rate than for larger *Aedes*. This was not the cases for *Daphnia*, where mortality per day was not significantly affected by prey body size

The distinctly different total prey mortality curves for the two prey species suggests that the behaviourally different prey types differ in their vulnerability to predation by *Dugesia*. The failure of the *Daphnia* total prey mortality curves to fit the traditional Holling's functional response curves suggests an additional mechanism is operating in the *Dugesia-Daphnia* interaction not included in the Holling's equations. Two mechanisms may partly account for this: partial prey ingestion and/or non-consumptive prey mortality.

Cook and Cockrell (1978) developed a model for a notonectid (Hemiptera)

predator with prey mortality curves qualitatively similar to those obtained for *Daphnia*. Increased partial prey ingestion at high prey densities by the notonectid shifted the prey mortality curve from a decelerating slope to an accelerating slope. Partial prey ingestion may occur when predators exposed to high prey densities feed only on the most easily extracted portions (optimization model), or when minimal search times due to high prey densities allow the animal to repeatedly top up its gut with many small meals from a number of different prey individuals (gut-filling model) (Cook and Cockrell 1978, Calow 1981, Wrona and Calow 1988). Both behaviours have the effect of producing a relative increase in prey mortality at high prey densities. Similar prey mortality curves, with partial prey ingestion invoked as the causal mechanism, have been obtained empirically for damselfly naiads (Johnson *et al.* 1975) and two species of predatory mites (Sandness and McMurtry 1970).

Alternatively, the increase in mortality of *Daphnia* at high prey densities could result from non-consumptive prey mortality. Scrimgeour *et al.* (1991), working on a mayfly-diatom system reported predator induced algal depletion rates that were qualitatively similar to the *Daphnia* curves (Fig. 4.1). Diatom depletion rates were a result of mayfly ingestion (consumptive mortality), best described by a Type II functional response, combined with a function describing non-consumptive losses produced by the dislodging of diatoms by the feeding activity of the mayfly. Scrimgeour *et al.* (1991) produced models combining a type II functional response (consumptive losses) with grazing induced non-consumptive diatom losses that were either an increasing or a

constant proportion of prey density. The model incorporating non-consumptive diatom mortality as an increasing proportion of total prey density produced curves similar to the *Daphnia* total mortality experiments. Incorporation of non-consumptive mortality as a constant proportion of total prey density produces curves qualitatively similar to the *Aedes* total prey mortality results.

Discriminating between the functional response and total predator induced prey mortality is necessary to model *Dugesia-Daphnia* or *Dugesia-Aedes* interactions. Coupling of predator and prey biology is usually through the functional response and is based on the assumption that all predator induced prey mortality is consumptive. Failing to include significant non-consumptive mortality would result in an under-estimation of prey mortality, while utilizing total prey mortality would over estimate the predators' rate of energy intake. Thus, whether non-consumptive mortality should be included in one or both of the predator-prey equations must be determined. Hence, the functional response and the role partial prey ingestion and/or non-consumptive prey mortality play in total predator induced mortality must be identified.

Consumptive mortality of *Aedes* and *Daphnia* by *Dugesia* follows a Holling's Type II functional response irrespective of prey size and species. Morphological and behavioural differences among prey species and sizes account for the significant effects of both prey type and density on the functional response. Observations showed that both size classes of *Aedes* grazed on mucus trails and on the triclads. Thus, *Aedes* larvae

frequently encounter mucus, but, in contrast to *Daphnia*, are usually able to break free and remove mucus from their bodies by grooming. It is postulated that the discrepancy in vulnerability (a′) and daily predator induced mortality between *Aedes* size classes is due to the greater success exhibited by the physically stronger large *Aedes* at breaking mucus entanglement and grooming off larger amounts of mucus. A similar relationship was found for *Aedes vexans* exposed to predatory *Chaoborus* larvae, where vulnerability was inversely related to *A. vexans* size class, despite greater encounter rates between the larger instars and the predator (Helgen 1989).

The greater variation in handling times (T_h) rather than attack coefficients (a′) explains for the lower asymptote in the functional response experienced by the large size classes of both prey species. For predators feeding on large prey items the limiting factor on consumption is often the rate of digestion of the prey (Spitze 1985, Moore 1988). In comparison, smaller prey have lower handling times (more rapidly digested), resulting in additional time available for searching and attacking new moving or entrapped prey, thereby increasing the number of prey ingested per unit time.

Non-consumptive mortality accounts for 0 - 83% of total *Dugesia* induced prey mortality, with large *Aedes* the least vulnerable to non-consumptive mortality and large *Daphnia* the most vulnerable. Total prey mortality curves are a combination of consumptive and non-consumptive predator induced mortality. Non-consumptive mortality clearly accounts for the qualitative nature of the *Daphnia* total mortality curves

and to a lesser degree to the total mortality curves for small *Aedes*. Increases in non-consumptive mortality throughout the course of the experiment (i.e. significant time effect) are the result of the time-delay involved in death after mucus entrapment. The length of time between entrapment and death is shorter for *Aedes* due to their need for atmospheric oxygen. For *Daphnia*, non-consumptive mortality increases more slowly with time as individuals trapped at the beginning of the experiment die much later. To equate total prey mortality to the functional response, as assumed in coupled predator-prey models, mucus trapped dead prey must be eventually ingested if they are to ultimately contribute directly or indirectly to the predators reproductive output. The daily removal of dead prey trapped in mucus may prevent *Dugesia* from scavenging and incorporating a proportion of the indirect predation into consumptive mortality. However, the extremely low ingestion rate of dead prey by starved *Dugesia* combined with the high coefficient of preference ($\propto$) for live prey in the preference experiment suggests dead prey are not a major dietary component for *Dugesia*. This evidence for non-consumptive mortality joins only a few other cases such as the belostomatid *Lethocerus sp.* feeding on tadpoles (Hollings 1961) and damselfly naiads feeding on *Daphnia* (Johnson *et al.* 1975).

Understanding the relationship between non-consumptive mortality and total prey density can only result from close examination of the behaviour of both predator and prey. The combination of a Type II functional response with the appropriate non-consumptive mortality for each prey class (fixed proportion of prey density: *Aedes* and

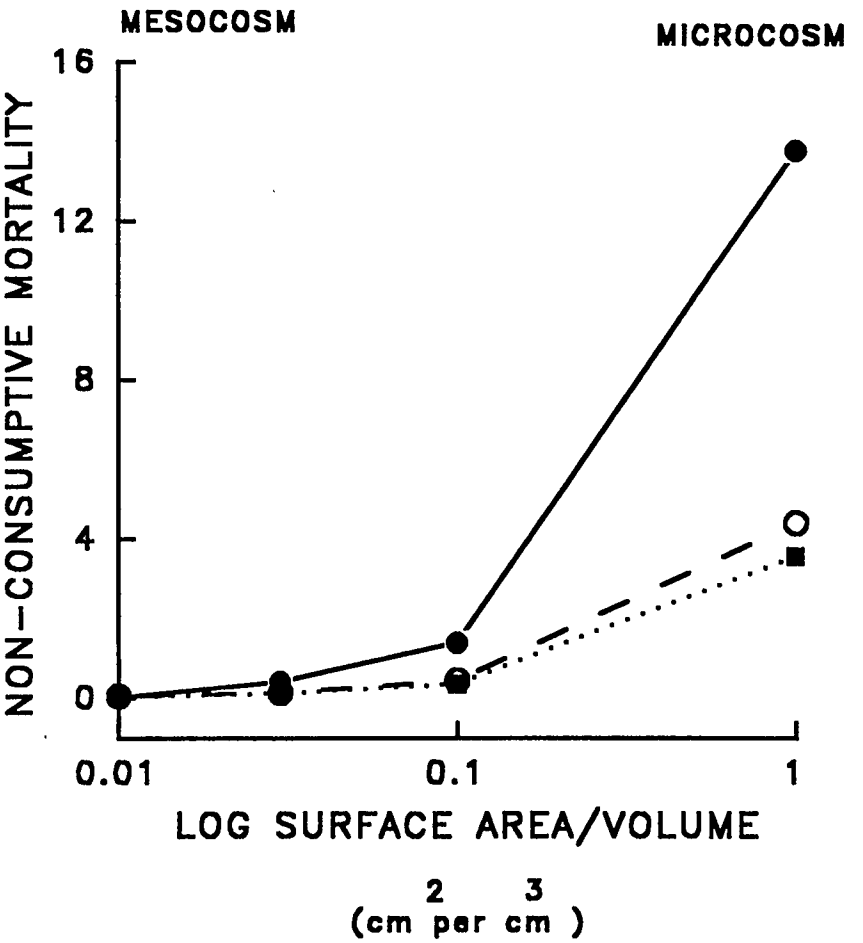
increasing proportion of prey density: *Daphnia*), closely approximate the results obtained from the total prey mortality experiments. *Aedes* seem to be at greatest risk of mucus entrapment when actively struck or contacting a *Dugesia* subduing or feeding on another prey. The lower ability of small *Aedes* to break free from mucus together with their lower grooming efficiency places them at greater risk of entanglement than large *Aedes*. Thus, *Aedes* may be vulnerable only when *Dugesia* are actively foraging, while *Daphnia* are vulnerable at all times in these microcosms due to their susceptibility to mucus trails. Higher movement rates (Thompson 1978) and the greater relative crowding effects experienced by large *Daphnia* will increase their probability of encountering a mucus trail in comparison to small *Daphnia*. Smaller individuals were also observed to retain more mobility when mucus-hampered than the large-size class. In addition, the longer digestive period of *Dugesia* feeding on large *Daphnia*, means fewer *Daphnia* trapped in mucus elicit attacks which may result in a density-dependent increase in non-consumptive mortality. Hence, non-consumptive mortality of small *Aedes* is dependent on the predator's feeding cycle, while *Daphnia* vulnerability is independent of the feeding cycle but dependent on *Daphnia* size.

Predatory behaviour measured at the individual level may not accurately reflect interactions when habitat scale is increased. The current interest in the modelling of population and community-level dynamics based on the biology of individuals (Gurney *et al.* 1983; Metz and Diekmann 1986; Caswell 1989; Nisbet *et al.* 1989), raises questions on how predator-prey interactions scale at different levels? In individual based

models, variables are usually empirically estimated under controlled laboratory microcosm conditions. Results from these microcosm or mesocosm studies are often applied directly to the larger scales of natural systems. Consequently, a fundamental understanding of how ecological processes scale with increasing habitat size and complexity is necessary before results can be generalized from micro- and meso-cosm experiments to natural systems (Addicott *et al.* 1987). Thus, it must be determined whether non-consumptive mortality is an experimental artifact resulting from the microcosm nature of the study.

Predicting the significance of non-consumptive mortality with increasing habitat scale is difficult. The ecological neighbourhood (Addicott *et al.* 1987) at which *Dugesia*-prey interact may be considered a function of the surface area to volume ratio of the habitat. This provides a relative measure of potential habitat overlap of the benthic forager *Dugesia* with zooplankton prey. Extrapolation of non-consumptive mortality obtained under high densities in the microcosms to the surface area:volume ratios for larger experimental mesocosms provides predictions on the effect of scaling on non-consumptive mortality (Fig. 4.3). Assuming non-consumptive mortality scales linearly with surface area to volume ratios, these results indicate a rapid decrease in non-consumptive mortality should occur with increasing habitat scale, theoretically declining to zero in larger mesocosms ($0.01 cm^2 * cm^{-3}$). Increasing scale from bowls ($1.0 cm^2 * cm^{-3}$) to small aquaria ($0.1 cm^2 * cm^{-3}$) results in a predicted decline in non-consumptive mortality for large *Daphnia* from approximately 14 prey per day to one prey per day.

Figure 4.3. Predicted non-consumptive prey mortality (No./day) for small *Aedes aegypti* (■), and small (○) and large *Daphnia magna* (●) at different habitat scales based on surface area:volume ratios. Estimates were determined utilizing non-consumptive mortality obtained at prey densities of 84 individuals/bowl and extrapolating the corresponding mortality to the new habitat surface area to volume ratio.


MESOCOSM
MICROCOSM
16
12
8
4
0
NON-CONSUMPTIVE MORTALITY
0.01
0.1
1
LOG SURFACE AREA/VOLUME
(cm 2 per cm 3)

Augmentation, of the experimental system with additional realism by increasing habitat scale, the addition of complexity to the habitat (eg. increased surface area provided by macrophytes), and group foraging by *Dugesia* (Cash *et al.* 1993) will doubtless alter the significance of non-consumptive mortality to the predator-prey interactions.

At the microcosm scale, total prey mortality is a combination of non-consumptive prey mortality and a Type II functional response. Thus, the models proposed by Scrimgeour *et al.* (1991) for a mobile predator (grazer)-sessile prey (algae) system, are applicable to this distinctly different predator-prey interaction. The significance of non-consumptive mortality to overall prey mortality was mediated by both behavioural and morphological prey characteristics such that it is both prey species and size specific. Correlating non-consumptive mortality with a relative measure of overlapping habitats (ratio of the surface area/volume), results in predictions that non-consumptive prey mortality would play a significant role only under microcosm conditions. In these microcosms non-consumptive mortality need only be addressed as a component of prey dynamics and does not contribute to the predators reproductive output. Further experiments are required to test the predictions on scaling effects and to determine the impact additional vertical complexity representative of macrophytes would have on non-consumptive mortality. Regardless of the outcome of these experiments, the effect of spatial scale on parameter estimates needs to be considered when applying values obtained at one spatial scale to another.

4.4 IMPLICATIONS FOR BIOLOGICAL CONTROL

Murdoch and Bence (1987) argue that most freshwater predators exhibit time-delays in their developmental and numerical responses in relation to their prey, therefore, only the functional response has the potential to be a stabilizing force in a predator-prey interaction. *Dugesia* exhibit a Type II functional response when feeding on *Aedes* or *Daphnia*. This means the functional response is a destabilizing force in this predator-prey interaction. *Dugesia* is a freshwater predator capable of a increasing in density with with the prey population when reproducing by fissioning. However, this is not a stabilizing numerical response owing to the time delay that *Dugesia*'s resistance to starvation imposes on the corresponding decrease in density when prey decline. Thus, *Dugesia* predation on mosquito larvae, like *Notonecta* and *Gambusia* (Murdoch *et al.* 1985, and Murdoch and Bence 1987) would likely be destabilizing tending to drive populations to extinction in simple communities where there are a few alternative prey choices. *Dugesia* could be an important component in an IPM program but would likely only be effective as the sole controlling agent in very simple communities where alternative prey choices are limited. The effect of alternative prey on *Dugesia* prey choice and the *Dugesia*-mosquito larvae functional response need further investigation.

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Appendix I. Standard dry larval food mixture. Ingredients combined and ground to a fine powder using a mortar and pestle. Powder was kept cool and discarded after one week.

Alfalfa	2.5 g
Wheat Germ	2.5 g
Tetramin	5.0 g
Yeast Extract	2.5 g
Lecithin	0.5 g
Cholesterol	0.5 g
Wesson Salt Mix	0.5 g
Insect Vitamin Mix	0.5 g

Appendix II. Artificial pond water. Ingredients should be dissolved in distilled water prior to mixing and mixed in the order listed. This list is for 50 litres of distilled water.

$Ca(NO_3)_2 \bullet 4H_2O$	12.5 g
$NaHCO_3$	12.5 g
$MgSO_4 \bullet 7H_2O$	6.0 g
KCl	2.5 g