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THE IMPACT OF SELECTIVE OVIPOSITION, EGG HATCHABILITY, FOOD AVAILABILITY AND INFECTION WITH *PLAGIORCHIS ELEGANS* ON THE PRE-IMAGO POPULATION DYNAMICS OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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ABSTRACT

Cercariae of the digenean *Plagiorchis elegans* cause high levels of morbidity and mortality among larvae of *Aedes aegypti*. The impact of this parasite on the larval population dynamics of the experimental host as mediated through intraspecific competition, egg hatchability and ovipositional preference was assessed by calculating the probability of pre-imagos to develop from one stage to the next, or to die within consecutive 24 h intervals. Attractiveness of the water to ovipositing females in a dynamic larval population was not affected by exposure to the parasite, but varied significantly over time, regardless of food abundance. In optimally fed populations, these changes were positively correlated with pupal production, but were not affected by early instar development. Most of the entomopathogenic effects of the parasite were expressed in the pupal stage. Thus, exposure to the parasite significantly reduced adult emergence, but did not greatly impair pre-imago development. Nonetheless, exposure to various levels of the parasite significantly increased mortality of all larval stages. Suboptimally fed larval populations displayed severely impaired development and produced few adults. Exposure to *P. elegans* increased adult production slightly, suggesting depensatory mortality. In nutritionally stressed populations, no correlation was found between biomass and ovipositional preference, but attractiveness of the water was significantly increased by the removal of individuals by pupation or mortality. Egg hatchability was not significantly affected by population structure, but varied with the nutrient content of the water. This study provides new insight into the use of parasites as agents in the biological control of mosquitoes.

RÉSUMÉ

Le digénien *Plagiorchis elegans*, à l'état de cercaire, entraîne de sévère taux de mortalité et de morbidité chez les larves de *Aedes aegypti*. L'impact du parasite sur la dynamique des populations larvaires de cet hôte experimental, qui se fait à travers la compétition intraspécifique, le taux d'éclosion des oeufs et la préférence d'oviposition, a été évalué par calcul de la probabilité de mortalité ou de passage des immatures d'un stade à un autre, pour chaque interval de 24 heures. L'attraction des femelles gravides vers l'eau contenant les population de larves n'a pas été affectée par l'exposition des larves au parasite, mais a significativement varié dans le temps, quelque-soit la disponibilité en nourriture. Pour des populations nourries de façon optimale, ces changements furent positivement corrélés avec la production de pupes, mais n'ont pas été affectés par le développement des stades précédents. La plupart des effets entomopathogéniques ont été observés durant le stade pupal. En effet, l'exposition au parasite a significativement réduit l'émergence d'adultes, mais n'a pas eu d'effet considérable sur le développement pré-imaginaire. Néanmoins, divers taux d'exposition au parasite ont significativement augmenté la mortalité à tous les stades larvaires. Nourries de façon sub-optimale, les populations larvaires ont subi de sévères changements développementaux, et ont produit peu d'adultes. L'exposition à *P. elegans* a légèrement augmenté la production d'adultes, suggérant une mortalité dépensatoire Dans le cas de populations privées de nourriture, aucune corrélation n'a été observée entre la biomasse et la préférence d'oviposition, mais l'attraction de l'eau pour les femelles a significativement augmenté avec le retrait d'individus suivant la pupation ou la mort de ceux-ci. L'éclosion des oeufs n'a pas été significativement affectée par la structure de population, mais a varié avec le contenu de l'eau en elements nutritifs. Cette étudeprocure de nouvelles informations pertinentes à l'emploi de parasites comme agent de biocontrôle contre les moustiques.

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PREFACE

Chapter 1 provides an introduction to the practical and theoretical background of the subjects addressed in this thesis. It reviews the literature dealing with the biology and population dynamics of *Ae. aegypti*, and the behaviours associated with oviposition and egg hatchability. In addition, information on the life cycle of *P. elegans* and the impact of the parasite on *Ae. aegypti* are summarized.

In **Chapter 2** the interaction between the population dynamics of *Ae. aegypti*, the attractiveness of larval holding waters and infection with *P. elegans* are assessed in a dynamic larval population under conditions of optimal food availability.

In **Chapter 3** the interaction between the population dynamics of *Ae. aegypti*, the attractiveness of larval holding waters and infection with *P. elegans* are assessed in a dynamic larval population, under conditions of suboptimal food availability.

Chapter 4 describes how population dynamics under two different levels of food availability affects the hatchability of *Ae. aegypti* eggs in a dynamic larval population.

Chapter 5 is a general summary and discussion.

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In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement as to who contributed to such work and to what extent. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all the authors of the co-authored papers.

In this context, Professors Rau and Lewis have provided supervision in all aspects of my research and appear as co-authors in all publications resulting from this work.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Mosquitoes are vectors of some of the world's most devastating diseases. The control of these diseases has proven difficult in the past, due to problems such as drug and pesticide resistance, as well as difficulties in the development of vaccines. In addition, urbanization, changes in water resource management, increased conflict and reduced health services have led to a resurgence of vector-borne diseases (Molyneux, 1997). Vector control is an integral part of any disease management program. Accordingly, in the past, much effort has been invested in developing mosquito control methods.

Early methods of mosquito control consisted mainly of the introduction and enhancement of natural predators, such as larvivorous fish, and the application of plant oils (Mulla, 1994). However, since the discovery of DDT in the 1930s, mosquito control has focused primarily on the use of chemicals (Service, 1983; Laird, 1981). Today, problems such as pesticide resistance, increased costs of pesticide production, and rising environmental concerns (Sharma, 1989) have led to a renewed interest in biological control.

Effective use of biological control agents requires a thorough understanding of the ecology of the pest species, the biocontrol agent, and their interaction within the environment (Larkin *et al.* 1995). Controlling insect vectors differs considerably from controlling agricultural pests. Crop pests usually occupy uniform, stable environments, as characterized by monocultures, whereas mosquitoes often inhabit variable, complex and unstable habitats (Yap, 1985). This is especially true of container breeders, which are common in temporary habitats and are frequently exposed to flooding and drying cycles

(Laird, 1988). The establishment of predators or pathogens in such habitats is considerably more difficult (Service, 1983). Furthermore, damage by agricultural pests is usually associated with the larval stage, whereas disease vectors are harmful as adults. Since biological controls are generally aimed at larval stages (Yap, 1985), this implies that larval populations must be controlled to reduce adult numbers as much as possible, irrespective of larval numbers. In addition, mosquitoes have high fecundity, short generation times, high dispersal potential and are efficient colonizers. This further complicates the introduction of natural enemies, although it has little effect on chemical control. Finally, whereas agricultural pests can be kept below an economic threshold, transmission of disease is usually reduced only when extremely low mosquito numbers are attained (Service, 1983, 1995; Washburn, 1995).

In order to persist in the target population, pathogens must generally recycle. However, if pathogenicity is too high, the resulting scarcity of suitable hosts may drive the pathogen to extinction. This leads to attenuation (Anderson, 1976) and reduces the control potential of the organism. Attenuation may render the use of many such recycling organisms unsuitable for the biological control of vectors. As a result, there has been an increased interest in the use of biological insecticides such as *Bacillus thuringiensis israelensis*. These agents may maintain a high virulence, as they do not recycle through the target population. However, they generally require inundative, sequential applications, which are expensive and logistically difficult.

The effect of natural enemies on the pest population mortality may be modified by environment factors and takes one of several forms. If the numbers of the target population are reduced, as is the goal of biocontrol, mortality is considered to be additive.

If the control agent has no effect on the size of the pest population because it is killing individuals that would otherwise have been eliminated by other factors, this is considered to be compensatory mortality. In some cases, the introduction of natural enemies may actually lead to an increase in pest numbers; this is considered to be depensatory mortality (Washburn, 1995; Vanni, 1987).

Aedes aegypti breeds in natural and artificial containers (Christophers, 1960). It is believed that populations are controlled by intraspecific competition between immature stages (Southwood *et al*, 1972; Gilpin and McLelland, 1979). Container breeders are primarily limited by the availability of resources (Carpenter, 1983; Washburn, 1995), and developmental times seem to be directly linked to this (Frogner, 1980). Competitive status of the larvae is determined by size. Thus later instars demonstrate density independent mortality, whereas in early instars mortality is density dependent (Southwood *et al*, 1972). Older cohorts may further benefit from the presence of young larvae (Livdahl, 1983), as these may serve as an alternate food source (Koenekoop and Livdahl 1986). Consequently, in resource-poor environments, increased mortality of older larvae, induced by a single pulse control effort, may actually yield a larger number of adults, due to the reduction of competition leading to increased survival in the early larval stages (Frogner, 1980). There will be accelerated larval development, as well as increased size and fitness of the resulting adults (Renshaw *et al*, 1994). Larger females are longer-lived, are more successful at obtaining blood-meals and thus have greater vector potential (Nasci, 1986 a,b). The above considerations have important implications for biological control in that the larvicidal activity of biological agents aimed at later instars may fail to improve or may, in fact, aggravate the situation. Agudelo-Silva and

Spielman (1984) and Cermack (1998) have demonstrated this phenomenon in the laboratory.

Aspects of the biology of entomopathogenic digenean parasites, such as *Plagiorchis elegans* may help to avoid the problem of depensatory mortality. These parasites have complex life cycles involving a series of vertebrate and invertebrate hosts. The entomopathogenic larval stages (cercariae) of the parasite are produced asexually in the tissues of an aquatic first intermediate snail host (Blankespoor, 1973). Cercariae will penetrate and kill pre-imagos of *Ae. aegypti* (Dempster *et al*, 1986), particularly late larval instars (Dempster and Rau, 1991). Although the death of such instars may reduce competition between larval instars and enhance their development, the sustained nature of cercarial release may ensure that the larvae are killed as they develop through their later instars. The use of entomopathogenic cercariae in mosquito control was first proposed by Rao *et al* (1985) who examined the effect of *Prosthogonimus* sp. cercariae on *Culex quinquefasciatus* larvae.

Understanding the population dynamics of a pest species and its control agent is essential when evaluating the efficacy of biological control systems. Larval recruitment is an important part of the population dynamics of *Ae. aegypti*, and is affected by both ovipositional preferences and hatchability of the eggs. Gravid *Ae. aegypti* are known to oviposit preferentially into waters containing low numbers of conspecific larvae (Maire, 1984; Bentley *et al*, 1976, Allan and Kline, 1998). When larvae are stressed, due to overcrowding and/or starvation, such attraction is reduced (Ikeshoi and Mulla, 1970a). Lowenberger and Rau (1993) subsequently found that larvae infected with *P. elegans* produce a strong oviposition repellent. Zahiri and Rau (1998) elaborated on these

findings and reported that the production of such repellents may counteract ovipositional attraction.

Aedes eggs hatch in response to a reduction in oxygen tension, which usually follows a build-up of microorganisms in the environment (Gjullin *et al.* 1941; Judson, 1960). At high larval densities, feeding activity may remove these bacteria from the surface of the eggs, keeping oxygen levels relatively high, and reducing the hatching stimulus (Edgerly & Marvier, 1992). Thus, hatchability may be affected by larval density (Livdahl & Edgerly, 1987).

In the past, studies concerning the effect of *P. elegans* on the population structure of pre-imago *Ae. aegypti* have been conducted under conditions of constant recruitment of first instars into the population (Hartley, 1996; Nguyen , 1998; Cermack, 1998). However, factors affecting oviposition and hatchability may have a major impact on the recruitment of new individuals into natural populations. The objectives of this study were to examine the structure of *Ae. aegypti* pre-imago populations under conditions of recruitment that vary with oviposition attractancy/repellency or egg hatchability, as determined by the intensity of larval browsing, larval respiration and food availability.

It is hypothesized that both ovipositional behaviour and egg hatchability will be influenced by feedback mechanisms from the population itself. Though initially larvae in the water may attract ovipositing females, factors such as crowding, starvation and parasitic infection, may eventually render waters repellent, as the population develops. Egg hatchability is enhanced through respiration of larvae, yet their feeding activity may, in turn, decrease the hatching stimulus by removing respiring microorganisms. Although recruitment into the population may not necessarily decrease in parasitized populations,

the number of adults produced will be greatly reduced, due to high mortality of late instars. This study hoped to provide further insight into the mode of action of entomopathogenic digeneans in the biological control of mosquitoes.

LITERATURE REVIEW

Biology of *Aedes aegypti*

Aedes aegypti is the principal Yellow Fever vector, and may also transmit Dengue and Dengue Hemmorrhagic Fever (Harwood and James, 1979). Dengue is currently the second most important tropical disease globally, and Yellow Fever is expanding northward into the United States, underscoring the importance of monitoring the numbers of its vector (Gubler, 1998). *Aedes aegypti* is widely distributed throughout the tropics and subtropics, mainly in and around human habitation. It is also a species that is extremely easy to rear in the laboratory (Kirkwood, 1961). As a result, the biology and ecology of this species have been well studied.

Aedes aegypti, which is believed to have bred originally in natural containers such as tree holes (Laird, 1988), now breeds mainly in artificial containers. Like all Diptera, *Ae. aegypti* is holometabolous. Its life cycle consists of the egg, four larval instars, the pupa and the imago. Larvae feed on microorganisms, particulate organic matter, and detritus (Clements, 1992). Feeding mechanisms are primarily browsing (termed collecting-gathering by Clements, 1992) and some filter-feeding (Christophers, 1960).

Larval development usually requires 5 - 7 days. The insect then pupates, and the adult emerges approximately two days later (Shannon and Putnam, 1934). Larval development is influenced by a number of factors, the most important being temperature, the degree of crowding and food availability (Christophers, 1960; Fay, 1964). The Optimal temperature is approximately 28°C. At significantly higher temperatures there is a decrease in developmental time, but a reduction in the size of the imago. Larvae of *Ae. aegypti* do not usually develop at temperatures below 16°C and above 34°C

(Christophers, 1960; Headlee, 1940, 1942). Development is favoured by mildly variable temperatures (Fay, 1964).

Overcrowding and low food availability also seem to have adverse effects on the survival and development of larvae (Wada, 1965; Christophers, 1960; Barbosa *et al*, 1972), as does an overabundance of food (Lewis, 1933). Up to a density of 100 larvae/l larvae pupate within 5-7 days and mortality is 5%. A larval density of 400/l, 1000/l and 4000/l results in an extension of the developmental periods to 5-10, 7-16 and 10-60 days, and mortalities of 15%, 21% and 48% respectively (Fay, 1964). This may be due to decreased food availability (Carpenter, 1983; Fish and Carpenter, 1982), mechanical interference (Shannon and Putnam, 1934; Dye 1984) or chemical interference (Ikeshoji and Mulla, 1970a; Moore and Fisher, 1969; Moore and Whitacre, 1972). However, Dye (1981, 1984), Carpenter (1983) and Broberg and Bradshaw (1995) have questioned the existence of chemical interference in the form of a growth retardant. Under conditions of starvation, older larvae may cannibalize earlier instars (Koenekoop and Lidvahl, 1986). As well, the carcasses of larvae killed by the parasite may also provide an additional source of food for survivors (Cermack, 1998).

Larvae of *Ae. aegypti* are negatively phototactic (Omardeen, 1957) and fairly active. Their vertical distribution in the water column is usually divided between the bottom, where foraging occurs, and the surface, which allows respiration (Gilchrist, 1994). As food availability decreases, larvae spend progressively more time foraging at the bottom (Paul, 1994). When alarmed at the surface, larvae usually react by diving (Mellanby, 1958).

The pupa is a non-feeding stage, which is anatomically and behaviorally distinct from the larva. Pupae generally rest at the water surface unless disturbed (Shuey *et al*, 1987). Emergence of adult *Ae. aegypti* is arrhythmic (Haddow *et al*, 1959), and takes approximately 15 minutes (Clements, 1992).

Males and females feed on sugars, such as plant nectar, throughout their lives. However, in order for oogenesis to occur, females must have a blood (Christophers, 1960). Females usually do not blood-feed for the first two days after emergence (Bowen and Davis, 1989; Davis, 1984;). They are attracted to their hosts by physical and chemical cues such as heat, CO_2, lactic acid, and volatile fatty acids, as well as visual cues such as colour and size (Klowden, 1995; Sutcliffe, 1987). Once blood fed, host-seeking behaviour is usually inhibited by factors such as abdominal distension due to feeding (Klowden and Leah, 1978), and the release of a humoral factor produced by the fat body (Klowden and Leah, 1979). Mating may occur before or after blood feeding. Males swarm around hosts in search of females. Females are characteristically monogamous, which is induced by a product of the male accessory gland (Klowden, 1995). This product may also inhibit further host-seeking behavior (Fernandez and Klowden, 1995). Size and nutritional status of the female greatly influences size and the number of bloodmeals taken (Klowden *et al*, 1988, Klowden, 1986; Xue *et al*, 1995; Nasci, 1986b). Oocyte development may be stimulated by products of the male accessory gland (Klowden and Chambers, 1991). Naksathit and Scott (1998) found that sugar-starved females showed increased fecundity and survivorship. Egg laying capacity also seems to be influenced by genetics (Gillett, 1956). Steinwascher (1984) found that large

females of *Ae. aegypti* produce larger eggs, which in turn tend to develop into large females creating a positive feedback cycle.

Oviposition

Selection of an oviposition site is an important factor affecting the reproductive success of mosquitoes. Females able to recognize sites favourable to larval development have an obvious selective advantage. *Aedes aegypti* females choose to oviposit mainly in small, artificial containers (Laird, 1988). Eggs are usually attached to the sides of the container, just above the water line (Christophers, 1960). Direct contact of females with water is necessary to initiate oviposition (Kennedy, 1942). Oviposition usually occurs in the late afternoon (Gillett *et al*, 1958a,b; Chadee and Corbet, 1987). *Aedes aegypti* displays skip oviposition: eggs are distributed among several oviposition sites (Corbet and Chadee, 1993).

Factors governing the choice of oviposition sites include a variety of physical, chemical and biological factors. Colour, optical density, texture, temperature and reflectance of the oviposition site are of primary importance (Bentley and Day, 1989). Generally, dark colours are preferred (Fay and Perry, 1965; Yap *et al*, 1995), presumably due to the dark appearance of water sources from above. From a close range, stimulation by reflectance and humidity probably further helps to guide mosquitoes to the site (Kennedy, 1942). Chemical properties of the water subsequently influence oviposition, although this is secondary to visual cues. Chemicals may be referred to as attractants or stimulants, and repellents or deterrents. These act as additional signals to stimulate and inhibit certain behaviours respectively (Foster and Harris, 1997).

Certain bacteria and their metabolites seem to act as oviposition attractants (Benzon and Apperson, 1988; Hazard *et al*, 1967; Hasselschwert and Rockett, 1988). *Culex quinquefasciatus* was found to be attracted to protein hydrolases of certain bacteria (Beehler, 1994). Organic infusions, such as oak leaf or grass infusions have proven to be attractive to many mosquitoes (Trexler *et al*, 1998, Kramer and Mulla, 1979). Mosquitoes may also produce pheromones that act as oviposition attractants. Many *Culex* spp. are known to be attracted to a substance from the apical droplets of egg rafts (Hwang *et al*, 1987); such attractions are very species specific (Ikeshoji and Mulla, 1970b). The oviposition pheromone has been synthesized in the laboratory (Dawson *et al*, 1990).

Many culicids are attracted to oviposit on waters containing conspecific larvae, which may indicate conditions favourable for larval survival and development (Allan and Kline, 1998; Soman and Reuben, 1970; Maire, 1984, 1985; Onyabe and Roitberg, 1997, McDaniel *et al*, 1979). As well, occupied habitats tend to be less transient and are less likely to dry out (Srivastava and Lawton, 1998). Benzon and Apperson (1988) questioned attraction due to larval products, and suggested that the observed attraction was attributable to bacterial contamination.

Knight and Corbet (1991) isolated some of the attractive compounds and, like Maire (1985 a, b), found a dose-dependent reversal of attractant activity. In 1994, Lowenberger and Rau observed the production of a repellent by *Plagiorchis elegans* infected larvae. Zahiri *et al* (1997a), and Zahiri and Rau (1998) re-examined this phenomenon, and found that such a repellent is also produced when larvae are stressed due to confinement, starvation or crowding, although at much lower levels. Dilution of repellent waters does not restore their attractive properties (Zahiri and Rau, 1998). It was

shown that the location and intensity of the infection of larvae also affects the repellency of their waters (Zahiri *et al*, 1997c). Analysis of the serum composition of larvae indicated that starved, crowded and infected larvae are nutritionally stressed and that this may be linked to the production of the repellent (Zahiri and Rau, 1998). The attractant/repellent effects show some species specificity, as females of *Aedes atropalpus* are responsive to waters containing their own species, but not *Ae. aegypti*. Female *Ae. aegypti*, however, respond to waters containing both conspecifics and *Ae. atropalpus*. This may be due to the broad environmental tolerance of *Ae. aegypti* in contrast to the rather specialized requirements of *Ae. atropalpus* (Zahiri *et al*, 1997b).

Other factors may reduce oviposition into certain environments. *Aedes taeniorhynchus* is repelled by waters containing fish (Ritchie and Laidlaw-Bell, 1994), and other mosquito species may avoid ovipositing in waters occupied by notonectids (Chesson, 1984) and tadpoles (Petranka and Fakhoury, 1991). Edgerly *et al* (1998) found that the presence of the predator *Anopheles barberi* did not affect the oviposition choice of *Aedes triseriatus*. Chadee *et al* (1990) found that *Ae. aegypti* females oviposit less frequently into waters where conspecific females have already oviposited, although Edgerly *et. al.* (1998) did not observe this for *Ae. triseriatus* females in the field.

Edgerly *et. al.* (1998) also found that females of *Ae. triseriatus* show a seasonal difference in egg laying behaviour. Individuals were more attracted to habitats containing a low density of larvae early in the season to avoid competition, and to habitats with higher densities of larvae in late summer, as this is an indicator of habitat permanence, which is required for eggs entering diapause to overwinter. However, this species occupies a more northern habitat than *Ae. aegypti*, which does not require egg diapause

during the winter months, and therefore does not require such seasonal changes in behaviour.

Egg Hatchability

Aedes aegypti eggs are small, black, elongate oval, torpedo shaped, and under a millimetre in length (Christophers, 1960). On the lower surface of the egg, the epichorion, when submerged, swells to form the gelatinous, sticky chorionic pad, which aids to anchor eggs ventral side up to the substrate.

Immediately after being deposited, eggs require moisture for a period of 2-3 days to allow the development of the larvae to the first instar stage; this is termed conditioning. Eggs dried immediately after oviposition collapse and die. In contrast, conditioned eggs are fairly resistant to desiccation (Putnam and Shannon, 1934; Fay, 1964). If submerged immediately after oviposition, eggs manifest reduced hatching success (Christophers, 1960). Males commonly hatch before females (Elzinga, 1961).

The hatching stimulus for eggs of *Ae. aegypti* is submersion (Christophers, 1960) and a decline in the oxygen content of the water. After flooding, the growth of microorganisms in the environment reduces the oxygen tension in the water and induces eggs to hatch (Judson, 1960; Gjullin *et al*, 1941). Gillett (1955) also found that individual variation in egg hatchability may be pre-determined genetically in *Ae. aegypti*.

Several authors have noted that hatching of *Aedes* eggs may be influenced by larval population density. Larvae grazing on eggs remove microorganisms; this prevents the decline in oxygen tension and impedes the hatching stimulus (Edgerly and Marvier, 1992; Livdahl and Edgerly, 1987). Edgerly *et al* (1993) found that eggs of *Ae. aegypti* did not hatch when eggs were exposed to a larval density of 0.96 larvae/ml Small numbers of

larvae provide a positive hatching stimulus, as their respiration causes a reduction in environmental oxygen tension. With a reduction in food availability, increased grazing on eggs by larvae prevents a reduction in oxygen levels, and ultimately inhibits egg hatching (Livhdal *et al*, 1984). Although Gillett (1959) suggests the possibility of a chemical hatching inhibitor, this has yet to be demonstrated in the laboratory.

The phenomenon where eggs of an age group do not all hatch at the same time is termed "instalment" or "erratic hatching", and results in generation overlap (Koenekoop and Livdahl, 1986; Livdahl, 1984). Increased mortality of younger cohorts due to cannibalism by older conspecifics, and retarded development under conditions of crowding and starvation, make such an inhibition of egg hatching a selective advantage. Since females may continue to oviposit into such habitats, there may be a build up of an egg bank over time. A decline in the larval population and the resulting removal of hatching inhibition may cause such eggs to hatch, and allow a quick recovery of the mosquito population.

Plagiorchis elegans

Plagiorchis elegans is a digenean trematode of the family Plagiorchiidae (Olsen, 1962). It was first described from a house sparrow in Germany (Braun, 1945). It is a cosmopolitan, ubiquitous parasite, affecting both birds and small mammals. Development of the parasite requires two intermediate hosts, the first a lymnaeid snail, the second an aquatic arthropod (Blankespoor, 1977).

The monoecious adults are found in the posterior portion of the host's small intestine (Blankespoor, 1977). Adult morphology and longevity is influenced by the definitive host and shows great variability (Blankespoor, 1974). Adults may be found in

the intestinal tract as early as four days post-infection in sparrows (Blankespoor, 1974), or six days in laboratory mice (Genov and Samnaliev, 1984), or 8 days in Syrian hamsters (Zakikhani and Rau, 1998). The definitive host passes unembryonated eggs into the environment with the feces of the host (Genov and Samnaliev, 1984; Zakikhani and Rau, 1998).

The first intermediate host, a lymnaeid snail, ingests embryonated eggs. Miracidia hatch, penetrate the gut lining and form mother sporocysts on the outer surface of the intestine. These produce daughter sporocysts, which migrate to the hepatopancreas and release large numbers of xiphidiocercariae, or stylet-bearing cercariae (Blankespoor, 1977). Infection in the snail host may last 10 months, and results in the asexual production of more than 4 million cercariae (Blankespoor, 1973).

The xiphidiocercariae possess a well-developed sucker, and a strong stylet (Styczynska-Jurewicz, 1962). Cercariae are usually first released about 49 days post-infection (Blankespoor, 1977). Cercarial shedding is initiated by a change from light to darkness and may continue over a period of 2-3 hours (Webber *et al*, 1986 a). Prior to shedding, the snail undergoes behavioural changes, moving to the surface of the water column. Cercariae disperse and settle to the bottom of the water column, where they reach maximum infectivity (Lowenberger and Rau, 1994). Cercariae infect a variety of aquatic invertebrates, such as odonates, dipterans, caddisflies, mayflies and amphipods (Blankespoor, 1977) by penetrating the host cuticle using their stylet and histolytic enzymes (Bock, 1989). In host tissue they encyst to form metacercariae. The time required to reach infectivity is 3-5 days, and is influenced by temperature (Lowenberger and Rau, 1994). The life cycle is completed when the second intermediate host is

ingested by the definitive host. Metacercariae hatch by means of proteinacious enzymes (Bock, 1989) and develop into monoecious adults (Blankespoor, 1977).

Interactions between *Plagiorchis elegans* and *Aedes aegypti*

Plagiorchis infections have a major impact on the development and survival of *Ae. aegypti* larvae. Infection results in high mortality, delayed development, interference with pupation, and the emergence of malformed adults (Dempster *et al*, 1986). Late instars are more susceptible to the infection than earlier instars and pupae, as parasite acquisition is largely a function of host size and activity (Dempster and Rau, 1987). However, if infected, early instars manifest greatly increased mortality.

The location of the infection in the body of the host is also significant; infections of the head are more damaging than abdominal or thoracic infections (Dempster and Rau, 1990). Increased environmental temperatures do not decrease the incidence of infection. Although increased temperatures shorten developmental time, thus reducing the duration of exposure to the parasite, this may be compensated by increased host activity, with a concomitant increase in contact with cercariae (Dempster and Rau, 1991).

Plagiorchis elegans induces behavioural changes in *Ae. aegypti* larvae. Gilchrist, 1994 observed that in the presence of cercariae released the night before, uninfected individuals may avoid feeding at the bottom of the water column and remain at the surface during the daylight hours. As darkness sets in, however, this avoidance reaction fades, and individuals shift to the bottom to feed. As cercariae are released during the night, massive infections may be acquired. This may be due to a generalized defence mechanism of the mosquitoes geared towards other parasites, from which *P. elegans* benefits. Reduced feeding due to parasite avoidance extends developmental time, which,

in turn, increases exposure time (Dempster and Rau, 1991). Individuals bearing low levels of infection display defence reactions, such as grooming and looping and as a result become more active and spend less time at the surface of the water (Webber *et al*, 1986a, Gilchrist, 1994). This may increase the probability of acquiring higher levels of infection (Webber *et al*, 1986a). Highly infected individuals manifest reduced activity and spend more time at the surface during the daytime (Webber *et al*, 1986a). Such larvae may become entangled in the vegetation and show increased susceptibility to predation by small mammals foraging at the water's edge (Webber *et al*, 1986b). Nocturnal shedding of cercariae may further benefit the parasite, as larvae will disperse during the night, increasing chances of host-parasite contact (Webber *et al*, 1989).

The parasite also affects the reproductive success of *Ae. aegypti*. Infected adults show reduced fecundity and fertility; there is also reduced longevity, which may reduce vectorial capacity of the females (Kimoro, 1990). The production of an oviposition repellent by infected larvae may also affect the reproductive success of adults (Lowenberger and Rau, 1994; Zahiri and Rau, 1998).

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CONNECTING STATEMENT 1

Chapter 1 reviews the literature concerning several aspects of *Aedes aegypti* biology, the phenomenon of oviposition and hatchability, and the interaction of the mosquito with the entomopathogenic digenean *Plagiorchis elegans* and erects the framework in which the study was undertaken. The choice of an ovipositional site by females as well as the rate at which eggs hatch are well known to be influenced by a number of factors such as crowding, starvation and parasitic infection. Population dynamics as well are influenced, to some extent, by these same factors, and by the recruitment of individuals into the population, which is, in turn, determined by oviposition and egg hatchability. The following chapters deal with the interaction of these factors.

The attractive properties of larval holding waters and the repellency of waters containing larvae infected with *P. elegans* have been well studied as separate phenomena. Population dynamics of *Ae. aegypti* exposed to *P. elegans* has been examined under conditions of constant recruitment into the larval population. It is likely, however, that in a natural situation all of these factors interact to form a number of feedback mechanisms. Attractive properties of the water are likely determined by a blend of attraction and repellency determined by environmental conditions. The resulting effect on recruitment influences population dynamics, which, in turn, have an impact on ovipositional preference. In chapter 2, the interaction of these factors is examined in a dynamic larval population, which mimics a natural system.

CHAPTER 2

THE IMPACT OF SELECTIVE OVIPOSITION AND INFECTION WITH *PLAGIORCHIS ELEGANS* ON *AEDES AEGYPTI* PRE-IMAGO POPULATION DYNAMICS AT OPTIMAL FOOD AVAILABILITY

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ABSTRACT

Progressive changes in the attraction of waters harbouring pre-imago populations of *Aedes aegypti* exposed to different levels of the entomopathogenic digenean *Plagiorchis elegans* to ovipositing conspecific females was assessed under conditions of optimal food availability. The impact of ovipositional preference and parasitic infection on population structure and development was investigated. Probabilities that larvae progress from one stage to the next or die within 24 h were calculated for all life stages. Exposure to *P. elegans* did not significantly affect the attractiveness of larval holding waters. Ovipositional preference increased significantly with growing biomass of the larval population, with the event of pupation and in some cases with late instar mortality. Exposure to various levels of the parasite significantly increased mortality of all instar stages, however most of the damage caused by the parasite occurred in the form of increased pupal mortality and decreased adult emergence. Exposure to the parasite significantly reduced the number of adults produced, yet did not impair larval development. Thus, larval recruitment into environments containing *P. elegans* remains high, the structure of larval populations remains relatively normal, but no adults are produced.

INTRODUCTION

Entomopathogenic cercariae of digenean parasites, among them *Plagiorchis elegans*, are highly infective to larvae of the yellow fever mosquito, *Aedes aegypti*. Parasitized mosquito populations experience elevated pre-imago mortality, greatly reduced adult emergence and severe developmental abnormalities (Dempster *et al.* 1986). Asexual production of the parasite occurs within the tissues of its molluscan first intermediate host and results in the daily release of large numbers of the entomopathogenic cercariae into the aquatic environment (Blankespoor, 1974). These cercariae penetrate and kill mosquito larvae, thus acting like a persistent, potent biological insecticide. Container breeding mosquito habitats do not contain other target organisms that may be affected by *P. elegans*. The action of these parasites as natural enemies of mosquito larvae and its application as a biological agent in their control is being investigated (Rau *et. al.*, 1990). Assessments of this nature require a detailed knowledge of the population dynamics of the pest species, and how these interact with the control agent.

Populations of container breeding mosquitoes, such as *Ae. aegypti* are regulated primarily by intraspecific competition among larvae. The structure of larval populations is also greatly affected by periodic recruitment of new individuals into the population, which, according to Soman and Reuben (1970), depends largely on patterns of female oviposition into the larval habitat. Location of a suitable oviposition site greatly influences the survival and development of the female's offspring (Bentley and Day, 1989). Females of *Ae. aegypti* oviposit preferentially into waters harbouring moderate numbers of conspecific larvae (Soman and Reuben, 1970). However, if such larvae are

stressed due to crowding or starvation, they produce a potent oviposition repellent, which discourages conspecific females from ovipositing into such environments (Zahiri, 1997). Such repellency was also observed in waters containing larvae bearing sublethal infections of *P. elegans* (Lowenberger and Rau, 1994; Zahiri and Rau, 1998). Lowenberger and Rau (1994) found that the presence of cercariae in the water by itself had no effect on ovipositional behaviour, but that repellence was clearly caused by infected larvae. Similar chemical communication has been reported for *Culex* (Hudson and McLintock, 1967) and for *Anopheles* (Reisen and Siddiqui, 1978).

In light of these factors, it is evident that infection with *P. elegans* may affect the population dynamics of *Ae. aegypti* in a number of ways which must be considered simultaneously in order to assess the potential of this parasite as a natural enemy of mosquitoes and as an agent in their biological control. Population dynamics of infected *Ae. aegypti* have been examined by Hartley (1996) and Nguyen (1998) under conditions of constant, daily recruitment into the population. It was the objective of the present study to examine the population structure and development of *Ae. aegypti* pre-imagos infected with *P. elegans* under conditions of variable recruitment as determined by direct oviposition into the population. It is expected that this will create a more realistic model of mosquito pre-imago population dynamics.

MATERIALS AND METHODS

Aedes aegypti

A colony of *Ae. aegypti* was maintained in the laboratory at 27±1° C under a photoperiod of 16 h light and 8 h dark. Adults were kept in nylon mesh and glass flight cages measuring 21cm × 21cm × 35cm. Adult females were blood-fed twice weekly and provided with 10% saturated sucrose solution. Oviposition sites consisted of 1liter plastic food containers lined with filter paper and filled with 500 ml of distilled water to a depth of 4 cm. Females deposited eggs on the filter paper, usually at the water line. Filter papers were removed every few days, and eggs were kept moist for conditioning. Eggs were hatched in a suspension of 2g/l brewer's yeast in water, which also served to feed the larvae.

Plagiorchis elegans

The life-cycle of *P. elegans* was maintained in the laboratory. A colony of the snail first intermediate host, *Stagnicola elodes,* was kept at 20±4°C and photoperiod of 16 h of light and 8 h of darkness. Snails were fed fresh lettuce and chalk ad libitum. Syrian hamsters, *Mesocricetus auratus,* served as experimental definitive hosts and were fed *P. elegans*-infected mosquito larvae by gavage to establish the adult infection. Parasite eggs are passed with the feces of the hamster hosts, and once embryonated, were fed to snails. *Plagiorchis* cercariae emerge from their host at dusk. Thus, infected snails were placed into 20 ml of aerated tap water just prior to scotophase in order to collect large numbers of cercariae. Mosquito larvae were then exposed to *P. elegans* by adding cercariae to the larval holding water. The cercariae penetrated their tissues to form metacercariae. Details

of these methods have been described previously (Lowenberger and Rau, 1993; Zakikhani and Rau, 1998).

Experimental Method

Experimental flight cages identical to those used for laboratory colonies were furnished with two oviposition sites each. Both sites consisted of 1 liter plastic containers lined with white filter paper, and filled with 200 ml of distilled water. One of the initially identical sites in each cage served as a larval habitat once the first eggs hatched (the larval holding site). The other site contained only distilled water, an environment considered to be neutral to ovipositing females (the neutral site). Forty male and 40 female adult *A. aegypti* were introduced into each flight cage. This was done in batches of ten, over a period of 4 days, to obtain a mixed-age population of egg laying females. Females thus had a choice between ovipositing into the neutral or the larval holding site. Each day, the (ovipositional) attractiveness of each larval holding site relative to that of distilled water was determined as the percentage of the total number of eggs laid in the corresponding flight cage that had been deposited into the larval holding site. Larval holding sites were considered attractive if this value was above 50%, and repellent if it was below 50%. In this manner, an attractiveness index was defined.

Mosquitoes were blood-fed daily and provided with 10% saturated sucrose solution on cotton wicks ad libitum. Experimental colonies in flight cages were maintained in the same manner as standard laboratory colonies. Brewers yeast at an amount of 0.1g/l, determined by Hartley (1996) to be near optimal for the development of larvae to the adult stage, was added daily to the larval holding sites as a source of food for larvae and a medium for bacterial growth. No yeast was added to the neutral sites.

Data collection began four days after the first females started to oviposit. Filter papers with attached eggs were removed daily from all sites, and eggs were counted and incubated at 100% R.H. and 27±1°C for two days to allow full embryonic development (conditioning) (Christophers, 1960). Liquid from larval holding sites was allowed to drip back through the old filter (lining the site) into the respective containers, which had been lined with fresh filter paper, to maintain continuity of the aquatic environment and its chemical properties. In contrast, water and filters in the neutral sites were renewed daily in order to maintain a constant neutral reference point.

After conditioning, eggs collected from the larval holding sites were hatched in a suspension of brewer's yeast. In order to generate realistic pre-imago populations, the number of larvae added daily to larval holding sites reflected a fixed proportion of the eggs that had been deposited into this particular site over the day preceding the conditioning period. To maintain a population of manageable size, this proportion was arbitrarily fixed at 21.25%, 1/4 of the hatchability of 85% determined in preliminary experiments. No larvae were added to neutral sites.

In addition, larval populations in the larval holding sites of nine flight cages were subjected to treatment with exposure to a low concentration of *P. elegans* cercariae (20/ml) and those of nine further flight cages to treatment with exposure to a high concentration of cercariae (40/ml). These were compared to the larval populations in the larval holding sites of nine further flight cages (controls), which were not exposed. Larvae were exposed to *Plagiorchis* by adding the appropriate number of cercariae to the larval holding sites, to generate the correct concentrations. The numbers of cercariae added were estimated volumetrically on the basis of ten 20 µl samples of cercarial

suspensions produced by laboratory-infected snails. No cercariae were added to the neutral sites of any flight cages.

A complete census of live and dead larval instars, pupae, and adults in each larval holding site was conducted each day in order to document progressive changes in the populations. Pupae were transferred to individual vials filled with distilled water to allow subsequent retrieval of the emerged adults. Adults were sexed and counted. Every two days, any dead larvae were removed, crushed and examined for infection under a compound microscope at a magnification of 100×, and carcasses were then returned to the corresponding sites. This was done to ensure infection had occurred. Dead larvae were returned to the holding sites, as they provide an alternate food source for the larvae.

Each flight cage represented one experimental unit. Data were collected daily for 14 days. The nine replicates were divided into three balanced blocks over time. The experiment thus followed a 1×3 factorial randomized complete block design with repeated measures in time.

Transition Probabilities

In order to quantify the progressive changes in the development of the population, transition probabilities were calculated. These calculations were based on an adaptation of a Markov-chain type model developed by Dr. Pierre Dutilleuil (McGill University, Department of Plant Science), and first applied to mosquito development by Nguyen (1998). A transition probability is the probability that one stage will develop to the next within a fixed time interval. For this study, probabilities were calculated from raw data for 24 hr periods (Figure 2.1.) All probabilities were calculated for each of the 14 days,

generating a time series for each probability. Calculations were performed using Microsoft Excel 97 spreadsheets.

Statistical Analyses

Mean time-dependent transition probabilities and ovipositional preference were calculated and graphed using Jandel Scientific Sigma Plot software. Analysis was then performed using an analysis of variance with repeated measures in time with days as the repeated factor using SAS 6.1 software (SAS Institute Inc., 1999). The general linear models procedure with its statement REPEATED was applied. Results of the univariate and multivariate approach were used as appropriate. When data failed to meet circularity conditions, an adjustment of the probabilities of significance of the univariate ANOVA F-tests was executed using the Huin-Feld Epsilon correction factor.

Repeated measures ANOVA was also used to analyse total biomass and individual instar abundance. Biomass was expressed in the form of fourth instar equivalents. The weight of individual fourth instars is 40 times greater than first instars, 12 times greater than second instars, and 2.2 times greater than third instars (Christophers, 1960). Total adult production was compared using ANOVA tests with SAS software. Post hoc comparisons were made by multiple t-tests using the Student-Newman-Keuls approach. Proportional data were transformed using arcsine square root transformation; population count data were transformed using a square root transformation (Zar, 1993).

Cross-Correlations of Time Series

To determine whether ovipositional preference of adult females was affected by the development of larvae in oviposition sites, cross-correlations between time series of ovipositional preference and transition probabilities were conducted. The procedure ARIMA in SAS economics and time series was used to perform these calculations. Proc ARIMA uses the Box and Jenkins (1976) method to create models of time series. Cross correlations were calculated using the 'identify' statement. Linear correlations between points were calculated between values of the different time series for different time lags. No attempts at model building were made in this study.

RESULTS

The development of first instars (Figure 2.2) changed significantly over time (P11: p=0.001; P12: p=0.02) and was characterized by periodic increases and decreases. The total number of first instars present in the population also showed significant variation over time (p=0.0026) (Figure 2.9). Infection with *P. elegans* had no significant effect on development. However, first instars showed significantly increased mortality with increasing levels of parasite exposure (p=0.001). Mortality was also affected by time (p=0.0003) (Figure 2.2).

Second instars also manifested significant differences in development over the 14-day experimental period (P22: p=0.001; P23: p=0.0001). Transition probabilities (Figure 2.3) rose to almost 50% on day 4 and then fluctuated between 25% and 40% for the remainder of the study. As with first instars, the total number of second instars showed a significant change with time (p=0.0006), increasing slightly but steadily over the two weeks (see Figure 2.9). Their transition probabilities were not affected by parasitic exposure. Mortality of second instars (Figure 2.3) was significantly affected both by time (p=0.0234) and by exposure to the parasite (p=0.0074). Control populations, in contrast, experienced very low mortality rates. Parasite exposed populations manifested mortality peaks on day 4 and 10. Heavily exposed populations showed the highest mortality of about 12% on day 10, whereas at light exposure, mortality never exceeded 7%.

Transition probabilities (Figure 2.4) revealed that third instar development differed significantly only over time (P33, P34: p=0.0001). Total numbers of third instars (Figure 2.9) increased significantly over time (p=0.0001) and decreased in the presence of the parasite (p=0.0222). However, the effects of exposure to high and low parasite

numbers did not differ significantly. Exposure to infection, regardless of its level, significantly increased mortality (p=0.004) (Figure 2. 4). Although not significant, a higher level of exposure appeared to increase third instar deaths. As with second instars, mortality peaked on day 4 and between days 8 and 10, but there appeared to be a second peak on day 14. The peak between days 8 and 10 is conceivably responsible for the decrease in P34 on day 9 (Figure 2.4). Exposure to *P. elegans* had no impact on third instar development (Figure 2.4). Thus, mortality associated with the various treatments remained statistically unchanged over time.

As in the case of third instars, development of fourth instars (Figure 2.5) changed significantly over time (P44, P4p: p=0.0001), with peaks in pupation on day 7, and again after day 9. Time did not significantly influence fourth instar mortality (Figure 2.5), and the number of fourth instars increased with time (p=0.0001). Exposure to parasites had no impact on development or on the number of individuals; however, it significantly increased mortality (p=0.0276). Highly exposed populations appeared to have the highest mortality, particularly on day 7, yet there were no effects attributable to the level of exposure, according to post hoc comparisons (Figure 2.5).

Adult emergence was significantly affected by time (p=0.001), whereas Ppp was not (Figure 2.6). There was a slight increase in Ppp in the absence of the parasite, although this difference was not statistically significant. The total number of pupae produced each day showed a significantly different pattern over time (p=0.002), decreasing at high levels of parasite exposure (p=0.0072). Exposure to parasites significantly reduced adult emergence (p=0.011). This was most pronounced in the case of heavy exposure. Pupal death (Figure 2.6) was also significantly different for all

treatments (p=0.0142). The highest mortality occurred in heavily exposed populations, whereas control populations experienced very low mortality. Standard ANOVA analysis revealed a significantly lower total level of pupal production over the 14-day period (p=0.0001) in heavily exposed populations and an apparent, but not statistically significant, difference in populations at light exposure (Figure 2.10).

The biomass of the larval population (Figure 2.7), expressed in fourth instar equivalents, increased significantly with time (p=0.0001). All three treatments yielded a significantly different biomass (p=0.0052), which was highest for the control populations, and lowest for the heavily exposed populations.

Adult production (Figure 2.10) differed significantly with all three treatments (p=0.0001). Thus, control populations produced almost eight times as many adults as population with heavy exposure and three times as many adults as populations with light exposure.

Total larval and pupal mortality over the fourteen-day period was significantly higher in heavily exposed populations than in control populations, and in populations exposed to low levels of the parasite (p=0.0014).

The ovipositional preference of females (Figure 2.8) varied significantly over time (p=0.0001). It remained low or neutral for the first seven days, and then peaked during the remaining days of the experiment; however, the presence of the parasite had no significant impact on the oviposition site selection of mosquitoes (p=0.3702). Populations exposed to high levels of the parasite remained below 50% preference during the first eight days (Figure 2.8). All P values obtained from repeated measures ANOVA are presented in Table 2.1 and table 2.2.

Cross-Correlations of Time Series

Oviposition was cross-correlated with pupation, biomass and late (third and fourth) instar mortality. In unexposed control populations, ovipositional preference correlated with pupation with a significant correlation factor of 0.585 without a time lag, and with a significant correlation factor of 0.636 with a time lag of two days. Significant correlations with biomass occurred with a factor of 0.696 when no time lag was considered, and with a factor of 0.579 at a time lag of −1 day. Ovipositional preference increased significantly with late instar death without time lag and a correlation factor of 0.573, and with a one-day time lag and a correlation factor of 0.584.

Populations exposed to low levels of *P. elegans* showed similar characteristics. In the absence of a time lag, ovipositional preference was significantly correlated with pupation with a factor of 0.594. With a two-day time lag, the correlation factor increased to a significant value of 0.618, whereas with a three-day time lag the correlation factor decreased to 0.544, which was only marginally significant. Correlation with biomass was significant in the absence of a time lag with a factor of 0.682, and with a −1 day time lag and a factor 0.544. No significant correlation was found between ovipositional preferences and late instar mortality at a low level of exposure. However, when infection was high, significant correlation between ovipositional preference and pupation occurred without a time lag and a factor of 0.581, with a −1 day time lag and a factor of 0.720, and with a −2 day time lag and a factor of 0.685.

Ovipositional preference correlated significantly with biomass with a factor of 0.798 and without a time lag, with a factor of 0.530 when the time lag was −1 day, and with a factor of 0.574 when the time lag was one day. The significant correlation factors

in the case of late instar mortality were 0.746 when no time lag was considered, and 0.530 when the time lag was 2 days.

DISCUSSION

This study documents the impact of *P. elegans* on larval recruitment, pre-imago development, mortality and adult emergence of *Ae. aegypti* under conditions of high food availability. Development of pre-imagos was not significantly affected by the presence of the parasite. Although the total biomass of parasite-exposed populations, particularly that of third instars, was significantly lower than that of controls, exposure did not influence the pattern of development. The remarkable similarity of transition probabilities between different time series suggests that the development of immature *Ae. aegypti* follows a distinct, and probably optimal, developmental pattern. Mortality and transition probabilities suggest a cyclical pattern, as reported by Hartley (1996).

Hartley (1996) and Nguyen (1998) suggested that in nutritionally poor environments, the presence of the parasite may enhance the development of early instars, as later instars are selectively killed and competition for resources declines. However, in the present study, food was abundant and competition for food was correspondingly low. Under such conditions, other factors, conceivably changing ovipositional preferences in response to changes in population structure may have been of primary importance to population development and structure. Thus, a large influx of first instars as a result of reduced crowding following late instar death may quickly negate any benefits that might accrue to surviving larvae.

The total biomass was reduced significantly, but not substantially by the parasite (Figure 2.7). This reduction in biomass is attributable to a somewhat higher mortality of all larval stages in parasitized populations. However, early instars were much less affected by the parasite than later instars. This supports the findings of Dempster and Rau

(1990), that third and fourth instars are more susceptible to entomopathogenic cercariae than are first and second instars. Mortality of second instars never rose above 12%, whereas pupal death reached almost 50 % in highly infected populations. Figure 2.3 shows that second instar mortality, although always remaining at low levels, was noticeably increased with exposure intensity, whereas third instar death (Figure 2.4) did not differ substantially with different levels of parasite exposure. Higher exposure did not increase mortality. Such findings may be attributed to the higher sensitivity of early instars to cercariae. Though they are infected less frequently, even a single cercaria kills such a small larva, whereas third instars may survive infection (Dempster and Rau, 1987, 1990). Death of late instars increased towards the end of the fourteen-day period, probably due to the effects of crowding (Wada, 1965).

Most *Ae. aegypti* mortality occurred in the pupal stage. This supports the results of Rau *et al* (1991). Since early instars and pupae are less susceptible to cercarial acquisition (Dempster *et al*, 1986), such high levels of mortality are most likely due to the accumulation of metacercariae during the late larval stages. The lethal effects are deferred until pupation and adult emergence. As a result, infected populations produced significantly smaller numbers of adults. Dempster and Rau (1990) found that infected pupae could still give rise to adults. However, in their study, the infections were administered only in the pupal stage, thus avoiding the cumulative effect of long term developmental damage.

Towards the end of the 14-day period of observation, emergence of adults increased in parasitized populations. This may be due to the fact that since the total number of cercariae added each day remained constant, the number of cercariae per larva

declined as the larval population rose, and more individuals were able to escape the infection and successfully complete their development.

The total number of adults produced throughout the 14-day period was significantly higher in control populations. In fact, populations exposed to a high level of *P. elegans* cercariae experienced an 88% reduction in the number of adults produced. Exposure to a lower level of cercariae resulted in a 66% reduction in adult emergence. These values are in line with reduction in the number of *Ae. provocans* reported from the field by Rau *et al* in 1991.

Thus, *P. elegans* targets primarily late instars of *Ae. aegypti*, and its effects are manifested in the form of pupal mortality and failure of adults to emerge. Such delayed effects are of some advantage since density-dependent competition may continue to act on early instars, and is not affected by the death of non-feeding pupae. Adult populations are thus successfully reduced while larval populations remain largely unaltered. This may minimize the impact on the ecological system as suggested by Lounibos *et al*, (1997). Furthermore, few cercariae are expended at killing early instar larvae that would normally succumb to density dependent factors. Such action may enhance the role of *P. elegans* and other digeneans as natural enemies of mosquitoes and may add to its usefulness as a potential agent in the biological control.

The attractive and repellent effects of larval holding water on ovipositing *Aedes* females are well documented (Maire, 1985 a,b, Allan and Kline, 1998; Soman and Reuben, 1970; Lowenberger and Rau, 1994; Zahiri *et al*, 1997). Ovipositional preference of females in this study varied significantly over time and is attributable, in part, to concomitant changes in population biomass. Attraction remained low over the first eight

days, and then increased to peak with over 75% on day 11. This was followed by a decline in attraction, but rose a second time after day 13. Initial increases in preference were most likely the result of increasing larval biomass (Figure 2.9), and were followed by a reduction attributable to the effects of crowding. Finally, attraction rose again when increasing mortality of late instars (Figure 2. 7) reduced crowding.

Ovipositional preference was significantly correlated with the biomass of the population. This correlation was strongest without a time lag. Thus, an increase in the larval population was characteristically accompanied by an increase in the attractiveness of the holding waters. This confirms reports by Bentley *et al* (1976) for *Aedes* species. No negative correlations between ovipositional preference and biomass were observed. Starved or crowded larvae may render holding waters repellent to ovipositing females (Zahiri *et al.* 1997). However, larvae in this study were well fed, and crowding was probably not severe enough to reduce attraction let alone to cause repellency.

Pupation was also positively correlated with ovipositional preference with time lags between zero and three days. Pupae no longer feed (Christophers, 1960) and probably no longer interfere with larval feeding. They may thus be removed from competition, allowing an increase in the overall fitness of remaining individuals. This, in turn, may have caused waters to become more attractive, and led to enhanced recruitment of eggs and subsequent first instar larvae.

Similarly, in populations exposed to large numbers of *P. elegans* cercariae, pupation was correlated with ovipositional preference with negative time lags. However such populations also showed a highly significant positive correlation between ovipositional preference and the death of late instars. As parasite-exposed larvae pass

through successive instars and approach pupation, they are also more likely to die of accumulated parasite burdens. Larval death reduces crowding as individuals are removed from the population, which may further increases the fitness of surviving individuals. Further increases in fitness due to removal by pupation, may not have been significant enough to increase the attractiveness of the environment to ovipositing females.

The attractiveness of waters did not differ significantly with treatments. The overall pattern of ovipositional preferences was similar in the three levels of infection, and seems to follow a characteristic pattern. Although not statistically significant, there was a difference between the three curves in figure 2.8. Thus, highly infected populations experienced an initial decrease in oviposition attractiveness in comparison to the other curves. This confirms that repellency is exerted only by living, infected larvae (Lowenberger and Rau, 1994). In the present study, a consistent, significant repellency attributable to infection with *P. elegans* as described by Loewenberger and Rau (1994) and Zahiri and Rau (1998) was not observed. This may be due to several factors. The water contained a population consisting of infected and uninfected individuals of different ages. The attraction exerted by uninfected larvae may have been strong enough to override any repellent effects. Similarly, the death of infected larvae may have removed their repellent effect and may have caused a concomitant increase in the fitness of the population. This may have compensated for any repellency produced by surviving infected larvae. In addition, unlike earlier studies, larval waters in this experiment were not changed throughout the fourteen days. Thus, an accumulation of bacteria may have contributed to the attractive properties of the water. The presence of mosquito larvae has been found to increase total levels of bacteria in natural and artificial treeholes (Kaufman

et.al, 1999), and bacteria may serve as a strong ovipositional attractant (Hasselschwert and Rockett, 1988). Furthermore, Ae. *aegypti* display 'skip-oviposition' in the laboratory (Corbet and Chadee, 1993). Thus, females disperse their eggs over several sites, rather than depositing them all in one place. As only two choices were provided for females to oviposit, this may have forced females to lay more eggs in unattractive containers, thus minimizing differences between sites. The presence of conspecific eggs may also affect oviposition attractiveness. Thus, Chadee *et al* (1990) noted that *Aedes* species avoid waters containing eggs of conspecifics. In contrast, Edgerly *et al* (1998) found that female *Ae. triseriatus* were attracted to oviposit into habitats containing conspecific eggs. However, since eggs in the present study were removed daily, this is not likely to have a major impact on oviposition preference.

The sustained attractiveness of waters harbouring *P. elegans*-infected larvae enhances the suitability of these organisms as an agent in the biological control of mosquitoes. Thus, ovipositing females continue to be drawn to waters containing the entomopathogenic cercariae and infected conspecific larvae, instead of being deflected to other environments. As a result, there is an increased probability that they will lose their reproductive investment and will not contribute to the adult population.

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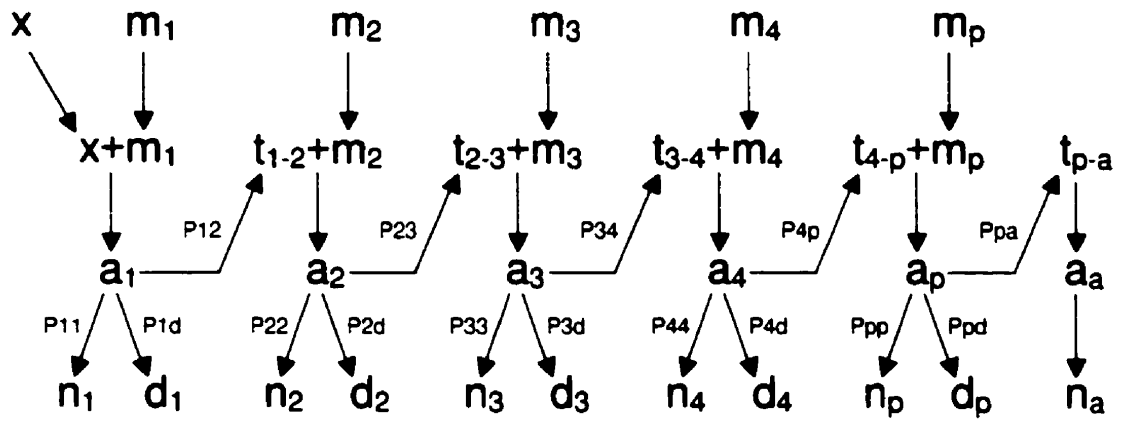
Figure 2.1: Time-dependent Transition Probabilities. **Pxx** represents the probability that an instar remains at stage **x** in 24 h; **Pxy** represents the probability that an instar will move from stage **x** to stage **y** in 24 h. **Pxd** represents the probability that an instar of stage **x** will die in 24 h. **X** is the number of 1^{st} instars added each day, **a** represents the total number of dead and live larvae on the present day, **m** is the larvae that were present the previous day, **n** is the live larvae counted the present day, and **d** is the dead larvae counted on the present day. **t** is the number of larvae that transformed from the previous stage (e.g. $\mathbf{t}_{3-4}$: number of larvae changed from 3^{rd} to 4^{th} instar).



Probabilities:

$P11=n_1/a_1$
$P12=t_{1-2}/a_1$
$P1d=d_1/a_1$
$P22=n_2/a_2$
$P23=t_{2-3}/a_2$
$P2d=d_2/a_2$
$P33=n_3/a_3$
$P34=t_{3-4}/a_3$
$P3d=d_3/a_3$
$P44=n_4/a_4$
$P4p=t_{4-p}/a_4$
$P4d=d_4/a4$
$Ppp=n_p/a_p$
$Ppa=a_a/a_p$

$a_1=x+m_1$
$a_2=t_{1-2}+m_2$
$a_3=t_{2-3}+m_3$
$a_4=t_{3-4}+m_4$
$a_p=t_{4-p}+m_p$
$a_a=n_a$

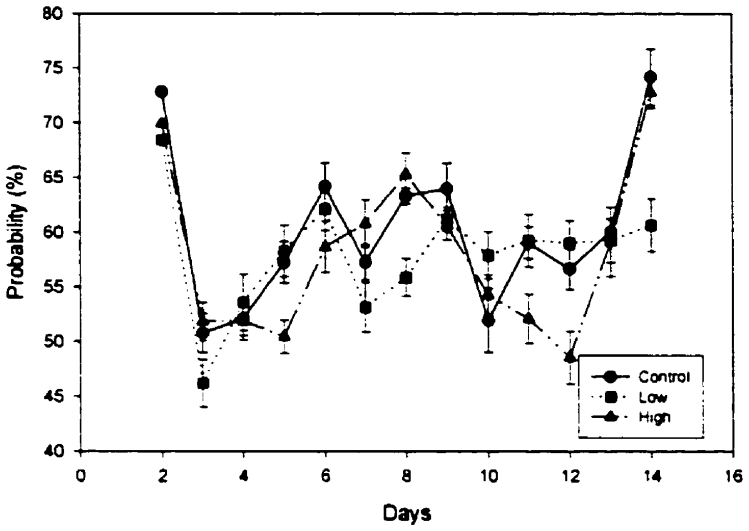
$t_{1-2}=(x+m_1)-n_1-d_1$
$t_{2-3}=m_2-n_2-d_2$
$t_{3-4}=m_3-n_3-d_3$
$t_{4-p}=m_4-n_4-d_4$

Figure 2.2: Time series of mean twenty-four hour transition probabilities of **A)** first instar A*edes aegypti* to remain at that stage (P11), **B)** to transform from the first to the second instar (P12) and **C)** of first instars to die (P1d), for control populations, populations exposed to low or high levels of *Plagiorchis elegans* at high food availability (0.1g yeast/l).

Error bars represent the standard error.

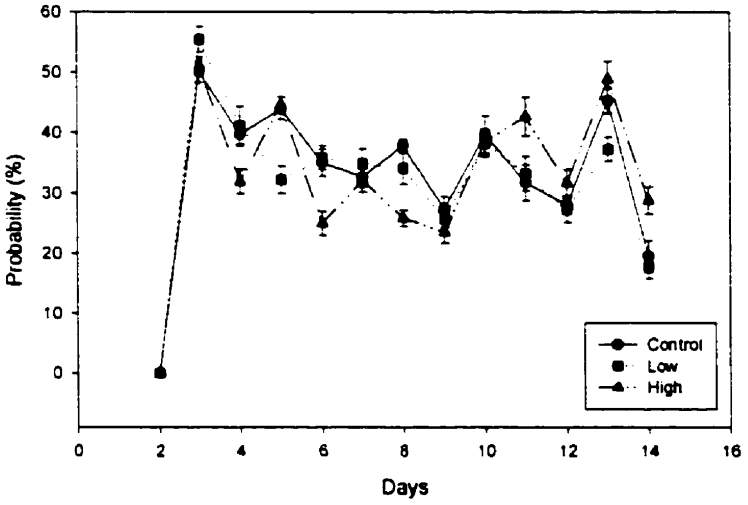
P11

A



P12

B



P1d

C

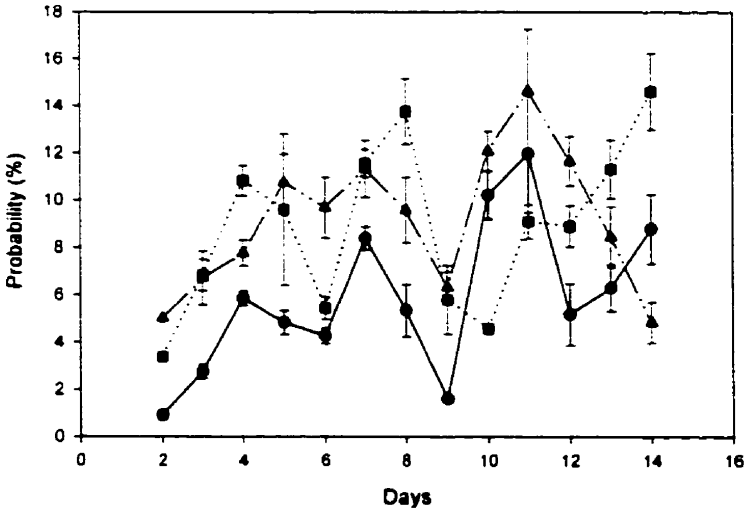
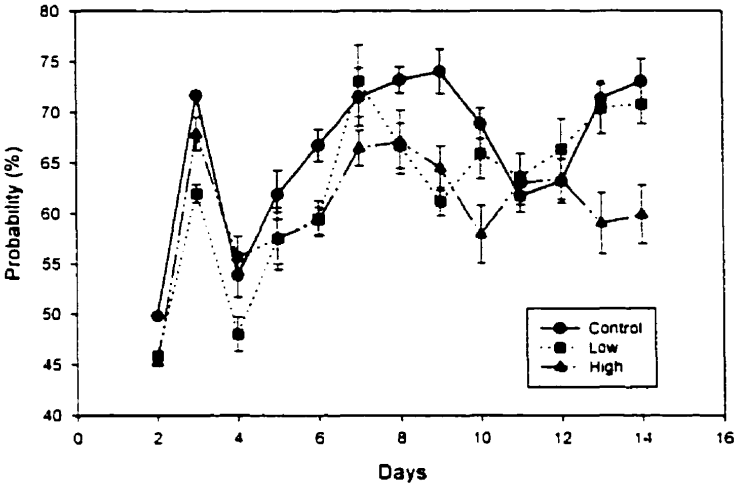


Figure 2.3: Time series of mean twenty-four hour transition probabilities of **A)** second instar *Aedes aegypti* to remain at that stage (P22), **B)** to transform from the second to the third instar (P23) and **C)** of second instars to die (P2d), for control populations, populations exposed to low or high levels of *Plagiorchis elegans* at high food availability (0.1g yeast/l).

Error bars represent the standard error.

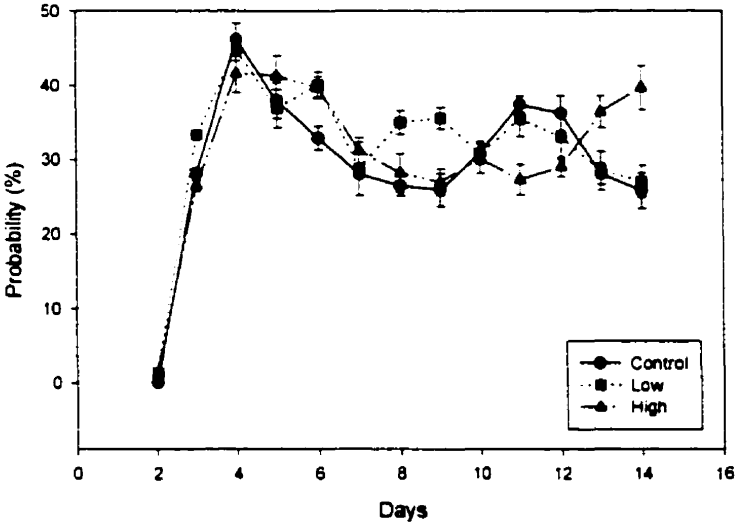
P22

A



P23

B



P2d

C

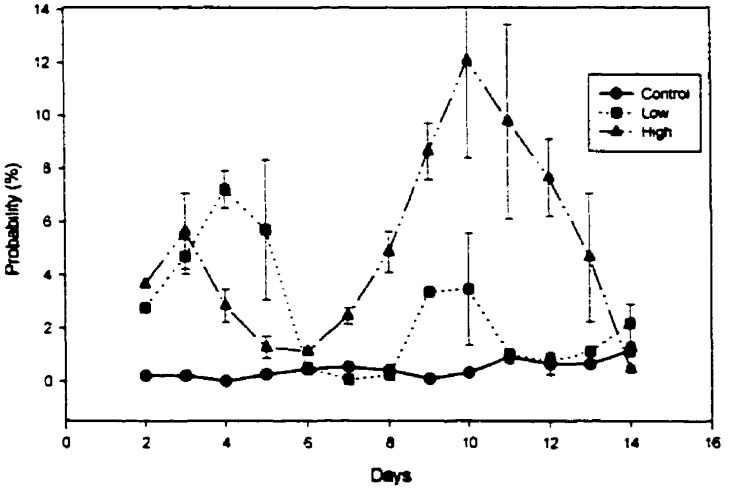
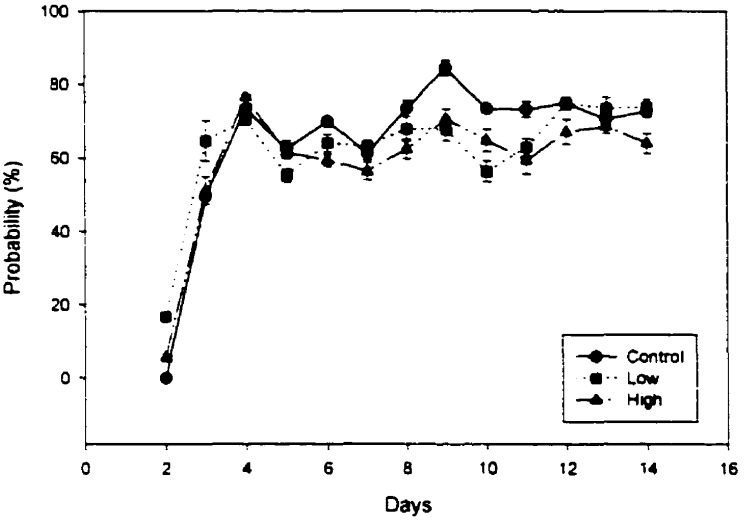


Figure 2.4: Time series of mean twenty-four hour transition probabilities of **A)** third instar A*edes aegypti* to remain at that stage (P33), **B)** to transform from the third to the fourth instar (P34) and **C)** of third instars to die (P3d), for control populations, populations exposed to low or high levels of *Plagiorchis elegans* at high food availability (0.1g yeast/l).

Error bars represent the standard error.

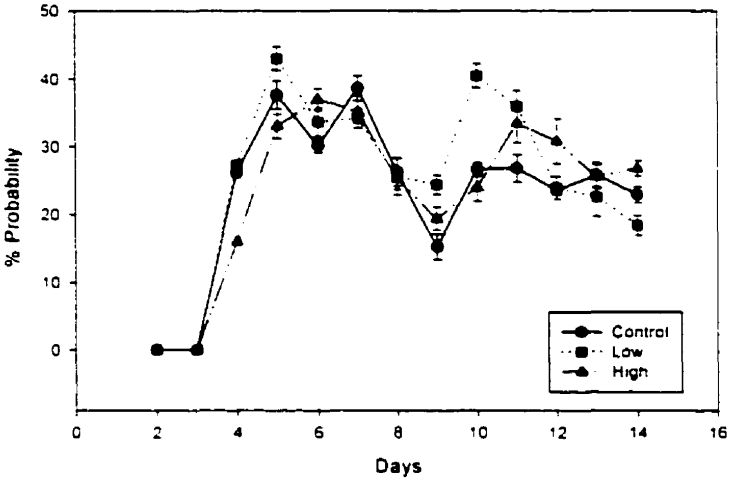
P33

A



P34

B



P3d

C

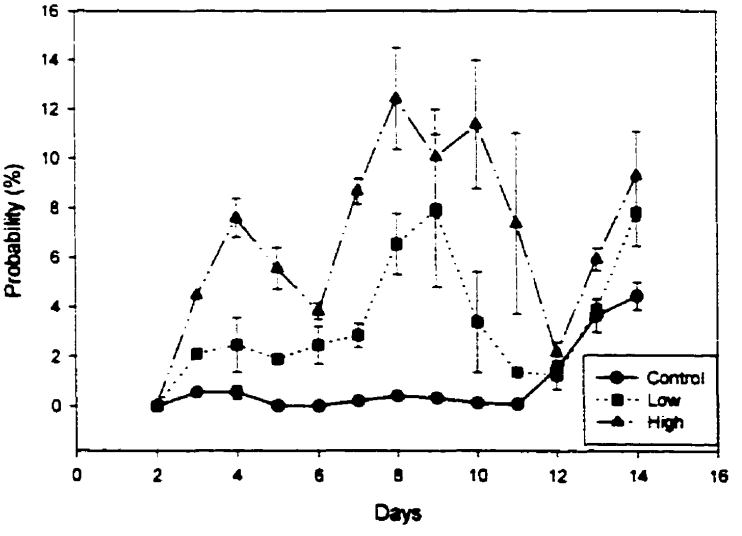
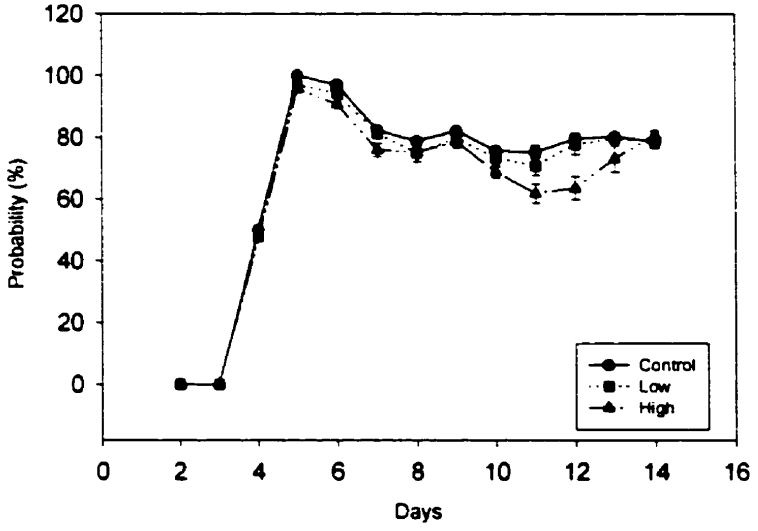


Figure 2.5: Time series of mean twenty-four hour transition probabilities of **A)** fourth instar A*edes aegypti* to remain at that stage (P44), **B)** to transform from the fourth instar to pupa (P4p) and **C)** of fourth instars to die (P4d), for control populations, populations exposed to low or high levels of *Plagiorchis elegans* at high food availability (0.1g yeast/l).

Error bars represent the standard error.

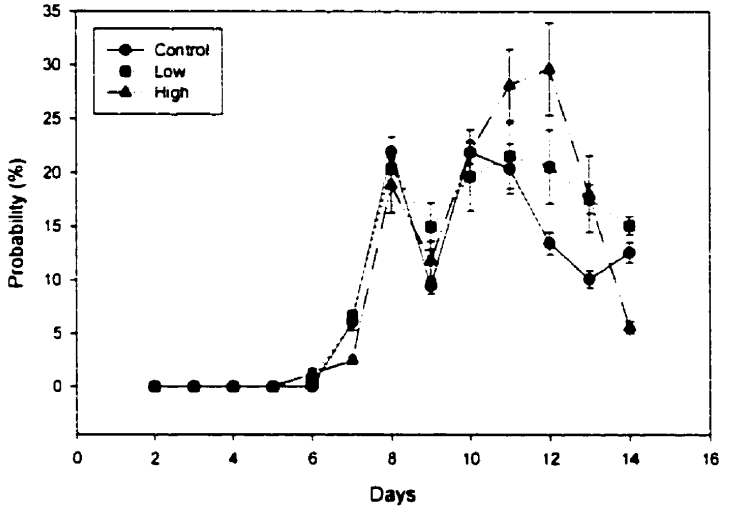
P44

A



P4p

B



P4d

C

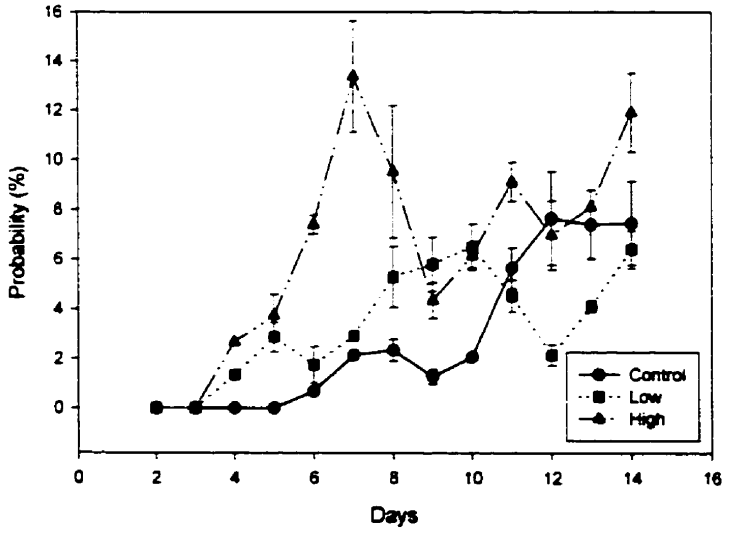
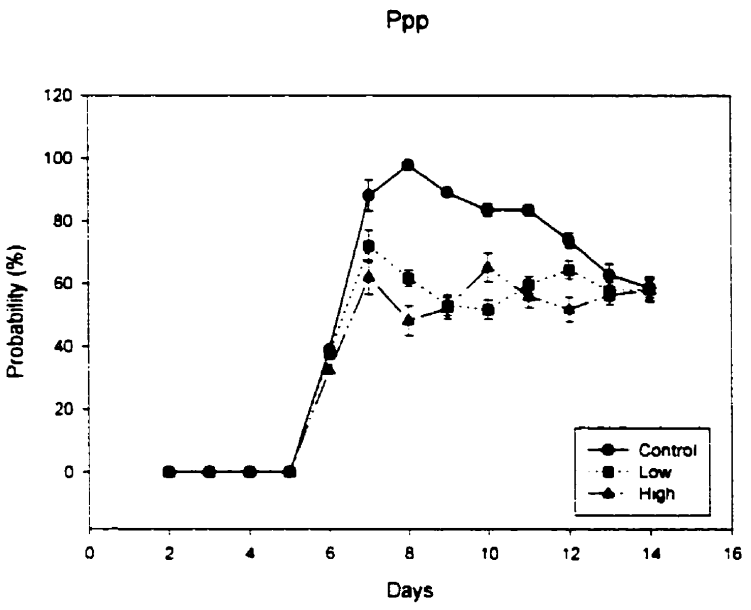


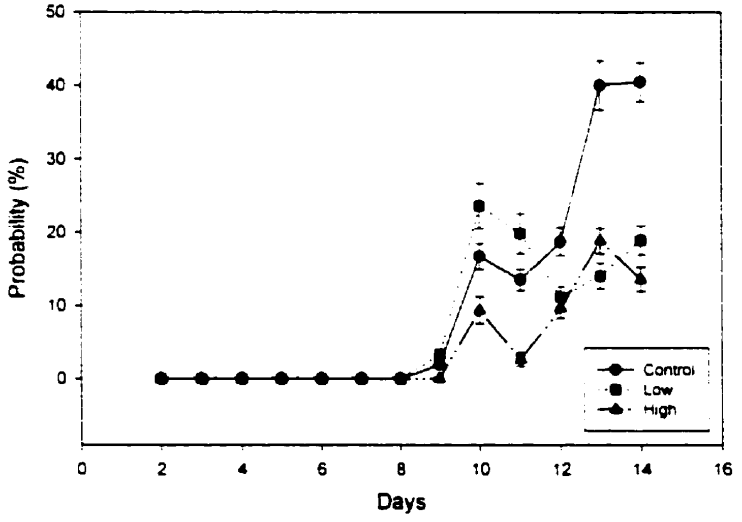
Figure 2.6: Time series of mean twenty-four hour transition probabilities of **A)** pupal A*edes aegypti* to remain at that stage (Ppp), **B)** to transform from pupa to adult (Ppa) and **C)** of pupae to die (Ppd), for control populations, populations exposed to low or high levels of *Plagiorchis elegans* at high food availability (0.1g yeast/l).

Error bars represent the standard error.

A


Ppp
Probability (%)
Days
Control
Low
High

B


Ppa
Probability (%)
Days
Control
Low
High

C

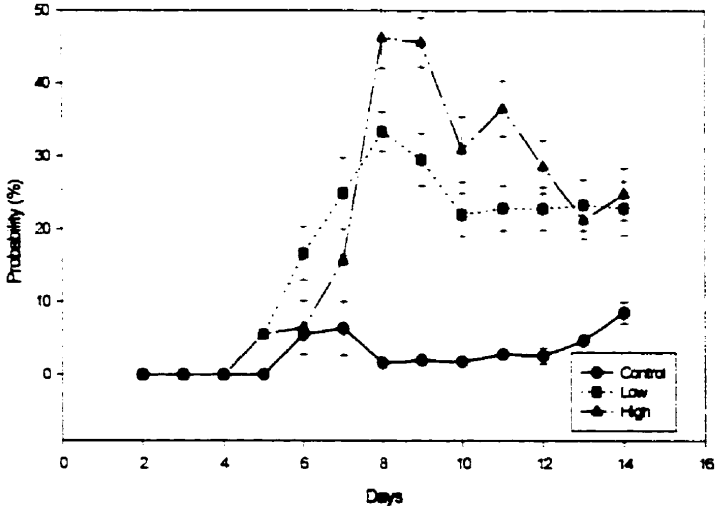

Ppd
Probability (%)
Days
Control
Low
High

Figure 2.7: Biomass of *Aedes aegypti* larval populations in terms of fourth instars for control populations, populations exposed to low or high levels of *Plagiorchis elegans* at high food availability (0.1g yeast/l). One unit biomass represents the equivalent of one fourth instar.

Error bars represent the standard error.

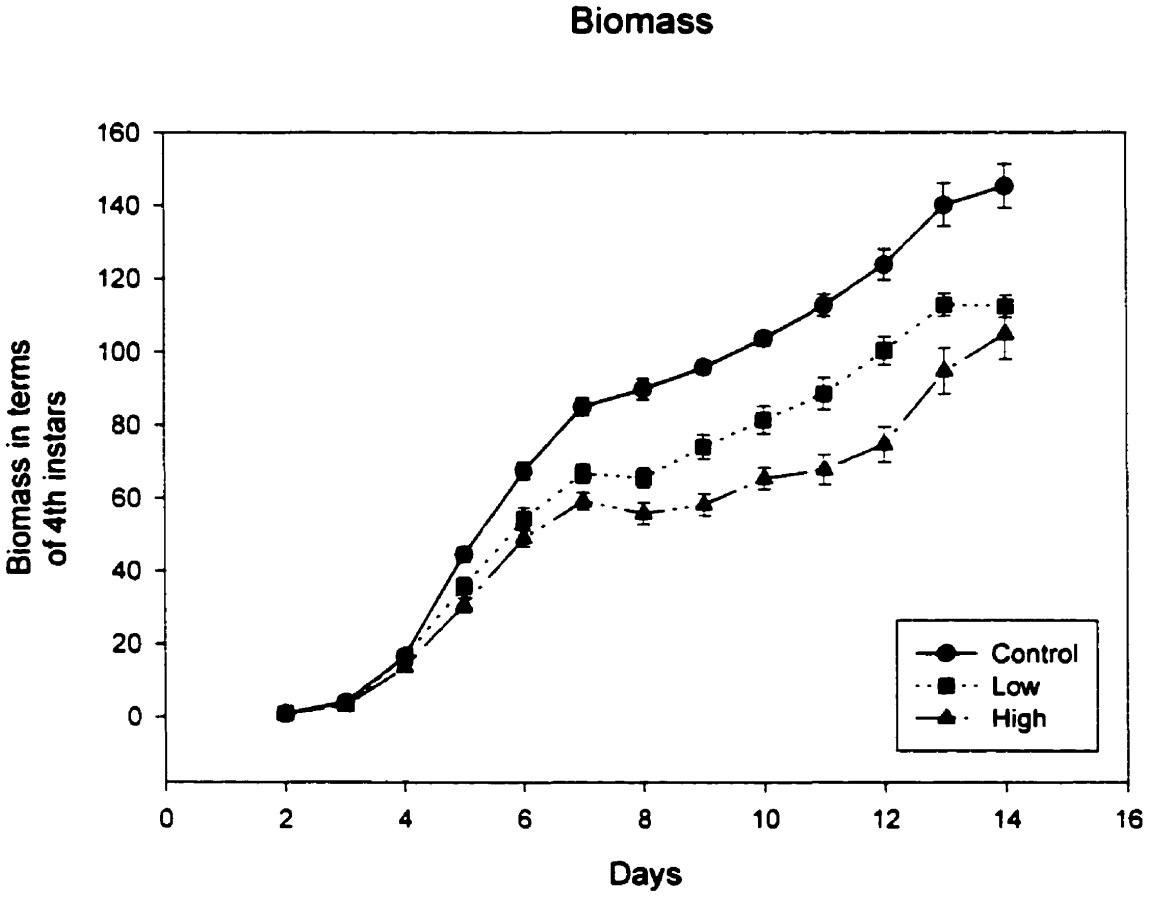

Biomass
Biomass in terms of 4th instars
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
Days
Control
Low
High

Figure 2.8: Percentage of *Aedes aegypti* eggs deposited by females into waters containing conspecific control populations, populations exposed to low or high levels of *Plagiorchis elegans* at high food availability (0.1g yeast/l).

Error bars represent the standard error.

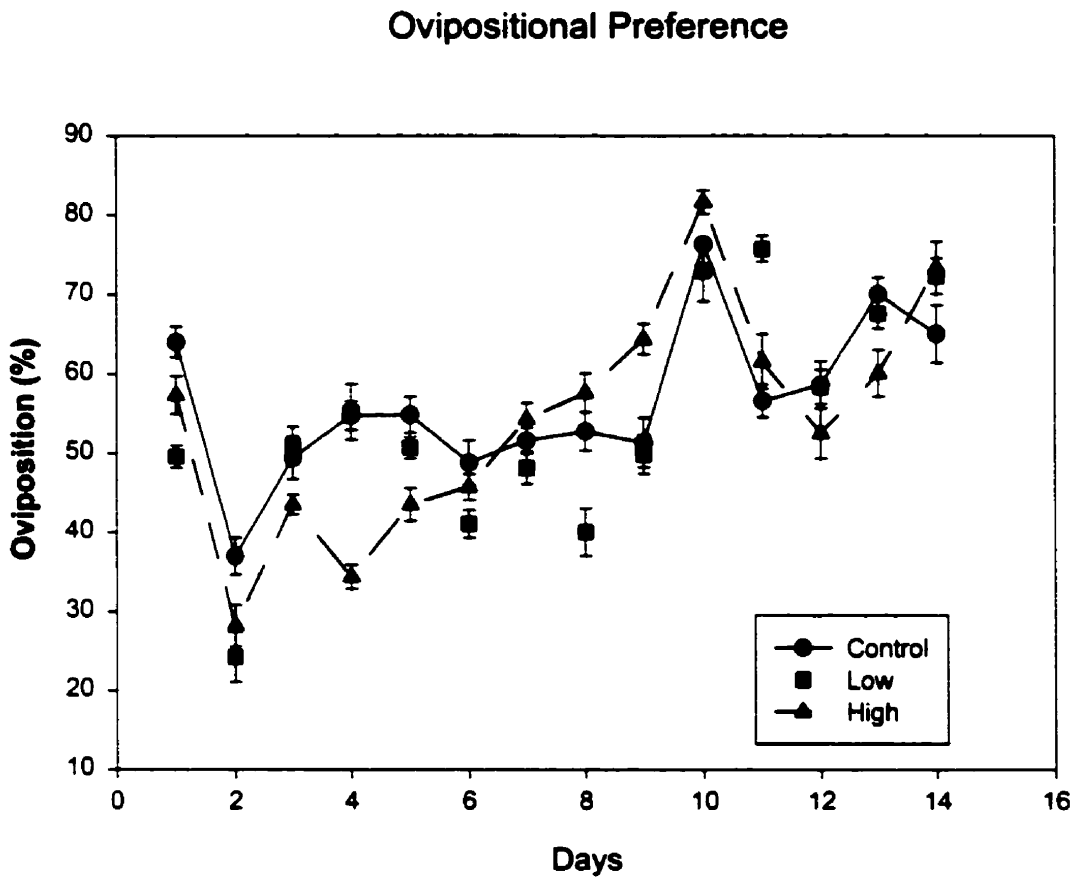
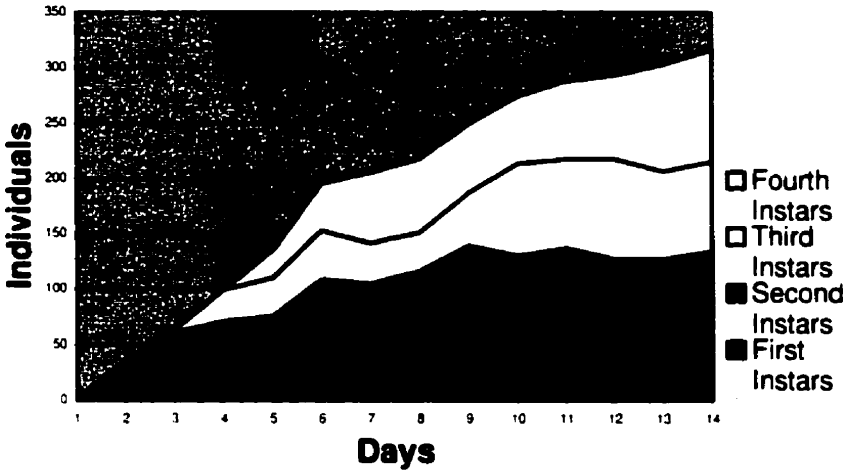
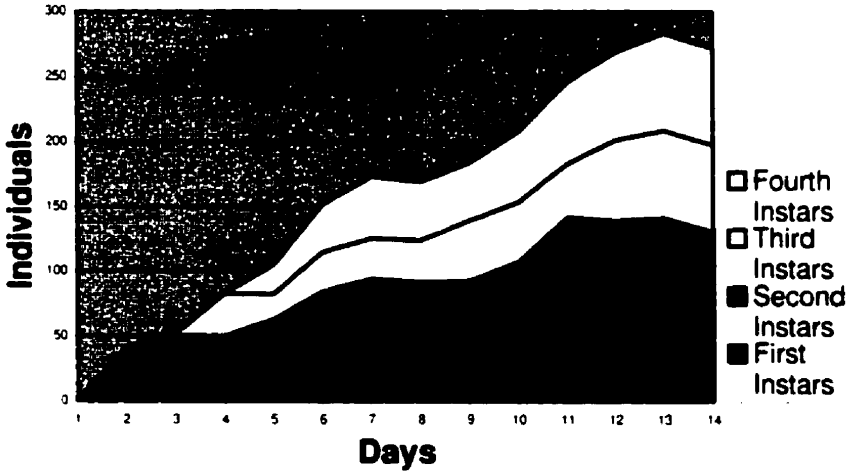

Ovipositional Preference
Oviposition (%)
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
Days
Control
Low
High

Figure 2.9: Population structure of *Aedes aegypti* larvae at high food availability (0.1g/l yeast) of **A)** control populations, **B)** of populations exposed to a low level of *Plagiorchis elegans* and **C)** of populations exposed to a high level of *Plagiorchis elegans* .

A


Control 0.1g/l Yeast
Individuals
Days
Fourth Instars
Third Instars
Second Instars
First Instars

B


Low Infection 0.1 g/l Yeast
Individuals
Days
Fourth Instars
Third Instars
Second Instars
First Instars

C

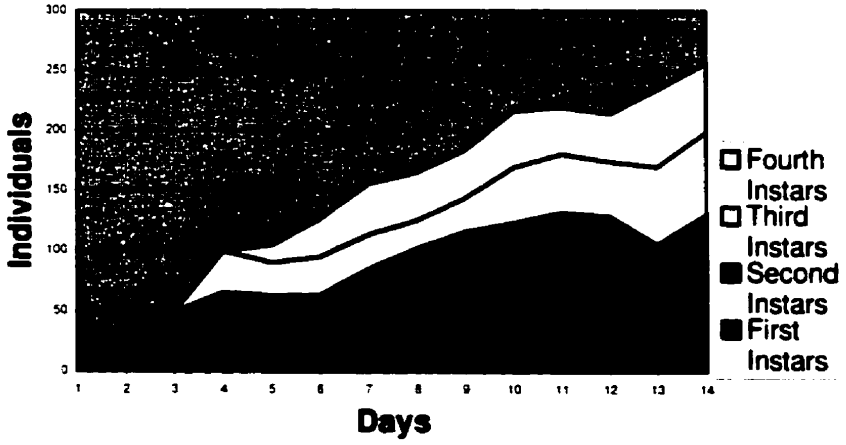
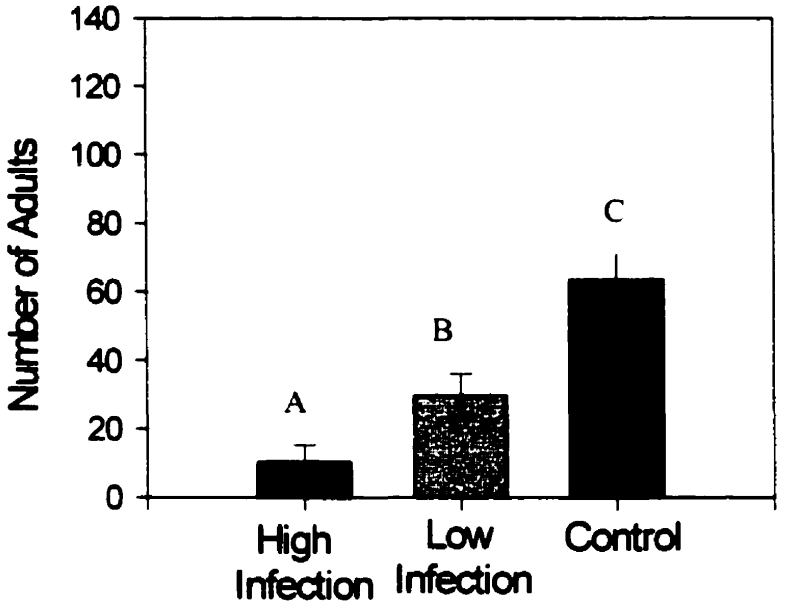

High Infection 0.1 g/l Yeast
Individuals
Days
Fourth Instars
Third Instars
Second Instars
First Instars

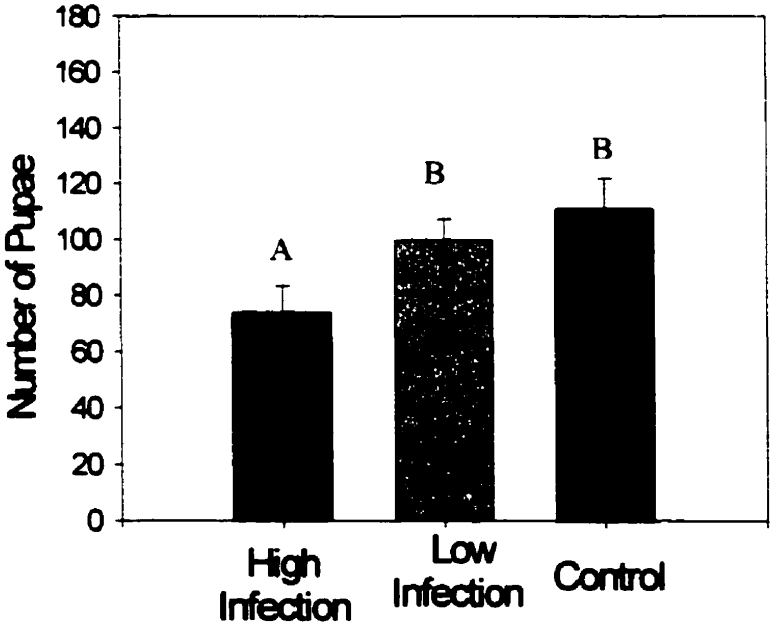
Figure 2.10: Number of total *Aedes aegypti* **A)** adults and **B)** pupae produced over the entire 14-day experimental period.

Error bars represent the standard error.

A



B



Bars with different letters are significantly different

Table 2.1*: Results of analysis of variance with repeated measures in time for time-dependent transition probabilities of *Aedes aegypti* populations exposed to different levels of *Plagiorchis elegans*..

Pr>F	P11	P12	P1d	P22	P23	P2d	P33	P34	P3d
Day	0.0010	0.0205	0.0003	0.0001	0.0001	0.0234	0.0001	0.0001	0.6436
Day × Infect	0.8180	0.4104	0.5767	0.5150	0.3050	0.7701	0.1770	0.1170	0.0676
Infect	0.07363	0.9298	0.0010	0.5703	0.5403	0.0074	0.1319	0.1700	0.0040

Pr>F	P44	P4p	P4d	Ppp	Ppa	Ppd
Day	0.0001	0.0001	0.0899	0.0726	0.0001	0.0880
Day × Infect	0.5858	0.3434	0.3349	0.1176	0.0576	0.6270
Infect	0.2700	0.2096	0.0276	0.6010	0.0110	0.0142

* Probabilities are significant if they are below 0.05.

Table 2.2*: Results of analysis of variance with repeated measures in time for relative abundance of *Aedes aegypti* at different instars in populations exposed to different levels of *Plagiorchis elegans*.

Pr>F	1st Instars	2nd Instars	3rd Instars	4th Instars	Pupae	Biomass
Day	0.0026	0.0006	0.0001	0.0001	0.0020	0.0001
Day × Infect	0.0666	0.0702	0.0101	0.5422	0.1261	0.0212
Infect	0.1168	0.9291	0.0222	0.1246	0.0072	0.0056

* Probabilities are significant if they are below 0.05.

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CONNECTING STATEMENT 2

In Chapter 2 it was demonstrated that at optimal food availability, exposure of *Aedes aegypti* to *Plagiorchis elegans* results in a significant reduction in adult production. This was due mainly to increased pupal mortality and reduced adult emergence. Ovipositional preference was not affected by exposure to the parasite, but was positively correlated with biomass. Container habitats are often poor in resources, and larvae may not develop optimally. Under such conditions, ovipositional repellence may be substantially lower than in the well-fed populations of the previous chapter. This may significantly affect recruitment into such populations. In addition, the effects of parasite exposure on population dynamics may differ in resource poor environments, as the death of individuals may decrease intraspecific competition. *P. elegans* may conceivably benefit larval development, and this may provide some insight into its efficacy as a potential natural enemy and biological control agent of mosquitoes. In Chapter 3, the interaction between pre-imago population dynamics and attractiveness of the water to ovipositing females is examined under conditions of suboptimal food availability.

CHAPTER 3

THE IMPACT OF SELECTIVE OVIPOSITION AND INFECTION WITH *PLAGIORCHIS ELEGANS* ON *AEDES AEGYPTI* PRE-IMAGO POPULATION DYNAMICS UNDER CONDITIONS OF SUB-OPTIMAL FOOD AVAILABILITY

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ABSTRACT

Larvae of *Aedes aegypti* were reared at two suboptimal levels of food availability and exposed to different concentrations of cercariae of the entomopathogenic digenean *Plagiorchis elegans*. Population structure, pre-imago development in terms of their probabilities of developing from one stage to the next or to die within 24h, as well as the progressive changes in the attraction/repellency of holding waters to ovipositing females, were documented, and their interaction was assessed. Starved larval populations displayed severely impaired development and produced very few adults. Exposure to *P. elegans* had little effect on pre-imago development and population structure, but increased adult production marginally, but not significantly, conceivably due to depensatory mortality. No correlation was found between total biomass of the larval population and ovipositional preference. However, the attractiveness of the water was increased significantly by pupation and pre-imago mortality. Nutritionally stressed populations, with or without exposure to the parasite did not attract conspecific ovipositing females. In natural habitats, which are frequently resource poor, adult production may be very low, and *P. elegans* may not reduce adult abundance of *Ae. aegypti* further.

INTRODUCTION

This study assesses the impact of the entomopathogenic digenean parasite *Plagiorchis elegans* on the development and pre-imago population structure of *Aedes aegypti*. Cercariae are produced asexually in the tissues of a molluscan first intermediate host, providing for sustained, daily releases of large numbers of these entomopathogenic organisms into the aquatic environment (Blankespoor, 1974). When cercariae encounter mosquito larvae they adhere, penetrate and accumulate in the tissues of this second intermediate host. The consequences of infection of mosquito larvae with the cercariae of entomopathogenic digeneans are severe (Dempster *et al*, 1986; Rao *et al*, 1985). Infection may cause developmental delays, debilitating physical abnormalities, elevated pre-imago mortality and greatly reduced adult emergence (Webber *et al*, 1986). Habitats of *A.aegypti* contain few non-target organisms, which might suffer from *P. elegans* introduction (Washburn, 1995)

When food is abundant, exposure of pre-imaginal *Ae. aegypti* populations to infection with *P. elegans* cercariae is characterized by severe pupal mortality and adult emergence but has little impact, either directly or indirectly, on the development of the larval population (Chapter 2). However, the habitat of *Ae. aegypti* and other container-breeding mosquitoes is frequently impoverished, and larvae must therefore compete for both food (Copeland and Craig, 1992) and space (Washburn, 1995). Such competition is primarily intraspecific and occurs during larval development (Washburn, 1995; Southwood *et al*, 1972; Lounibos *et al*, 1997). Since many species of mosquitoes hatch in instalments, competition tends to occur among cohorts (Livdahl, 1983). Later instars are superior competitors and their development tends to be density independent, whereas

earlier instars display density dependent growth (Southwood *et al*, 1972). In addition, later instars will frequently cannibalize younger conspecifics when food resources are scarce (Koenekoop and Livdahl, 1986).

Aedes aegypti larvae are remarkably resistant to starvation due to their ability to accumulate food reserves in the form of lipids (Timmerman and Briegel, 1999), and may thus survive much longer in resource-poor environments than groundwater-breeding species (Barrera, 1996). However, starvation may prevent pupation, defer development and reduce the size of emerging adults (Brust, 1968; Barrera, 1996). Larval nutrition also affects adult fitness. Large females are frequently more efficient at finding blood meals, and their blood meals are larger, giving them greater vector potential as well as higher fecundity (Novak et al, 1993; Nasci, 1986 a,b, 1988). Such females also exhibit greater reproductive success (Steinwascher, 1982).

Plagiorchis spp. infect and kill primarily late instars (Demster and Rau, 1990). When food resources are limited, late instar death may reduce competition with earlier instars and enhance the development of the latter, giving rise to more fit adults with greater vectorial capacity (Agudelo-Silva and Spielman, 1984; Hare and Nasci, 1986; Grill and Juliano, 1996). Furthermore, starvation, crowding and sublethal infection of *Ae. aegypti* larvae with *P. elegans* may render their waters repellent to ovipositing conspecific females of Ae. aegypti, whereas well-fed, uninfected individuals at moderate densities may render waters attractive (Soman and Reuben, 1970; Allan and Kline, 1998; Lowenberger and Rau, 1994; Zahiri and Rau, 1998; Maire 1984, 1985). Lowenberger and Rau (1994) found that the presence of cercariae in the water by itself had no effect on ovipositional behaviour, but that repellence was clearly caused by infected larvae.

The present study explores the impact of exposure to *P. elegans* cercariae on the pre-imago development and population structure of *Ae. aegypti* populations at suboptimal levels of food availability and at levels of larval recruitment that reflect the oviposition attraction/repellency of prevailing experimental conditions over time.

MATERIALS AND METHODS

Aedes aegypti

A colony of *Ae. aegypti* was maintained in the laboratory at 27±1° C with a photoperiod of 16 h light and 8 h dark. Adults were kept in nylon mesh and glass flight cages measuring 21cm × 21cm × 35cm. Adult females were blood-fed twice weekly and provided with 10% saturated sucrose solution. Oviposition sites consisted of 1liter plastic containers lined with filter paper and filled with 500ml of distilled water to a depth of 4 cm. Females deposited eggs on the filter paper, usually at the water line. Filter papers were removed every few days, and eggs were kept moist to allow full embryonic development. Eggs were hatched in a suspension of 2g/l brewer's yeast in water, which also served to feed the larvae.

Plagiorchis elegans

The life-cycle of *P. elegans* was maintained in the laboratory. A colony of the snail first intermediate host, *Stagnicola elodes,* was kept at 20±4°C and photoperiod of 16 h of light and 8 h of darkness. Snails were fed fresh lettuce and chalk ad libitum. Syrian hamsters, *Mesocricetus auratus,* served as experimental definitive hosts and were fed *P. elegans*-infected mosquito larvae by gavage to establish the adult infection. Parasite eggs are passed with the feces of the hamster hosts, and once embryonated, were fed to snails. *Plagiorchis* cercariae emerge from their host at dusk. Thus, infected snails were placed into 20 ml of aerated tap water just prior to scotophase in order to collect large numbers of cercariae. Mosquito larvae were then exposed to *P. elegans* by adding cercariae to the larval holding water. the cercariae penetrated the mosquito larval tissues to form

metacercariae. Details of these methods have been described previously (Lowenberger and Rau, 1993; Zakikhani and Rau, 1998).

Experimental Procedure

Experimental flight cages identical to those used for laboratory colonies were furnished with two oviposition sites each. Sites consisted of 1 liter plastic containers lined with white filter paper, and filled with 200 ml of distilled water. One of the sites in each cage served as a larval habitat once the first eggs hatched (the larval holding site). The other site contained only distilled water, an environment considered to be neutral to ovipositing females (the neutral site). Forty male and 40 female adult *A. aegypti* were introduced into each flight cage. This was done in batches of ten, over a period of 4 days, to obtain a mixed age population of egg laying females. Females thus had a choice between ovipositing into the neutral or the larval holding site. Each day, the (ovipositional) attractiveness of each larval holding site relative to that of distilled water was determined as the percentage of the total number of eggs laid in the corresponding flight cage that had been deposited into the larval holding site. Larval holding sites were considered attractive if this value was above 50%, and repellent if it was below 50%. In this manner, an attractiveness index was defined.

Mosquitoes were blood-fed daily and provided with 10% saturated sucrose solution on a cotton wick ad libitum. Experimental colonies in flight cages were maintained in the same manner as standard laboratory colonies. Brewer's yeast in one of two sub-optimal amounts (0.02 g/l and 0.03 g/l, based on preliminary experiments), was added daily to the larval holding sites as a source of food for larvae and a medium for bacterial growth. No yeast was added to the neutral sites.

Data collection began four days after the first females started to oviposit. Filter papers with attached eggs were removed daily from all sites, and eggs were counted and incubated at 100% R.H. and 27±1°C for two days to allow full embryonic development (conditioning) (Christophers, 1960). Liquid from larval holding sites was allowed to drip back through the old filter (lining the site) into the respective containers, which had been lined with fresh filter paper, to maintain continuity of the aquatic environment and its chemical properties. In contrast, water and filters in the neutral sites were renewed daily in order to maintain a constant neutral reference point.

After conditioning, eggs collected from the larval holding sites were hatched in a suspension of brewer's yeast. In order to generate realistic pre-imago populations, the number of larvae added daily to larval holding sites reflected a fixed proportion of the eggs that had been deposited into this particular site over the day preceding the conditioning period. To maintain a population of manageable size, this proportion was arbitrarily fixed at 42.5%, 1/2 of the hatchability of 85% determined in preliminary experiments. A greater proportion than in chapter two was chosen to further increase the nutritional stress. No larvae were added to neutral sites.

In addition, larval populations in the larval holding sites of three flight cages per food level were subjected to treatment with exposure to a low concentration of cercariae (20/ml) and those of three further flight cages per food level to treatment with exposure to a high concentration of cercariae (40/ml). These were compared to the larval populations in the larval holding sites of three further flight cages per food level (controls), which were not exposed. Larvae were exposed to *Plagiorchis* by adding the appropriate number of cercariae to obtain correct concentrations to the larval holding

sites. The numbers of cercariae added were estimated volumetrically on the basis of ten 20 µl samples of cercarial suspensions produced by laboratory-infected snails. No cercariae were added to the neutral sites of any flight cages.

A complete census of live and dead larval instars, pupae, and adults in each larval holding site was conducted each day in order to document progressive changes in the population. Pupae were transferred to individual vials filled with distilled water to allow subsequent retrieval of the emerged adults. Adults were sexed and counted. Every two days, any dead larvae were crushed and examined for infection under a compound microscope at a magnification of 100×, and carcasses were then returned to the corresponding sites. This was done to insure infection had occurred. Dead larvae were not removed from the sites, as they provide an alternate food source.

Each flight cage represented one experimental unit. Data were collected daily for 12 days. Three replicates for each combination of food level (low, high) and parasitic treatment (low, high, control) were performed. The experiment followed a completely randomized design with repeated measures in time, with two treatment factors, one with two, the other with three levels.

Transition Probabilities

In order to examine the development of the population, transition probabilities were calculated. These calculations were based on an adaptation of a Markov-chain-like model developed by Dr. Pierre Dutilleuil (McGill University, Department of Plant Science), and first applied to mosquito development by Nguyen (1998). A transition probability is defined as the probability that one stage will develop to the next stage within a fixed time interval. For this study, probabilities were calculated from raw data

for 24-hr periods, as presented in Figure 3. 1. All probabilities were calculated for each of the 12 days creating time series for each probability. Calculations were performed using Microsoft Excel 97 spreadsheets.

Statistical Analyses

Mean time-dependent transition probabilities and ovipositional preference were calculated and graphed using Sigma plot software. Analysis was then performed using an analysis of variance with repeated measures in time with days as the repeated factor using SAS 6.1 software (SAS Institute Inc., 1999). The general linear models procedure with its statement REPEATED was applied. Results of the univariate and multivariate approach were used as appropriate. When data failed to meet circularity conditions, an adjustment of the probabilities of significance of the univariate ANOVA F-tests was done using the Huin-Feld Epsilon correction factor.

Repeated measures ANOVA was also used to analyse total biomass and individual instar abundance. Biomass was expressed as fourth instar equivalents. The biomass of fourth instars is generally 40 times greater than that of first instars, 12 times greater than second instars, and 2.2 times greater than third instars (Christophers, 1960). Total adult production was compared using ANOVA tests with SAS software. Post hoc comparisons were made using multiple t-tests using the Student-Newman-Keuls approach. Proportional data were arcsine square root transformed, and population count data were subjected to square root transformation (Zar, 1993).

Cross-Correlations of Time Series

To determine whether ovipositional preference was affected by development, cross-correlations between time series of ovipositional preference and probabilities were conducted. The procedure ARIMA in SAS economics and time series was used to perform these calculations. Proc ARIMA uses the Box and Jenkins (1976) method to create models of time series. Cross correlations were calculated using the 'identify' statement. Linear correlations between points are made between values of the different time series for different time lags. No attempts at model building were made in this study.

RESULTS

The development of first instars (P11, P12) was significantly affected by time (p=0.0118; p=0.0001), and P12, but not P11, differed with food level (p=0.001); there was no significant effect of infection (p>0.05 in all cases). First instar larval mortality remained unchanged regardless of levels of exposure, but differed significantly with food and with time (p=0.0025 and p= 0.0001 respectively) (Figures 3.1 and 3.2). The total number of first instars (Figures 3.15 and 3.15) did not differ significantly between treatments, but did change over time.

Second instar development (Figures 3.3 and 3.4) was affected by time (P22: p=0.0043; P23: p=0.001) and P23 was affected by nutritional levels (p= 0.0003). Mortality followed a similar pattern in response to time and food level (p=0.0148; p=0.0001), and both development (P23) and mortality of second instars was significantly affected by exposure to *P. elegans* (P23: p=0.0283; P2d: p=0.0336). The number of second instars (Figures 3.15 and 3.16) did not change significantly with treatments or with time.

The probability that third instars remained at this stage was significantly affected by time (p=0.0048), but not by food or by exposure to the parasite. The probability that third instars would change to the next stage was affected significantly by food level (p=0.0019) and by time (p=0.0372). As well, mortality differed significantly with time (p=0.0189), with food (p=0.002), but not with exposure to *P. elegans* (p= 0.1767) (Figures 3.5 and 3.6). The numbers of third instars did not differ significantly with treatments, but changed significantly over time (Figures 3.15 and 3.16).

Analysis of fourth instar development suggested a significant effect only in terms of the probability that they remain at that stage over time ($p=0.014$) of food level ($p=0.0027$). Fourth instar mortality and pupation (Figures 3.7 and 3.8) showed no significant differences over time with infection or with food availability. The number of fourth instars in the population (Figures 3.15 and 3.16) was significantly affected by food level and time, but not by infection. However, due to the scarcity of data from fourth instars these probabilities may be unreliable.

At a food level of 0.02g/l of yeast no emergence of adults occurred. For the slightly higher food level of 0.03g/l, adult emergence, as well as pupal death and Ppp could not be analyzed by repeated measures ANOVA, since the small size of the data set did not allow a test for circularity. Nevertheless only parasite-exposed populations produced adults (Figures 3.9 and 3.10), albeit in very low numbers. Highly exposed populations produced an average of three adults and populations exposed to a lower number of parasites produced an average of two adults. These numbers were too low to draw any statistically significant conclusions.

The overall biomass of the population (Figures 3.11 and 3.12) differed significantly with food levels ($p=0.0002$) and time ($p=0.0014$). There was no significant difference in biomass between populations exposed to different levels of the parasite or between those and the control.

There was no significant effect of food level, parasite load, or time on the ovipositional choice of A*e. aegypti* females ($p>0.05$ in all cases) (Figures 3.13 and 3.14). All P values obtained from repeated measures ANOVA are presented in Table 3.1 and Table 3.2.

Cross-Correlations of Time Series

No significant correlation was found between biomass and oviposition preference for any of the food level-parasite exposure combinations. Significant correlations were found between oviposition preferences and third instar death. At high levels of parasite exposure and 0.02g/l of yeast there was a significant correlation (factor: 0.659) with a time lag of one day. A significant correlation (factor: 0.593) without a time lag was also found at low levels of parasite exposure and 0.03g/l of yeast.

Pupation was significantly correlated with ovipositional preference on several occasions. Pupation of control populations supplied with 0.03g/l of yeast was significantly correlated at a time lag of -2 days (factor: -0.700). At high levels of parasite exposure and a food level of 0.03g/l, there was a significant correlation (factor: -625) without a time lag. At low levels of parasite exposure and a food level of 0.03g/l, a significant correlation (factor: 0.646) occurred with a time lag of one day.

DISCUSSION

Food availability has a strong impact on the dynamics of mosquito populations. In this study, population dynamics were examined under conditions of low food availability. Such populations showed reduced size, retarded development, high mortality, as well as low adult production, characteristics similar to those reported by Brust (1968). First instars (Figures 3.2 and 3.3) manifested near-normal development to the second instar over the first few days, but thereafter these transition probabilities declined and remained consistently low. The development of poorly fed first instars was considerably slower than that of the optimally fed individuals in the previous chapter. Populations fed 0.02 g/l of yeast per day manifested almost no development beyond the first few days. The development of such first instar populations was significantly slower than in populations fed 0.03g/l of yeast. As P11 was not significantly affected by food levels, it may be presumed that the reduction of first instar development (P12) at the lower food level was due to an increase in mortality (Figure 3.2 and 3.3). The presence of the parasite did not benefit the development of first or second instars, as reported by Nguyen (1998). *Plagiorchis* affects primarily large, late instars (Dempster and Rau, 1987). Since nutritional stress decreased the growth and development of larvae, fewer individuals may have acquired the infection and succumbed to the parasite. As a result, the increase in food resources due to the removal of competition and the concomitant increase of food in the form of dead larvae (Barbosa and Peters, 1973) may have been too slight to improve early instar development. Similarly, early larval mortality did not increase significantly in the presence of the parasite as in the previous chapter. Larval mortality due to starvation

was conceivably the primary cause of death, and may have overshadowed any effects of the parasite.

Exposure to the parasite significantly increased mortality of second instars (Figures 3.3 and 3.4). This mortality increased towards the end of the experimental period, concomitant with an increase in the development of second instars. As time progressed, late instars (Figures 3.5- 3.8) commenced to pupate or died. Such deaths may have increased resource availability and allowed for some development of survivors. The consequence of such accelerated development may have been a further increase in mortality. Larvae must attain a critical weight to initiate moulting (Chambers and Klowden, 1990); with improved resources, this critical weight may have been attained but individuals may have been too weak to moult successfully, and died in the process, as suggested by Nguyen (1998). However, Timmerman and Briegel (1999) question the theory of a moulting threshold, and argued that individuals will utilize lipid reserves until enough protein becomes available to moult.

Although not statistically significant, population structure (Figures 3.15 and 3.16) reveals that there is a larger number of third instars in populations exposed to low levels of *P. elegans*. It is conceivable that, at low levels of exposure, parasite-induced mortality increased food resources sufficiently to allow second instars to moult into thirds, at which stage they would acquire *P. elegans* infection. Sublethally infected individuals may not have been able to moult to the next stage. Consequently, in spite of a slight and statistically insignificant elevation of mortality, there was a noticeable accumulation of third instars.

At food levels of 0.02g/l of yeast, moulting from third to fourth instars occurred towards the end of the 12 days (Figure 3.5), whereas when 0.03g/l of yeast was provided, third instars developed early on, and mortality increased towards the end of the experiment (Figure 3.6). Perhaps increasing nutritional stress, as the number of late instars increased, led to unsuccessful moulting and death.

At the lowest food level, in the absence of the parasite, very few fourth instars developed, and fewer still pupated successfully (Figure 3.7). However, fourth instar mortality occurred only in parasite-exposed populations. When food was increased to 0.03g/l (Figure 3.8), fourth instar numbers increased significantly. Pupation was greater in infected than in control populations, although this difference was not statistically significant. Furthermore, although no repeated measures analysis could be done, it appeared that both pupal death and adult emergence were higher in infected than in control populations (Figure 3.10). This may have been due to a decline in feeding activity by heavily infected fourth instars (Webber *et al*, 1986; Zahiri and Rau, 1998), which may have decreased competition for limited food resources among uninfected and lightly infected fourth instars in the population, allowing some to pupate and later to emerge as adults. In contrast, later instars in control populations may have been more stressed nutritionally, and thus were less likely to pupate or emerge successfully. Parasite exposed populations yielded adult mosquitoes whereas uninfected populations did not. Such depensatory mortality has been reported by Agudela-Silva and Spielman (1984), Vanni (1987) and Washburn (1995). Adult emergence in the face of parasite exposure was low, with an average of only 3 adults produced over the 10-day period, substantially less than the mean of more than 60 adults produced in optimally fed populations over 14 days in

the previous chapter. Nevertheless this phenomenon should be taken into consideration when applying this or other biological agents for the control of mosquitoes.

The effect of the parasite in terms of late instar mortality and enhanced development of early instars, as observed by Nguyen (1998) and Hartley (1996) was not apparent in this study. Although there was a clear accumulation of first instars, it was somewhat less pronounced than in the studies cited above. Populations in this experiment were reared at much lower levels of food availability. Such extreme conditions may have increased mortality due to starvation and cannibalism, thus reducing the build-up of first intars that may occur under somewhat more favourable environmental conditions (Wada, 1976). Furthermore, the total biomass of the population (Figures 3.11 and 3.12) did not differ significantly between treatments, although the figures suggest a reduction in biomass as a result of exposure to parasites. Since only small numbers of late, large instars were produced, there were conceivably fewer targets for the parasite, and thus its impact may have been less severe. The biomass of the population increased slowly, and appeared to level off towards the end of the experiment. At 0.02 g/l of yeast, the population stabilized at a much lower level, as starvation was very severe.

Ovipositional preference in this study (Figures 3.13 and 3.14) was not significantly affected by time, food level or level of exposure to the parasite. This is a complex system, with numerous factors and interactions, which may potentially influence female oviposition. In this study, no correlation was found between ovipositional preference and biomass. Stressed populations have been shown to have a repellent effect on ovipositional preference (Zahiri *et al*, 1997). In the previous chapter, at high levels of food availability, a strong correlation between ovipositional preference and biomass was

found. Thus, it is likely that at low levels of food availability larvae were stressed and their holding waters were no longer attractive.

Significant correlations were found between third instar deaths and oviposition with a time lag of one day. Third instar deaths are likely the deciding factor, as, due to the absence (0.02 g/l yeast) or comparatively low numbers (0.03 g/l yeast) of fourth instars, third instars will be the largest larvae in the population, thus being most susceptible to infection, as well as consuming the greatest proportion of food. Deaths of third instars thus reflect best the overall severity of parasitic infection, and have the greatest effect on the food resources. In addition fourth instar deaths do not reflect levels of infection as well as third instar death, since fourth instars frequently survive infection.

At low food levels and low exposure, no correlation was found. The low level of infection may not have been high enough to influence the relatively small number of infectable later instars. At the higher level of exposure, however, a correlation was found between third instar deaths and oviposition. This may indicate that third instar deaths from infection were frequent enough to slightly relieve the remaining larvae from starvation, as they were thus removed from competition. This in turn may have increased the attractiveness of the water. At high food levels, a correlation between third instar deaths and oviposition was found at low levels of infection. Due to the greater overall number of third instars in this slightly more favourable environment, more individuals were susceptible to infection. In consequence, a lower level of exposure may have been needed to raise the death toll of third instars to a level at which it will begin noticeably to relieve other larvae from starvation. However, no correlation between third instar deaths and oviposition was found at the higher food level and high exposure. This may indicate

that the repellent released by sublethally infected third and fourth instars (presumably reflected in third instar deaths) began to outweigh the decrease in repellent production due to relief from starvation.

Some other correlations were found, but it is difficult to interpret with any degree of certainty as to which of the several interacting factors, such as starvation, infection and death, are of primary importance.

It becomes clear from these results that under conditions of nutritional stress, a number of factors may act on ovipositional preference, and that the outcome may be difficult to predict. However, under conditions of variable recruitment, when nutritionally stressed individuals are exposed to the parasite, there is the danger of compensatory and depensatory mortality. Further investigations in natural populations are necessary to assess the role of *P. elegans* as a natural enemy of mosquitoes and as a potential agent in their biological control.

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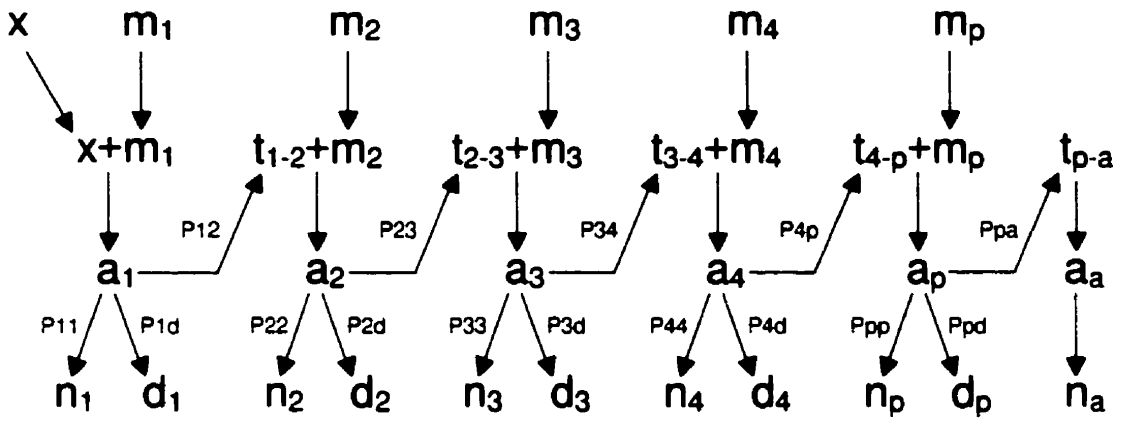
Figure 3.1: Time-dependent Transition Probabilities. **Pxx** represents the probability that an instar remains at stage **x** in 24 h; **Pxy** represents the probability that an instar will move from stage **x** to stage **y** in 24 h. **Pxd** represents the probability that an instar of stage **x** will die in 24 h. **X** is the number of 1^{st} instars added each day, **a** represents the total number of dead and live larvae on the present day, **m** is the larvae that were present the previous day, **n** is the live larvae counted the present day, and **d** is the dead larvae counted on the present day. **t** is the number of larvae that transformed from the previous stage (e.g. t_{3-4}: number of larvae changed from 3^{rd} to 4^{th} instar).



Probabilities:

$P11=n_1/a_1$
$P12=t_{1-2}/a_1$
$P1d=d_1/a_1$
$P22=n_2/a_2$
$P23=t_{2-3}/a_2$
$P2d=d_2/a_2$
$P33=n_3/a_3$
$P34=t_{3-4}/a_3$
$P3d=d_3/a_3$
$P44=n_4/a_4$
$P4p=t_{4-p}/a_4$
$P4d=d_4/a4$
$Ppp=n_p/a_p$
$Ppa=a_a/a_p$

$a_1=x+m_1$
$a_2=t_{1-2}+m_2$
$a_3=t_{2-3}+m_3$
$a_4=t_{3-4}+m_4$
$a_p=t_{4-p}+m_p$
$a_a=n_a$

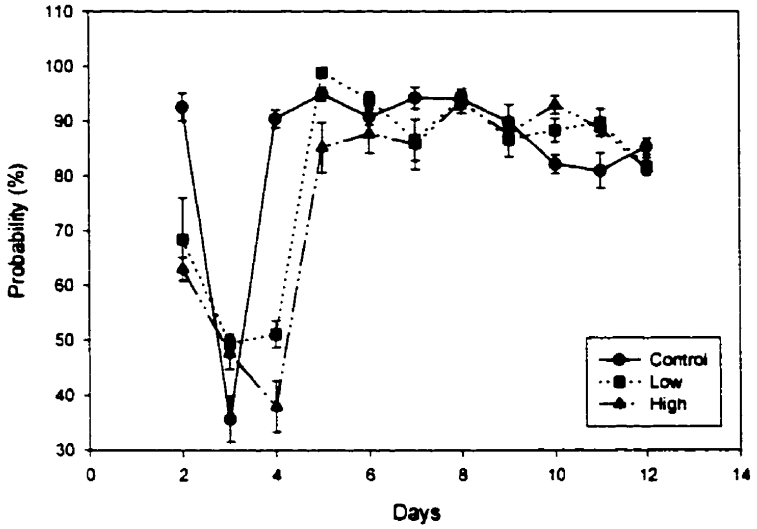
$t_{1-2}=(x+m_1)-n_1-d_1$
$t_{2-3}=m_2-n_2-d_2$
$t_{3-4}=m_3-n_3-d_3$
$t_{4-p}=m_4-n_4-d_4$

Figure 3.2: Time series of mean twenty-four hour transition probabilities of first instar *Aedes aegypti* **A)** to remain at that stage (P11), **B)** to transform from the first to the second instar (P12) or **C)** of first instars to die (P1d), for control populations, populations exposed to a low or a high level of *Plagiorchis elegans*, and fed 0.02g yeast/l.

Error bars represent the standard error.

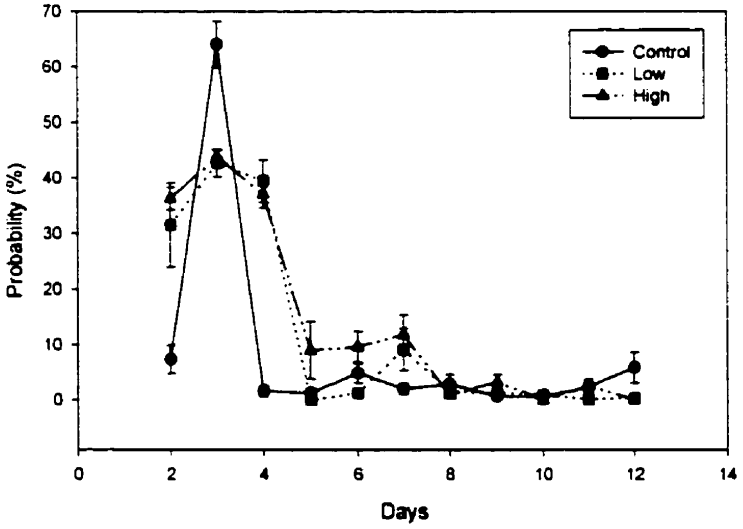
P11

A



P12

B



P1d

C

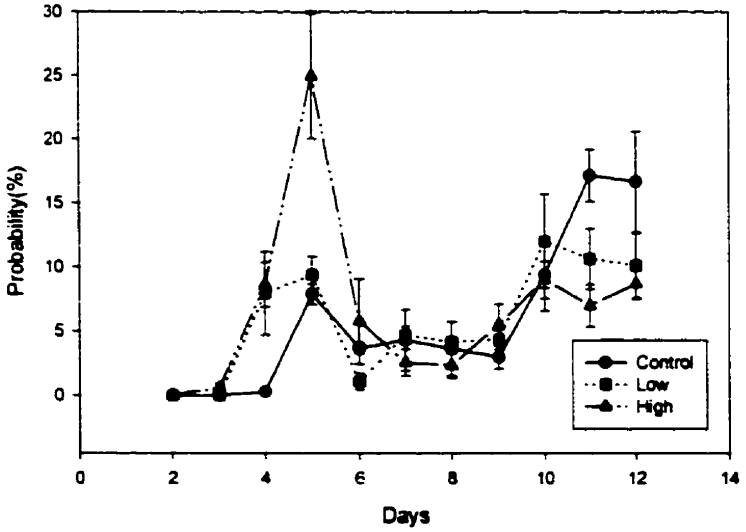
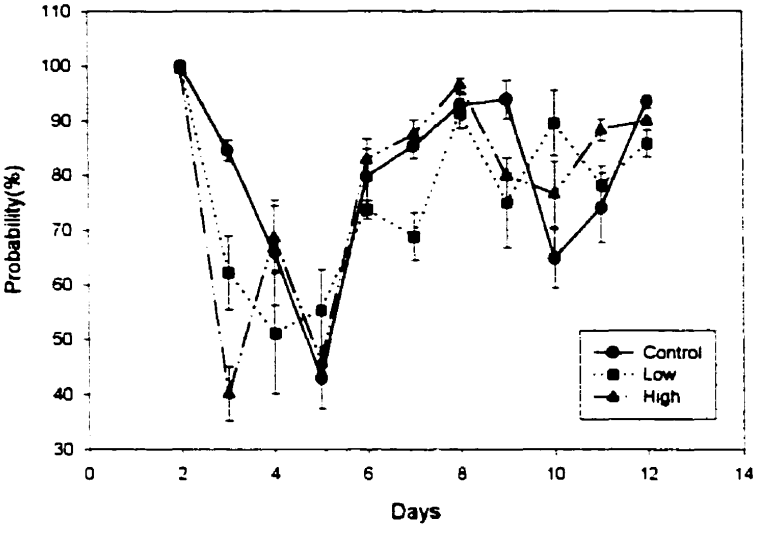


Figure 3.3: Time series of mean twenty-four hour transition probabilities of first instar *Aedes aegypti* **A)** to remain at that stage (P11), **B)** to transform from the first to the second instar (P12) or **C)** of first instars to die (P1d), for control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.03g yeast/l.

Error bars represent the standard error.

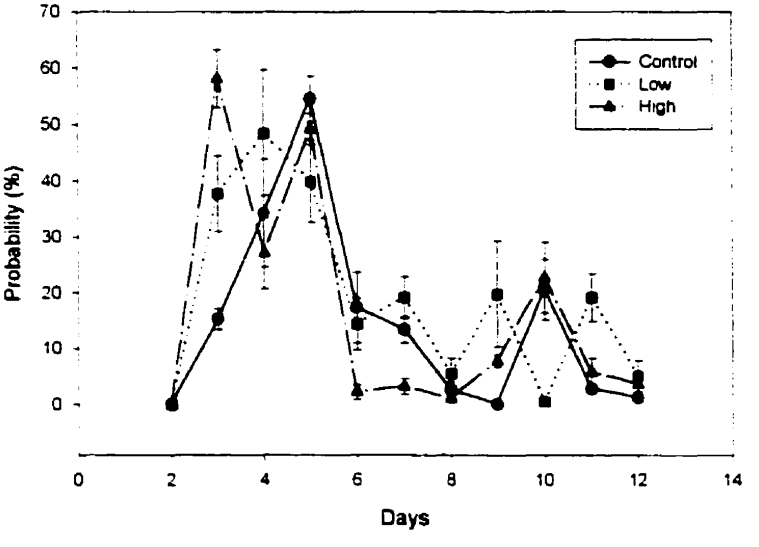
P11

A



P12

B



P1d

C

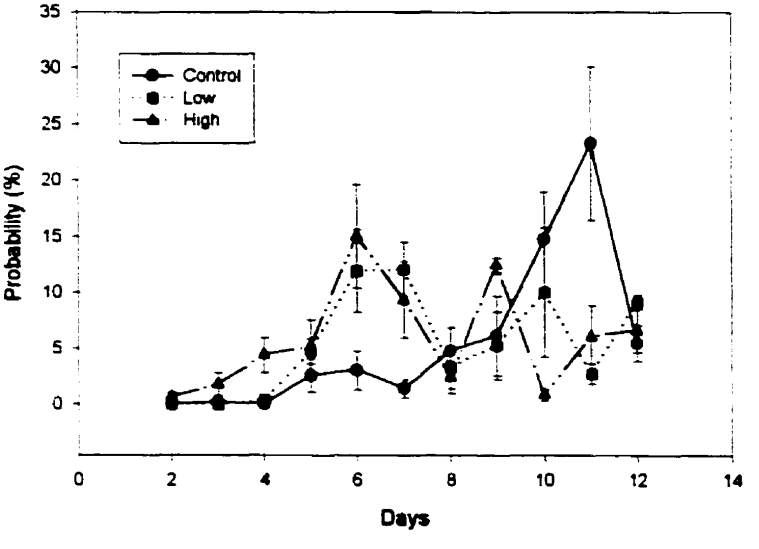
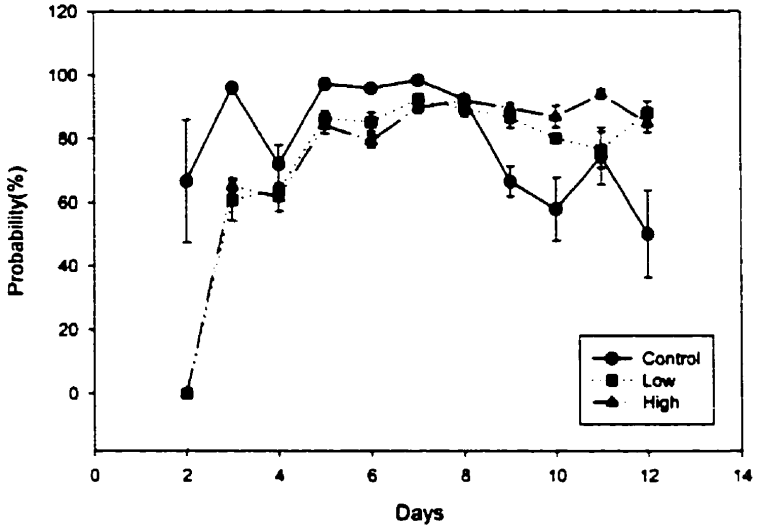


Figure 3.4: Time series of mean twenty-four hour transition probabilities of second instar *Aedes aegypti* **A)** to remain at that stage (P22), **B)** to transform from the second to the third instar (P23) or **C)** of second instars to die (P2d), for control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.02g yeast/l.

Error bars represent the standard error.

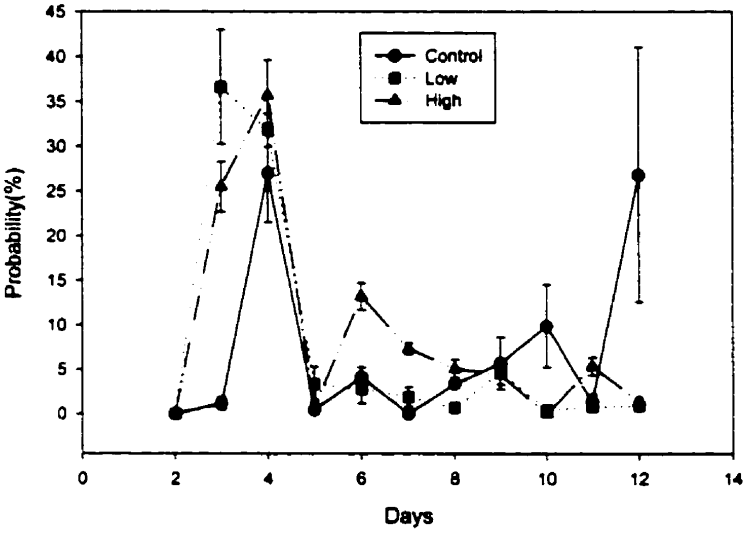
P22

A



P23

B



P2d

C

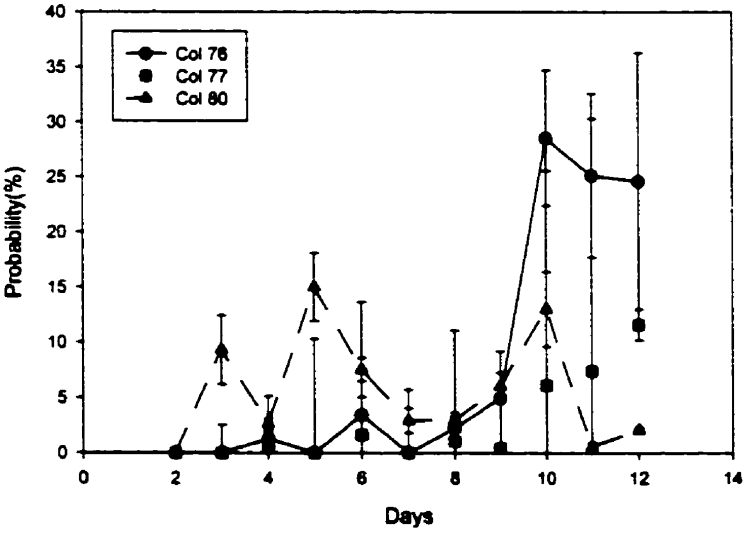
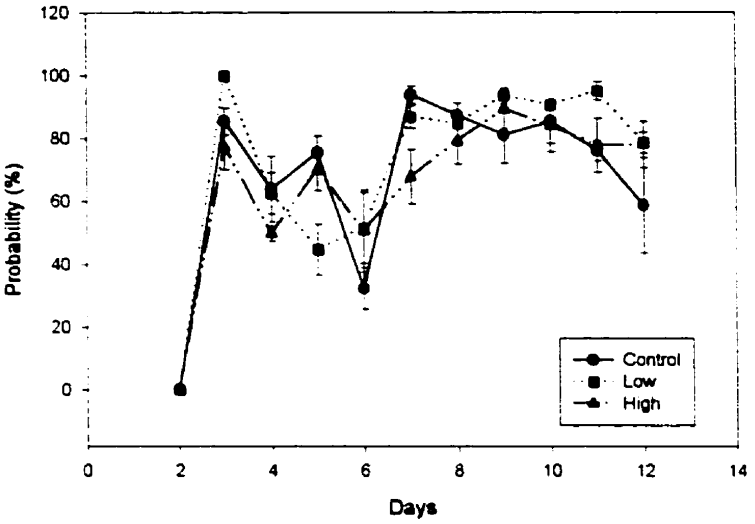
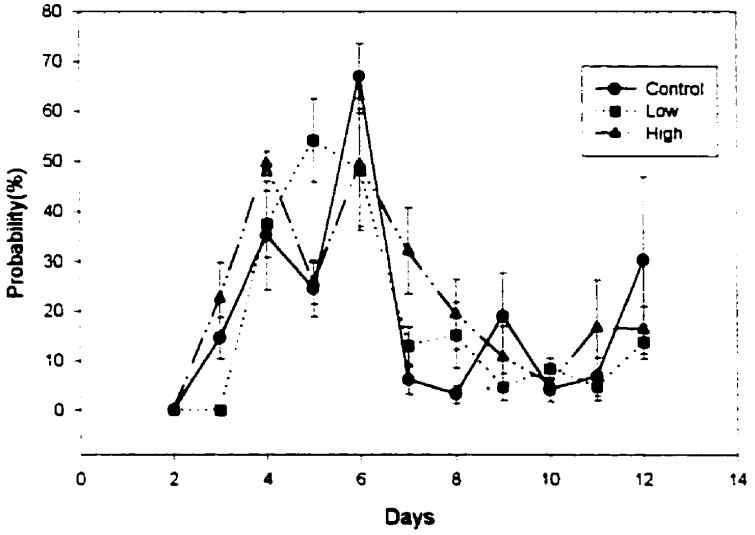


Figure 3.5: Time series of mean twenty-four hour transition probabilities of second instar *Aedes aegypti* **A)** to remain at that stage (P22), **B)** to transform from the second to the third instar (P23) or **C)** of second instars to die (P2d), for control populations, populations exposed to a low or to a low or high level of *Plagiorchis elegans*, and fed 0.03g yeast/l. Error bars represent the standard error.

A


P22
Probability (%)
Days
Control
Low
High

B


P23
Probability(%)
Days
Control
Low
High

C

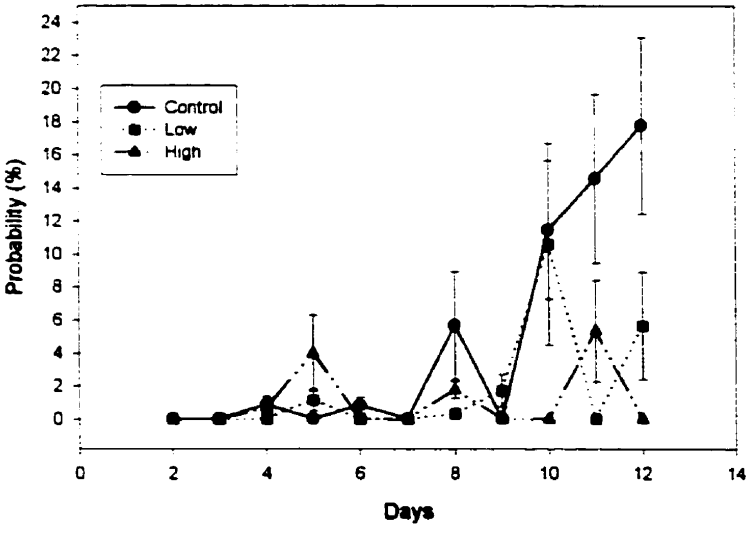
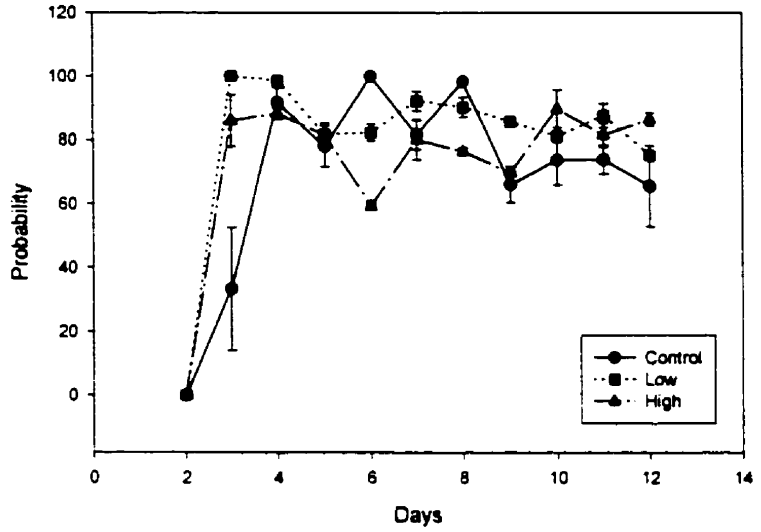

P2d
Probability (%)
Days
Control
Low
High

Figure 3.6: Time series of mean twenty-four hour transition probabilities of third instar *Aedes aegypti* **A)** to remain at that stage (P33), **B)** to transform from the third to the instar (P34) or **C)** of third instars to die (P3d), for control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.02g yeast/l.

Error bars represent the standard error.

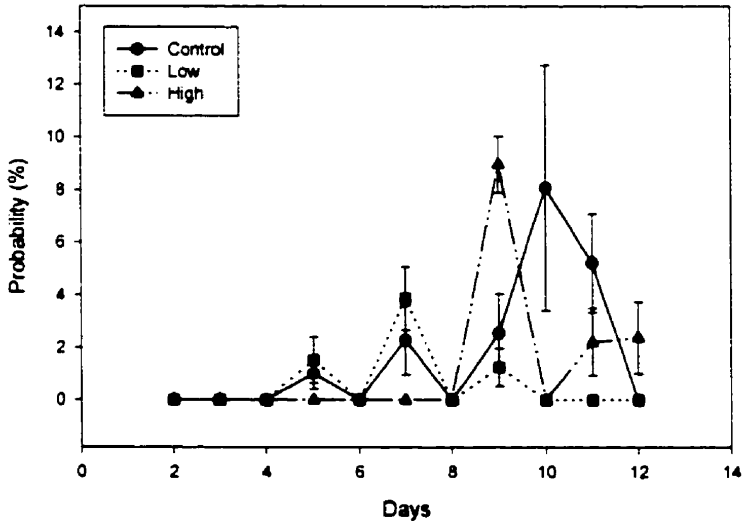
P33

A



P34

B



P3d

C

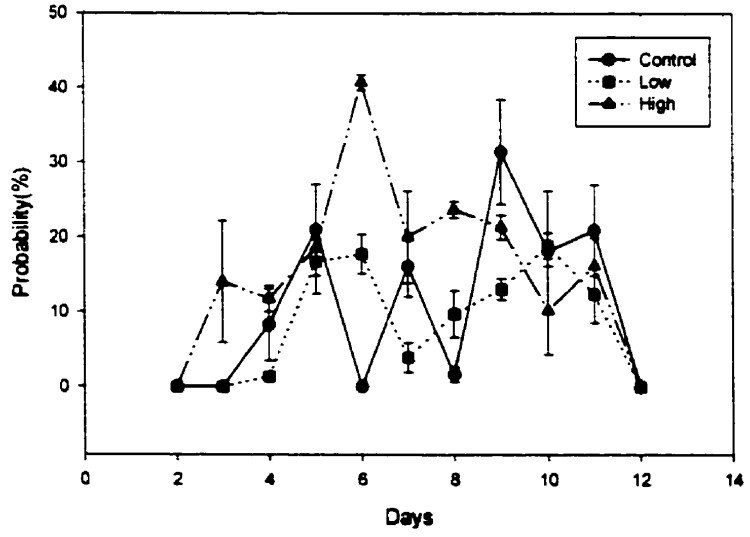
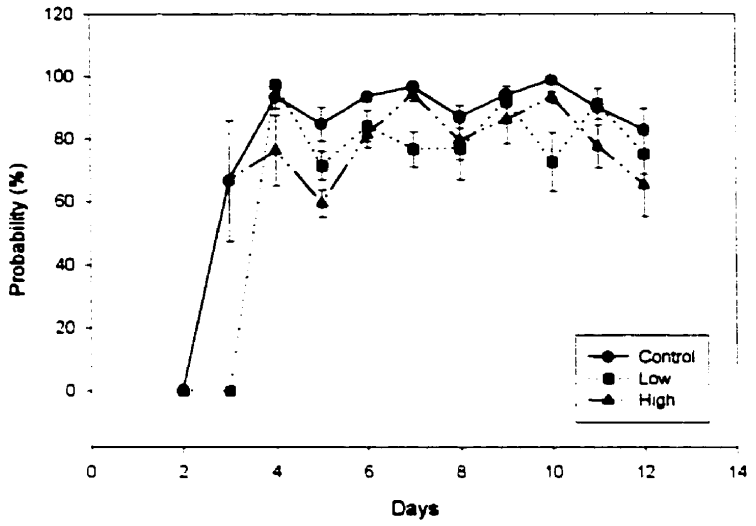


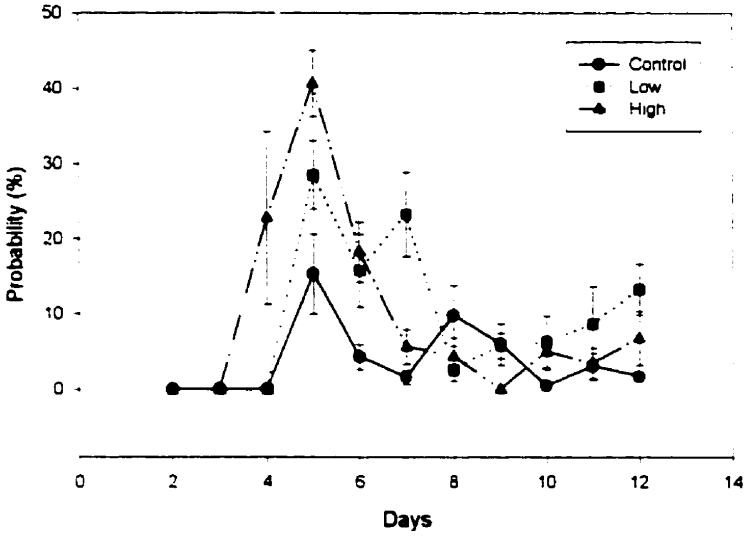
Figure 3.7: Time series of mean twenty-four hour transition probabilities of third instar *Aedes aegypti* **A)** to remain at that stage (P33), **B)** to transform from the third to the fourth instar (P34) or **C)** of third instars to die (P3d), for control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.03g yeast/l.

Error bars represent the standard error.

A


P33
Probability (%)
Days
Control
Low
High

B


P34
Probability (%)
Days
Control
Low
High

C

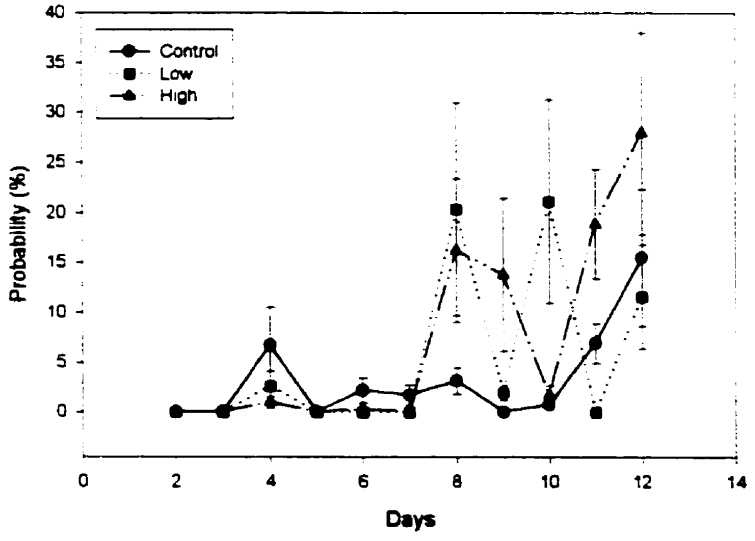
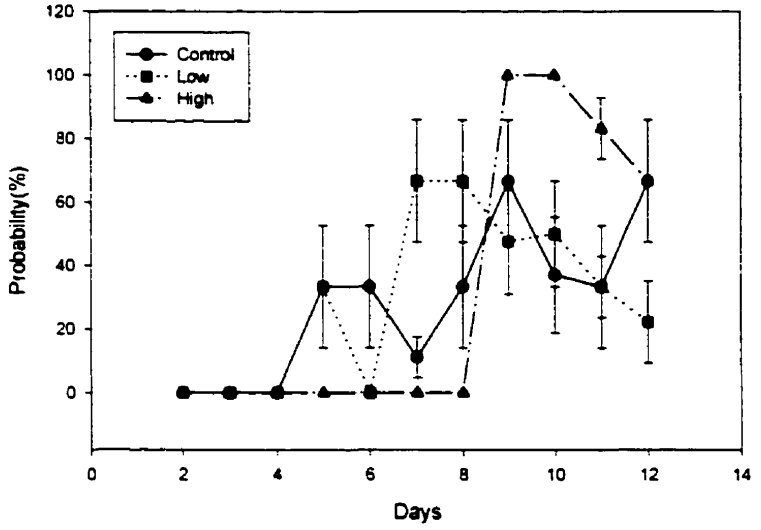

P3d
Probability (%)
Days
Control
Low
High

Figure 3.8: Time series of mean twenty-four hour transition probabilities of fourth instar *Aedes aegypti* **A)** to remain at that stage (P44), **B)** to transform from the fourth instar to pupa (P4p) or **C)** of fourth instars to die (P4d), for control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.02g yeast/l.

Error bars represent the standard error.

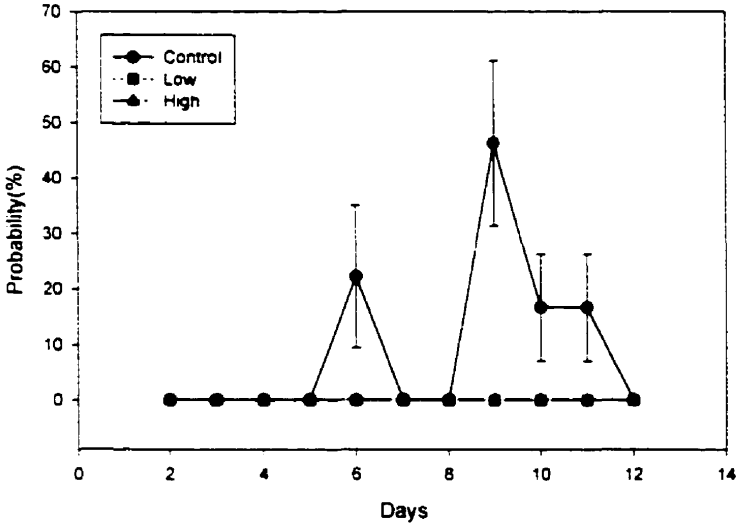
P44

A



P4p

B



P4d

C

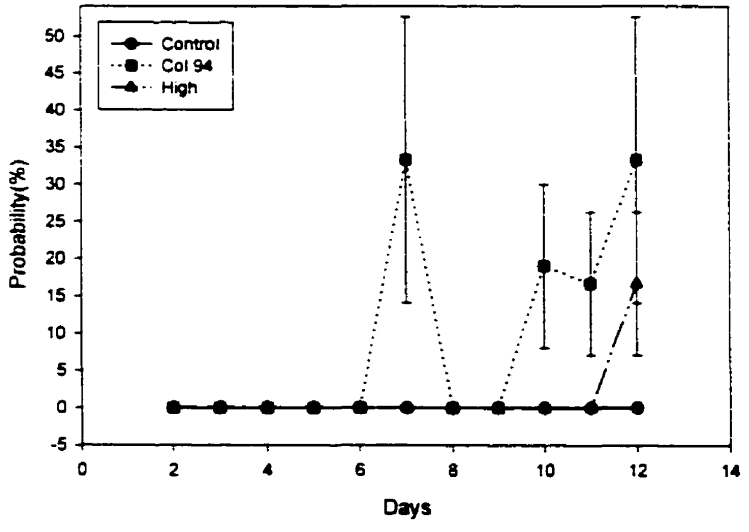
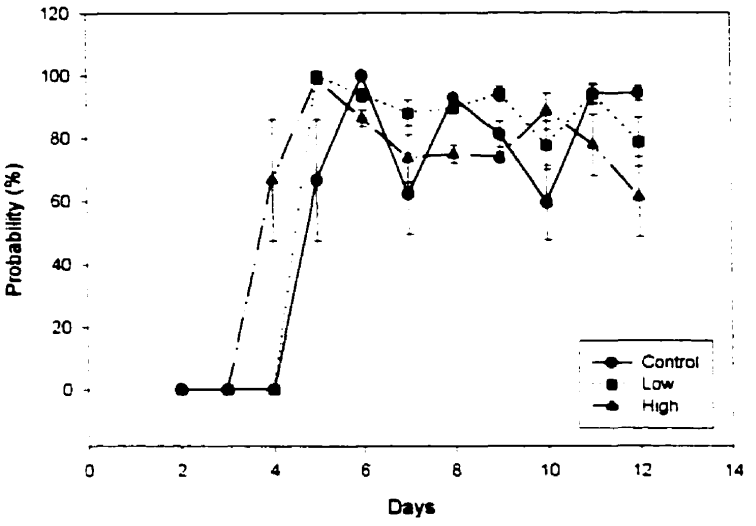


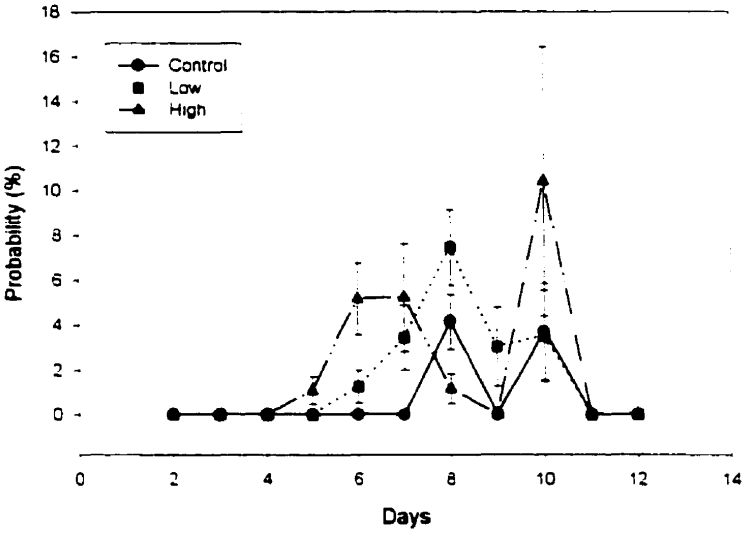
Figure 3.9: Time series of mean twenty-four hour transition probabilities of fourth instar *Aedes aegypti* **A)** to remain at that stage (P44), **B)** to transform from the fourth instar to pupa (P4p) or **C)** of fourth instars to die (P4d), for control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.03g yeast/l.

Error bars represent the standard error.

A


P44
Probability (%)
Days
Control
Low
High

B


P4p
Probability (%)
Days
Control
Low
High

C

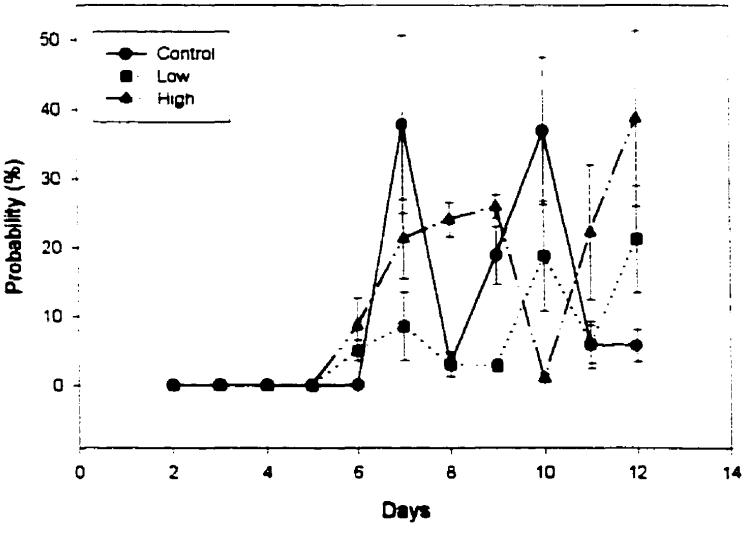
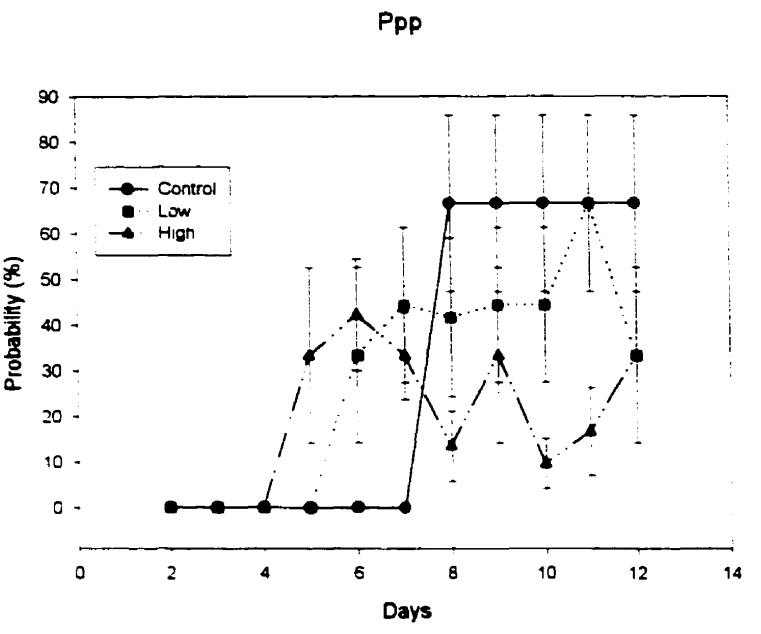

P4d
Probability (%)
Days
Control
Low
High

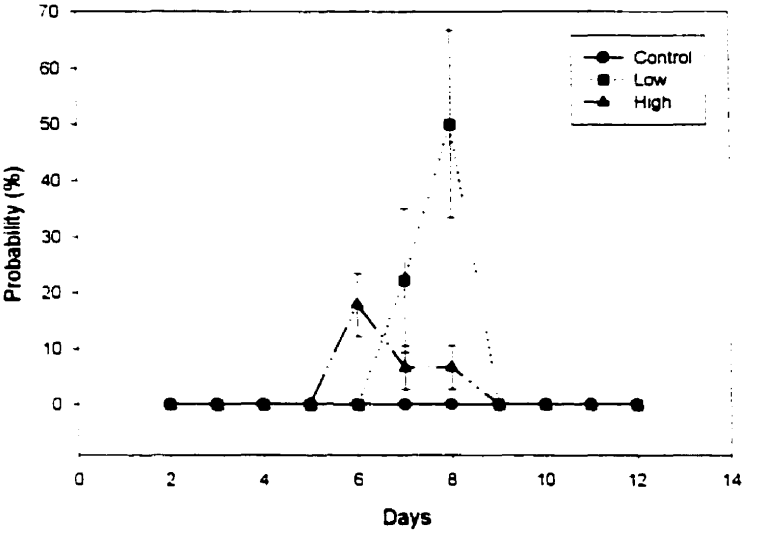
Figure 3.10: Time series of mean twenty-four hour transition probabilities of **A)** pupal *Aedes aegypti* to remain at that stage (Ppp), **B)** to emerge as adults (Ppa) or **C)** pupae to die (Ppd), for control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.03g yeast/l.

Error bars represent the standard error.

A


Ppp
Control
Low
High
Probability (%)
Days
0
10
20
30
40
50
60
70
80
90
0
2
4
6
8
10
12
14

B


Ppa
Control
Low
High
Probability (%)
Days
0
10
20
30
40
50
60
70
0
2
4
6
8
10
12
14

C

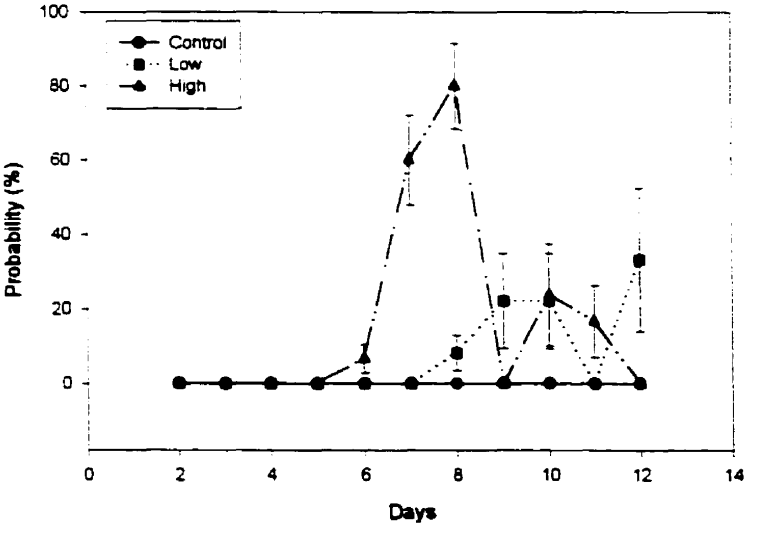

Ppd
Control
Low
High
Probability (%)
Days
0
20
40
60
80
100
0
2
4
6
8
10
12
14

Figure 3.11: Biomass of *Aedes aegypti* larvae of control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.02g yeast/l. One biomass unit represents the equivalent of one fourth instar.

Error bars represent the standard error.

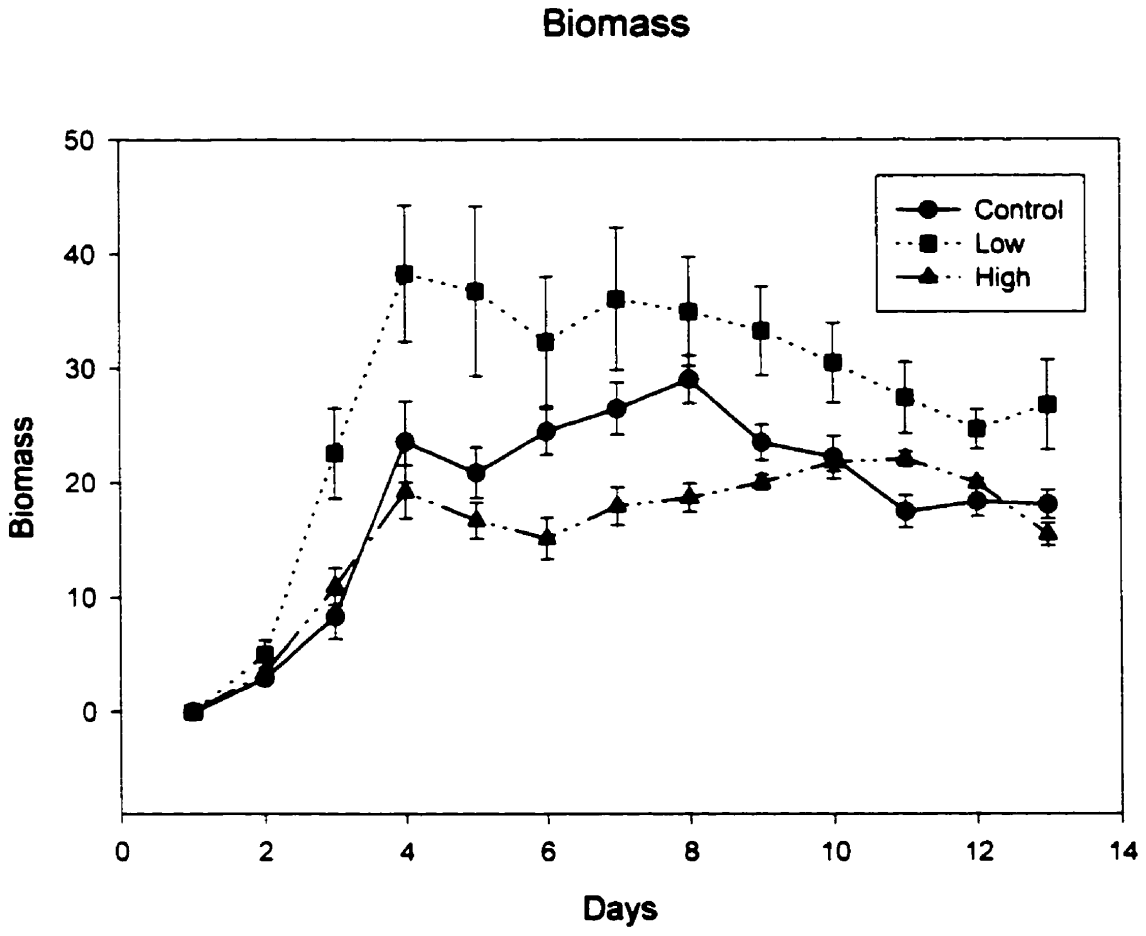

Biomass
Biomass
Days
Control
Low
High
50
40
30
20
10
0
0
2
4
6
8
10
12
14

Figure 3.12: Biomass of *Aedes aegypti* larvae of control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.03g yeast/l. One biomass unit represents the equivalent of one fourth instar.

Error bars represent the standard error.

Biomass

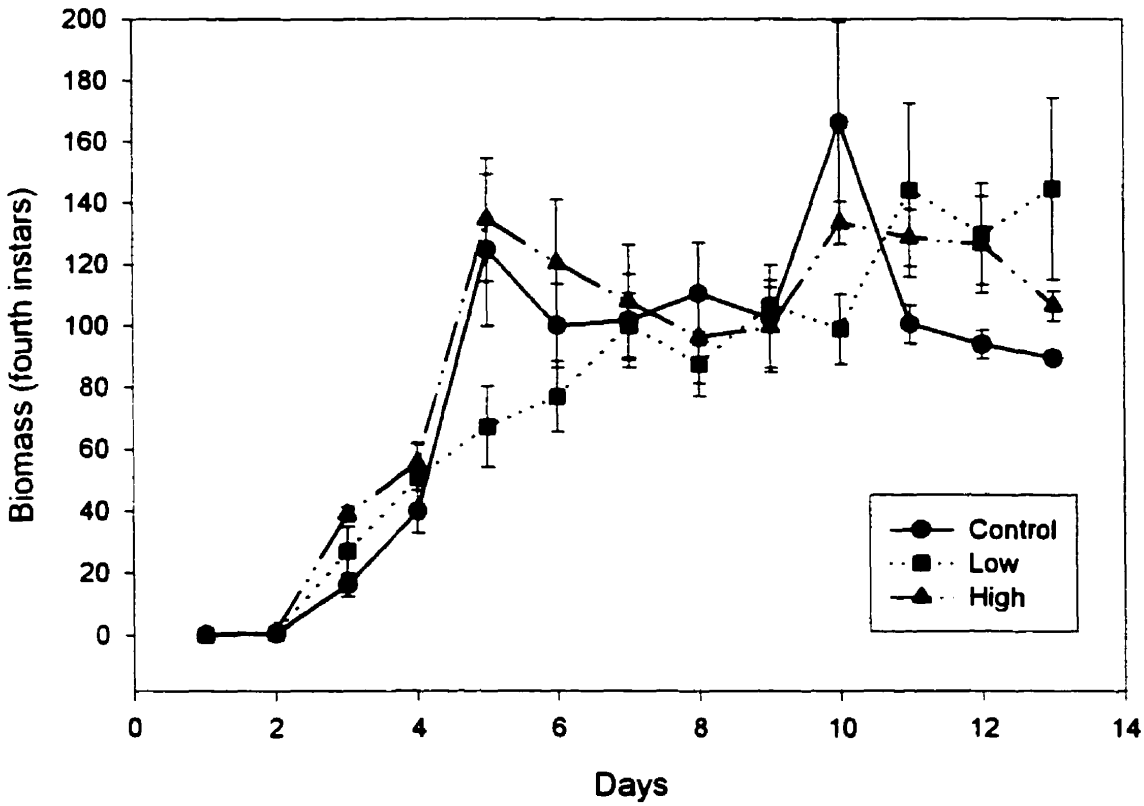

Biomass (fourth instars)
200
180
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Days
Control
Low
High

Figure 3.13: Percentage of *Aedes aegypti* eggs deposited into waters containing control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.02g yeast/l.

Error bars represent the standard error.

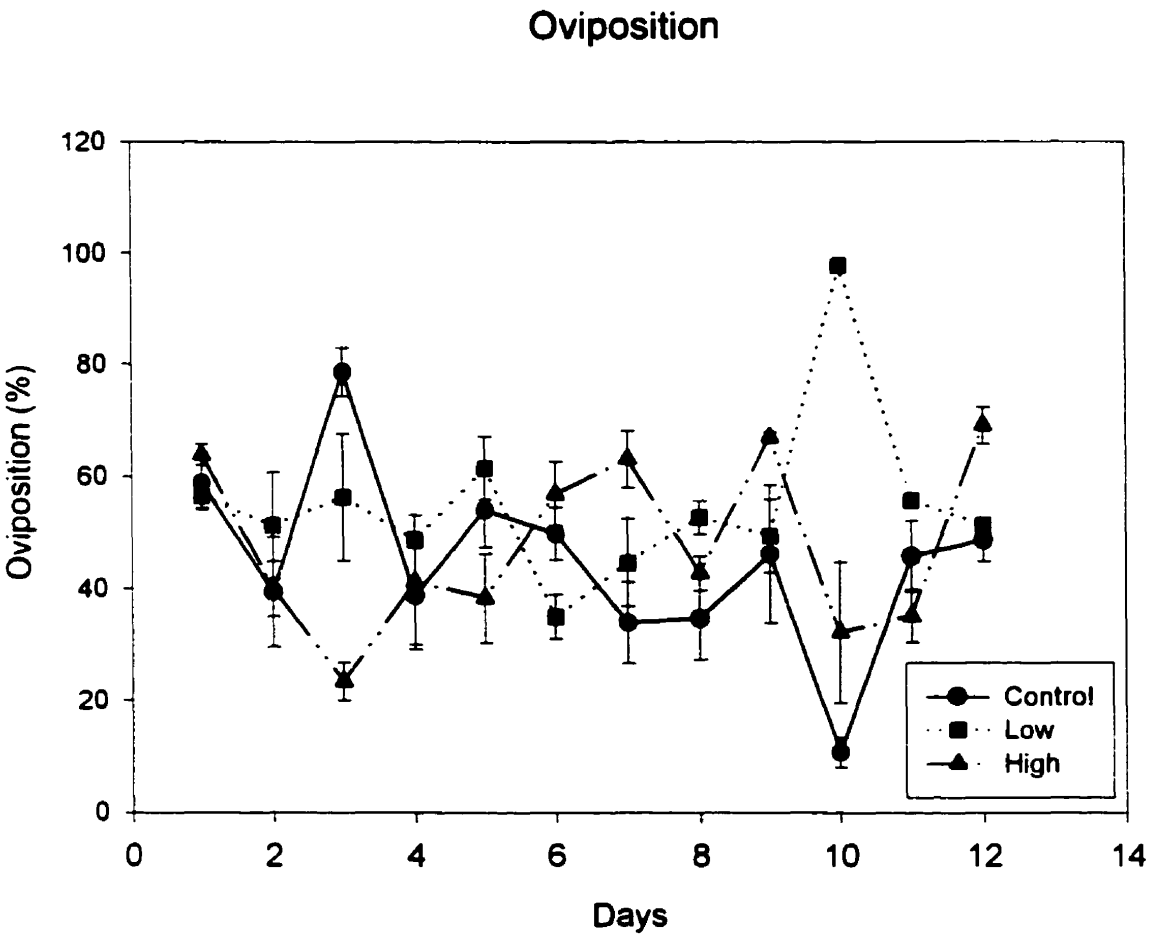

Oviposition
Oviposition (%)
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Days
Control
Low
High

Figure 3.14: Percentage of *Aedes aegypti* eggs deposited into waters containing control populations, populations exposed to a low or high level of *Plagiorchis elegans*, and fed 0.03g yeast/l.

Error bars represent the standard error.

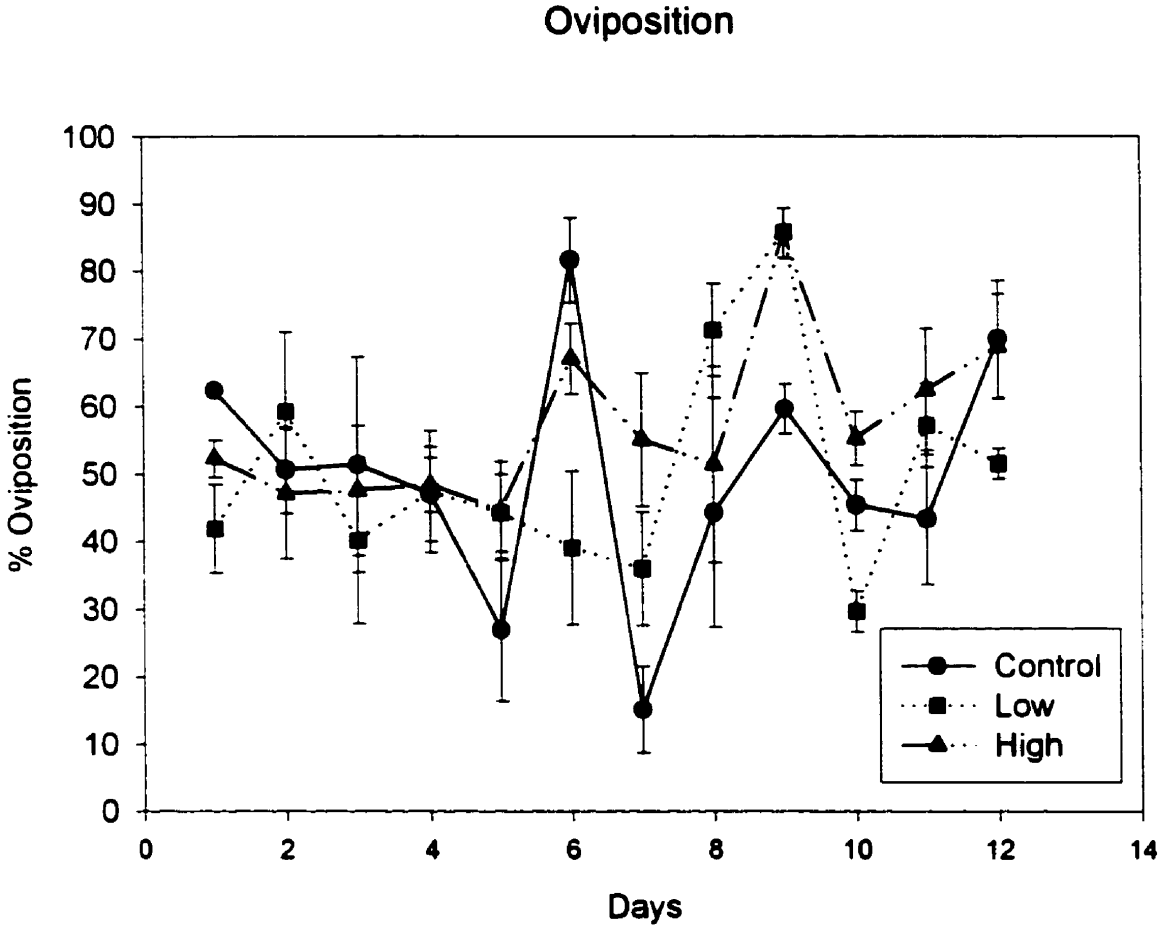
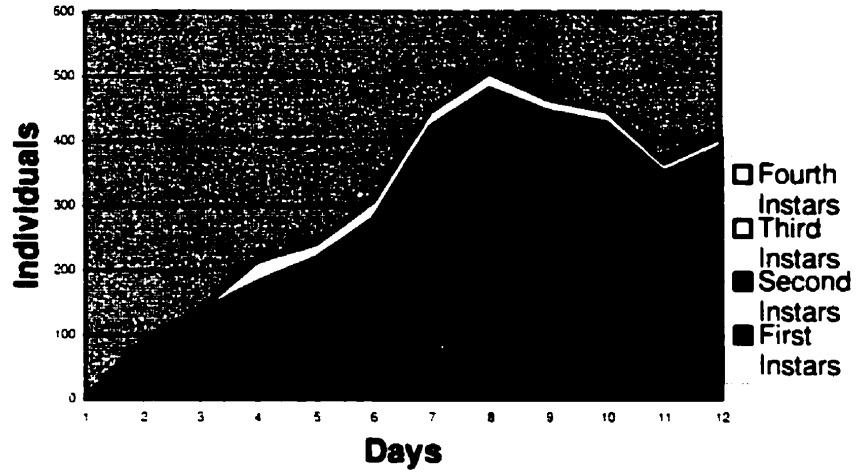
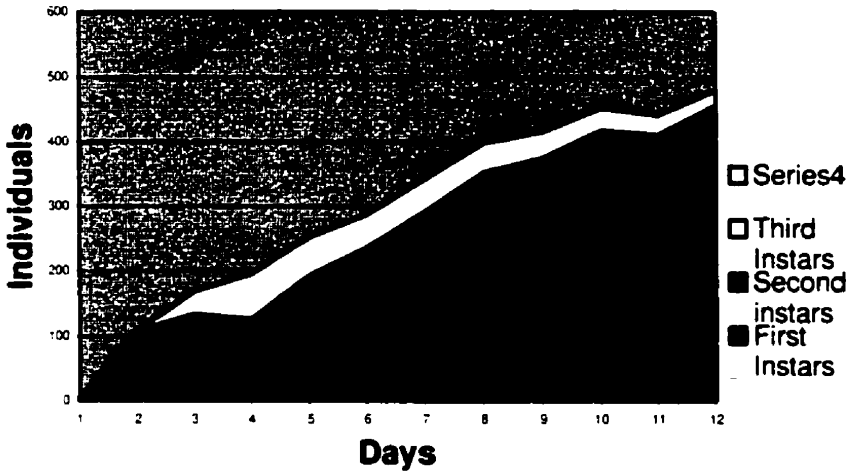

Oviposition
% Oviposition
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
Days
Control
Low
High

Figure 3.15: *Aedes aegypti* larval population structure at 0.02g yeast/l of **A)** control populations, **B)** populations exposed to a low level of *Plagiorchis elegans* and **C)** populations exposed to a high level of *Plagiorchis elegans*.

A


Control 0.02g/l Yeast
Individuals
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12
Days
Fourth Instars
Third Instars
Second Instars
First Instars

B


Low Infection 0.02g/l Yeast
Individuals
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12
Days
Series4
Third Instars
Second instars
First Instars

C

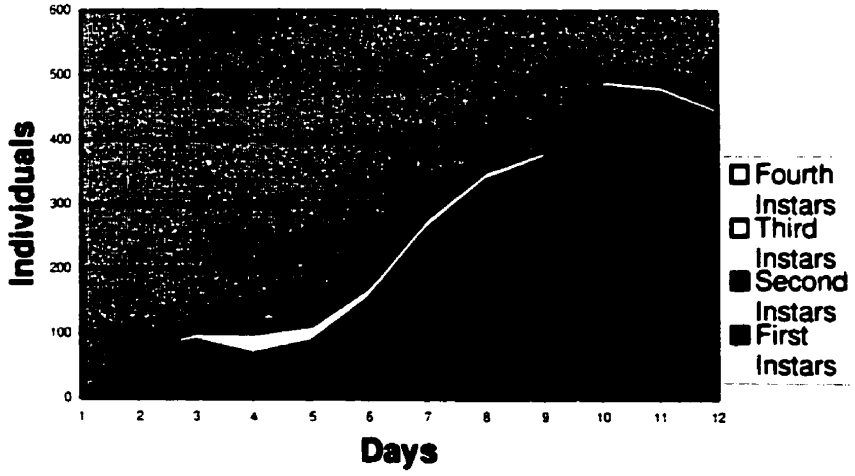

High Infection 0.02 g/l Yeast
Individuals
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12
Days
Fourth Instars
Third Instars
Second Instars
First Instars

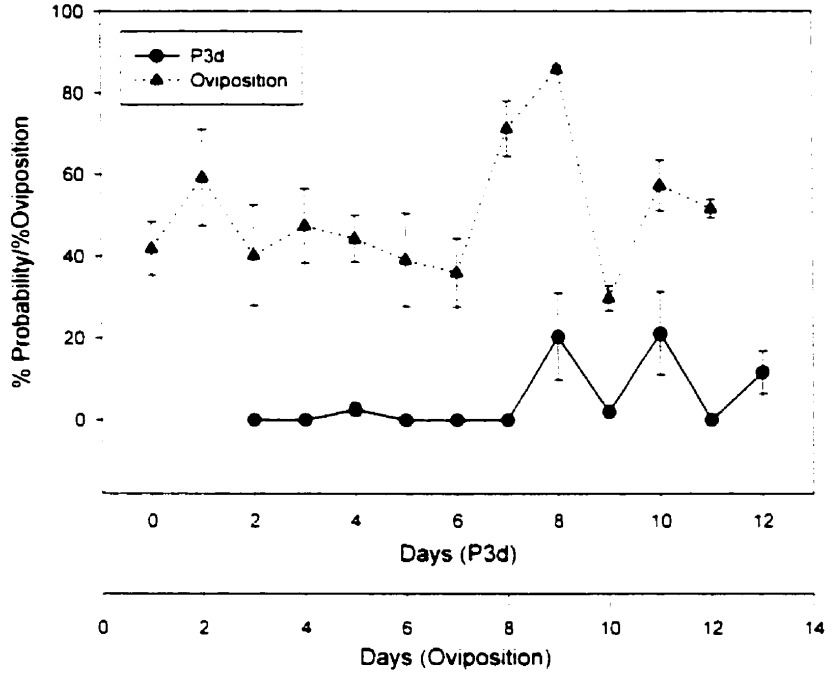
Figure 3.16: *Aedes aegypti* larval population structure at 0.03g yeast/l of **A)** control populations, **B)** populations exposed to a low level of *Plagiorchis elegans* and **C)** populations exposed to a high level of *Plagiorchis elegans*.

Figure 3.17: A) The probability that a third instar *Aedes aegypti* dies within a 24 hour interval and ovipositional preference at 0.03 g/l yeast and subjected to high exposure to *Plagiorchis elegans*. Oviposition is plotted with a time-lag of one day.

B) The probability that a third instar *Aedes aegypti* dies within a 24 hour interval and ovipositional preference at 0.03 g/l yeast and subjected to low exposure to *Plagiorchis elegans*. Oviposition is plotted with a time-lag of one day.

All error bars represent the standard error.

A


P3d and Oviposition at 0.03 g/l of Yeast and Low Infection
(Oviposition plotted with a time lag of 1 day)
P3d
Oviposition
% Probability/%Oviposition
Days (P3d)
Days (Oviposition)

B

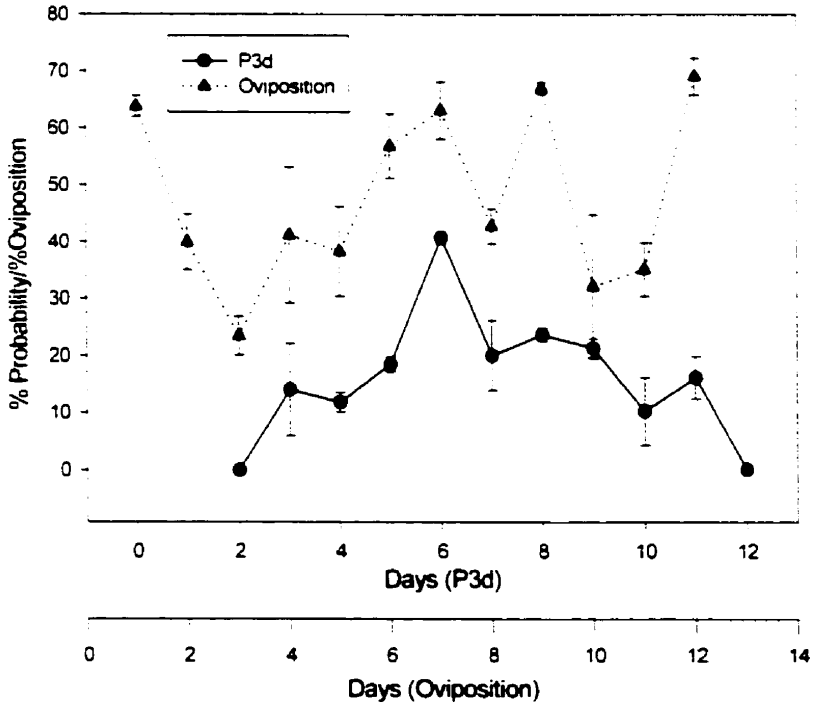

P3d and Oviposition at 0.02 g/l of Yeast and High Infection
(Oviposition plotted with a time lag of 1 day)
P3d
Oviposition
% Probability/%Oviposition
Days (P3d)
Days (Oviposition)

Table 3.1*: Results of analysis of variance with repeated measures in time for time-dependent transition probabilities of *Aedes aegypti* exposed to different levels of *Plagiorchis elegans* at two suboptimal food levels.

Pr>F	P11	P12	P1d	P22	P23	P2d	P33	P34	P3d
Day	0.0118	0.0001	0.0001	0.0043	0.001	0.0148	0.0048	0.0372	0.0189
Infect	0.0532	0.0591	0.1678	0.7475	0.283	0.0336	0.2643	0.0983	0.1767
Food	0.1721	0.001	0.0025	0.1768	0.0003	0.0001	0.8821	0.0001	0.002

Pr>F	P44	P4p	P4d
Day	0.014	0.7977	0.2077
Infect	0.9513	0.6900	0.1121
Food	0.0027	0.5075	0.1335

* Probabilities are significant if they are below 0.05

Table 3.2*: Results of analysis of variance with repeated measures in time for relative abundance of *Aedes aegypti* at different instars in populations exposed to different levels of *Plagiorchis elegans* at two suboptimal food levels .

Pr>F	1st Instars	2nd Instars	3rd Instars	4th Instars	Biomass
Day	0.1938	0.0737	0.0045	0.0001	0.0014
Food × Infect	0.3813	0.3452	0.1521	0.1039	0.0505
Infect	0.6668	0.6191	0.5811	0.0774	0.3340
Food	0.0037	0.2906	0.0663	0.0001	0.0002

* Probabilities are significant if they are below 0.05.

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CONNECTING STATEMENT 3

In chapters 1 and 2 it was shown that ovipositional preference of *Aedes aegypti* is not affected by exposure to *Plagiorchis elegans* cercariae. In Chapter 2, a positive correlation between biomass, pupation and ovipositional preference was observed, suggesting that at optimal food levels, an attractant was produced by larvae. Nevertheless, a mild repellent effect, conceivably due to crowding, was observed, since removal of fourth instars through pupation increased the attractiveness of the water to ovipositing females. In Chapter 3 it was suggested that at suboptimal food availability larvae were stressed and no longer produced sufficient attractant to support a significant correlation between biomass and ovipositional preference. These findings provide some insight into the major factors that govern the recruitment into pre-imago *Ae. aegypti* populations. An additional factor, egg hatchability, was briefly investigated in preliminary experiments, and was found to be constant around 85% under prevailing experimental conditions. Nevertheless, differential egg hatchability has been reported from a variety of studies. Chapter 4 integrates this phenomenon with the events that shape dynamic *Ae. aegypti* pre-imago populations.

CHAPTER 4

THE INTERACTION BETWEEN *AEDES AEGYPTI* PRE-IMAGO POPULATION DYNAMICS AND EGG HATCHABILITY AT VARYING LEVELS OF FOOD AVAILABILITY

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ABSTRACT

Eggs of *Aedes aegypti* hatch in response to a decrease in oxygen tension in their aquatic environment. An influx of nutrients and microorganisms as a result of flooding or rainfall may provide such a stimulus, as may the production of CO_2 through cutaneous respiration of larvae. However, as larvae feed, respiring microorganisms are removed, reducing hatchability. Furthermore, when larvae are nutritionally stressed, they may remove bacteria directly from the surface of the eggs, thus reducing hatching stimulus. This study assessed the hatchability of eggs in direct contact with a dynamic pre-imago population of *Ae. aegypti* provided with two different levels of food availability. In the presence of abundant food, hatchability was high when larval populations were low, but then decreased since respiring microorganisms were removed as the number of larvae increased. Thereafter, egg hatchability quickly regained high levels, as the first cohorts of later instars temporarily arrested feeding prior to moulting, while respiration remained high. This was followed by a second decline in hatching rates after the completion of moulting and the resumption of feeding. Finally, with the onset of pupation hatchability quickly regained high levels, as non-feeding pupae allowed the yeast concentrations to increase. As larval development became progressively less synchronized, egg hatchability stabilized at a relatively high level. At lower food levels hatchability was initially low, due to the absence of respiring microorganisms. After several days there was a sharp increase in hatchability, as the number of respiring larvae in the water became large enough to provide a strong hatching stimulus. With the onset of pupation, a concomitant decrease in cutaneous respiration caused a decline in hatchability. However, since more first instar larvae were introduced daily, hatchability quickly stabilized at high levels.

INTRODUCTION

Aedes aegypti is the vector of several viral diseases of global medical importance, among them yellow fever, Dengue and Dengue hemorrhagic fever (Gubler, 1998). Females of this species deposit their eggs on the walls of small artificial or natural containers, just above the water line (Christophers, 1960). Eggs must remain moist immediately after oviposition for a period of 2-3 days to allow complete embryonation (Putnam and Shannon, 1934; Fay, 1964).

The hatching stimulus for embryonated *Aedes* eggs is submersion (Christophers, 1960) and a decline in the oxygen content of the water. Hatching may occur when containers are flooded by rainfall, and a concomitant proliferation of microorganisms reduces water oxygen tension (Judson, 1960; Gjullin *et al*, 1941; Fallis and Snow, 1983). Gillett (1955) also found that individual variation in egg hatchability may be pre-determined genetically in *Ae. aegypti.*

Aedes eggs from the same batch do not all hatch at the same time. This phenomenon is known as "instalment" or "erratic hatching". This causes generation overlap (Koenekoop and Livdahl, 1986; Livdahl, 1983), and results in competition between larval cohorts (Edgerly and Marvier, 1992). Installment hatching is thought to be of advantage to species developing in temporary habitats (Andreanis, 1990). Such habitats are prone to drying out, but since eggs resist desiccation, they may hatch with the next rainfall (Christophers, 1960).

Hatching of *Aedes* eggs is also influenced by larval population density. Thus, Livdahl *et al* (1984) report that moderate numbers of larvae provide a positive hatching stimulus, as their respiratory activity reduces environmental oxygen tension. However, at

high larval densities (approximately 1 larva/ml) *Ae. aegypti* eggs failed to hatch. This was attributed to a density dependent reduction in food availability and a concomitant increase in the removal of bacterial overgrowth from eggs by foraging larvae, thus preventing a decline in oxygen tension (Edgerly *et al,* 1993; Edgerly and Marvier, 1992; Livdahl and Edgerly, 1987).

Increased mortality of younger cohorts due to cannibalism by older conspecifics (Koenekoop and Livdahl, 1986) and retarded development under conditions of crowding and starvation (Livdahl, 1983), render such inhibition of egg hatching a selective advantage (Livdahl *et al*, 1984). Since females may continue to oviposit into established pre-imago habitats, an egg bank may build up of over time. A decline in the larval population and the resulting removal of hatching inhibition may conceivably cause such eggs to hatch and allow a quick recovery of the mosquito larval population. This study examines population dynamics and its interaction with *Ae. aegypti* egg hatchability in a laboratory larval population simulating a natural population.

MATERIALS AND METHODS

Aedes aegypti flight cages, as described in previous chapters, were set up two weeks prior to the experiment and kept under the same experimental conditions as in chapters 2 and 3. Adults were provided with 10% saturated sugar solution in cotton wells ad libitum, and were blood fed twice weekly. Each cage was furnished with an oviposition site consisting of a 450 ml plastic container lined with brown filter paper and filled with 150 ml of distilled water.

Filter paper was removed daily from oviposition sites and eggs were conditioned at 27°C for two days (Christophers, 1960). Then, a sample of 10 eggs from each filter paper collected was crushed and examined under a dissection microscope (×50) to ensure that embryonation was complete. Larval habitats for the assessment of hatchability were established separate from the flight cages, consisting of 500 ml food containers filled with 250 ml of distilled water, which had been aerated overnight. To assess hatchability, 50 fully embryonated eggs were added to each larval habitat every day for a period of two hours. Eggs were then removed and examined under a dissection microscope (×50) to determine whether hatching had occurred. Hatched eggs can be easily identified, as the operculum has been separated from the egg. The proportion of eggs hatched from each sample represented hatchability in this habitat for that particular day. Larvae that had hatched from these eggs remained in the container to form a quickly growing pre-imago population.

Each day, a complete census of live larval instars, pupae, and adults was conducted in order to document progressive changes in the population. To facilitate

counting, all larvae were temporarily transferred to tissue culture wells (17 mm diameter) for easy separation.

Brewer's yeast at two levels, either high (0.1 g/l, determined to be optimal for larval development to the adult stage by Hartley (1996)) or low (0.04 g/l, thus sub-optimal), was added daily to the larval habitats, as a source of food for larvae and a medium for bacterial growth. As this was done after hatchability assessment and census, larvae were allowed to feed overnight before hatchability was tested again.

The experiment was conducted over a period of 9 days. It consisted of a completely randomized design with 1 treatment factor at 2 levels and three replicates per treatment factor.

Transition Probabilities

In order to characterize the development of the population, transition probabilities were calculated. These calculations were based on an adaptation of a Markov-chain-like model developed by Dr. Pierre Dutilleuil (McGill University, Department of Plant Science), and first applied to mosquito development by Nguyen (1998). A transition probability is defined as the probability that one stage will develop to the next stage within a fixed time interval. For this study, probabilities were calculated from raw data for 24 hr intervals, as presented in Figure 4.1. All probabilities were calculated for each of the 10 days creating time series for each probability. Calculations were performed using Microsoft Excel 97 spreadsheets. In this study no transition probabilities for mortality were calculated.

Statistical Analyses

Mean time-dependent transition probabilities and hatchability were calculated and graphed using Jandel Scientific Sigma Plot software. Analysis was then performed using an analysis of variance with repeated measures in time, with days as the repeated factor, using SAS 6.1 software (SAS Institute Inc., 1999). The general linear models procedure with its statement REPEATED was applied. Results of the univariate and multivariate approach were used where appropriate. When data failed to meet circularity conditions, an adjustment of the probabilities of significance of the univariate ANOVA F-tests was executed using the Huin-Feld Epsilon correction factor.

Repeated measures ANOVA was also used to analyse total biomass. The biomass was expressed in terms of fourth instar equivalence. Biomass of fourth instars is generally 40 times greater than first instars, 12 times greater than second instars, and 2.2 times greater than third instars (Christophers, 1960). Total adult production was compared using ANOVA tests with SAS software. Post hoc comparisons were made by multiple t-tests using the Student- Newman-Keuls approach. Proportional data were transformed using arcsine square root transformation; population count data was transformed using a square root transformation (Zar, 1993).

Cross-Correlations of Time Series

To determine whether ovipositional preference was affected by development, cross-correlations between time series of ovipositional preference and probabilities were conducted. The procedure ARIMA in SAS economics and time series was used to perform these calculations. Proc ARIMA uses the Box and Jenkins (1976) method to create models of time series. Cross-correlations were calculated using the 'identify'

statement. Linear correlations between points were made between values of the different time series for different time lags. No attempts at model building were made in this study.

RESULTS

The development of first instars changed significantly over time (P11: p=0.0074; P12: p=0.0005). Food availability had a significant impact on the development of first instars (P12), but not on the probability to remain first instars (P11) (P11: p>0.05; P12: p=0.0319) (Figure 4.2). Significant correlations were found between hatchability and first instar development at high food availability (correlation coefficients: 0.701 for P11 and 0.68798 for P12 without time lags).

Food availability did not significantly affect second instar development (P22, P23). However, a significant effect of time on P22 (p=0.0001), but not on P12 was observed. Hatchability did not correlate significantly with P22 or P23 at either food level (Figure 4.3).

There was a significant effect of time (P33: p=0.0001; P34: 0.0083), but not of food availability on third instar development (P33, P34) (Figure 4.4). Cross-Correlations performed between hatchability and P33 were significant at low food availability (Correlation coefficient of -0.76515 with a time lag of -1). Hatchability was significantly correlated with P34 at low food levels (correlation coefficients: 0.76515 with a -1 time lag), however no correlation with P34 at high food availability was observed.

Fourth instar development (P44, P4p) changed significantly with time (P44: p=0.0001; P4p: p=0.0004). Food level significantly affected the transition of fourth instars into pupae (P44: p=0.0085; P4p: p=0.0124), and populations provided with 0.04 g of yeast/l produced fewer pupae than those provided with 0.1 g/l (Figure 4.5). No significant correlations were found between hatchability and P44 or P4p at either food level. Adult emergence was not affected by food availability, however it changed

significantly with time over the 9-day period (Ppp: p=0.0016; Ppa: p=0.0001) (Figure 4.6).

ANOVA using repeated measures over time performed on hatch rates at two levels of food availability yielded significant results for time (p=0.056), and for food availability (p=0.0256) (Figure 4.8).

Repeated measures ANOVA was also performed on the total biomass of the larval population, expressed as fourth instar equivalents. Significant results were observed for time (p=0.0015). There was also an effect of food availability on larval biomass (p=0.0001) (Figure 4.7). Hatchability was not significantly correlated with larval population biomass, regardless of the amount of yeast added.

All P values obtained from repeated measures ANOVA are presented in Table 4.1 and Table 4.2.

DISCUSSION

The hatching of mosquito eggs is influenced by a reduction in the concentration of dissolved oxygen in the water (Judson, 1960). Two factors influence egg hatching through their affect on oxygen tension. These are the removal of bacteria from the eggs' surface by grazing larvae which exerts a negative hatching stimulus, and cuticular respiration by larvae, which acts as a positive hatching stimulus (Livdahl *et al*, 1984). Turbulence generated by swimming larvae may also influence the oxygen concentration directly in a positive manner (Edgerly *et al*, 1993).

Hatchability of *Ae. aegypti* eggs varied over time and with food availability as the larval population grew and developed (Figure 4.8). Initial levels of egg hatchability depended on the amount of suspended yeast. Under the condition of low food availability, the initially low number of larvae, their relatively low biomass (Figure 4.7) and their corresponding low level of respiration were insufficient to promote hatching. Livdahl *et al* (1984) observed an inhibiting effect of first instars, which was attributed to the inability of newly hatched first instars to utilize yeast as food, thereby causing them to resort to grazing on the surface of eggs. It should be noted that the study cited above used 35 ml vials in which eggs would be concentrated and thus more accessible to newly hatched larvae. In contrast, eggs dispersed in the 500 ml containers used in the present study would be less likely to encounter browsing larvae.

Hatchability was significantly affected by third instar development (Figure 4.4). Their increased consumption of yeast between days 2 and 3 may have contributed to the observed decline in hatchability. The increase in hatchability between days 4 and 5 may be attributed to an increase in the number of fourth instars in the population (Figure 4.9).

Their respiratory activity may have removed dissolved oxygen and stimulated egg hatching. A decline in hatchability was observed between days 6 and 7, which corresponds with the onset of pupation (Figure 4.6). Since pupae switch to non-cutaneous respiration (Christophers, 1960), the amount of dissolved oxygen removed by respiration may decrease. Finally, hatchability appeared to stabilize at a relatively high level. Conceivably as larval development became progressively less synchronized as subsequent cohorts were introduced, egg hatchability stabilized at a relatively high level determined by the average level of respiration.

Egg hatchability at high food availability followed a similar trend as that of low food availability (Figure 4.8). Initially hatchability was greater, presumably because the large amount of yeast added generated a positive hatching stimulus, and because the depletion of resources by the small larval population was negligible. Hatchability declined between days 3 and 4, conceivably with the appearance of third instars (Figure 4.4 and 4.9), which consume proportionately greater amounts of food (Christophers, 1960). Larvae arrest feeding prior to moulting, but continue to respire. This may cause the increase in hatchability between days 4 and 5, immediately prior to the development of fourth instars (Figure 4.5). Feeding recommences by day 6, and hatchability declines once again. As fourth instars moult to the non-feeding pupal stage there is a concomitant, slow increase in hatchability after day 7 (Figure 4.6). Thereafter, hatchability will likely stabilize at high levels of hatchability much like what occurred at lower food levels. Thus, at high food levels, the amount of yeast in the aquatic environment was the primary hatching stimulus, whereas at low food levels, with the absence of excess yeast, larval respiration became the most important factor determining hatchability.

In experiments conducted in chapters 2 and 3, larval populations rose to high levels very quickly. The resulting high respiratory rate may induce a consistently high level of hatchability.

Our findings are consistent with the reports that *Aedes* egg hatchability is governed by oxygen levels in the aquatic environment (Judson, 1960) and by both larval and microbial respiration and indirectly, food availability (Edgerly *et al.* 1993; Edgerly and Marvier, 1992; Livdahl and Edgerly, 1987). However, variability in egg hatching may be a transient phenomenon, only observed under conditions of low larval abundance. In dense, stable larval populations hatchability may remain high and relatively stable, fluctuating only if the system is disturbed.

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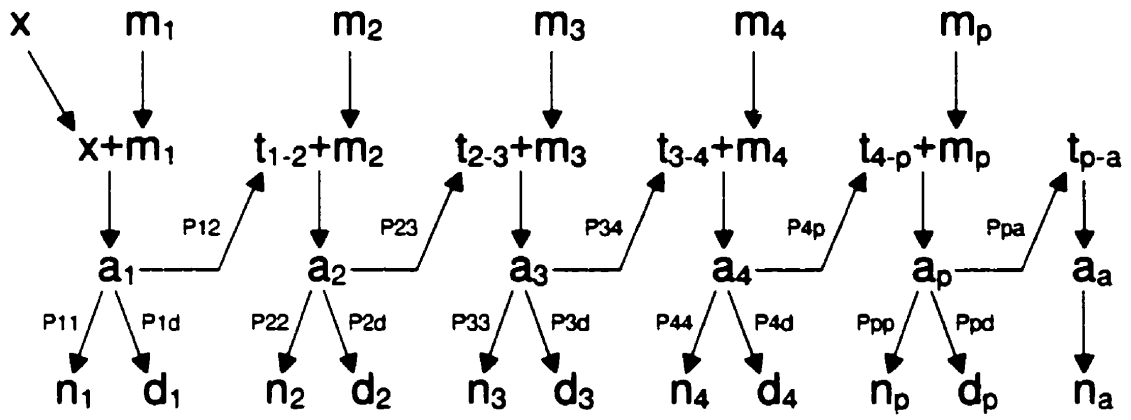
Figure 4.1: Time-dependent Transition Probabilities. **Pxx** represents the probability that an instar remains at stage **x** in 24 h; **Pxy** represents the probability that an instar will move from stage **x** to stage **y** in 24 h. **Pxd** represents the probability that an instar of stage **x** will die in 24 h. **X** is the number of 1^{st} instars added each day, **a** represents the total number of dead and live larvae on the present day, **m** is the larvae that were present the previous day, **n** is the live larvae counted the present day, and **d** is the dead larvae counted on the present day. **t** is the number of larvae that transformed from the previous stage (e.g. $t_{3\to4}$: number of larvae changed from 3^{rd} to 4^{th} instar).



Probabilities:

$P11=n_1/a_1$
$P12=t_{1-2}/a_1$
$P1d=d_1/a_1$
$P22=n_2/a_2$
$P23=t_{2-3}/a_2$
$P2d=d_2/a_2$
$P33=n_3/a_3$
$P34=t_{3-4}/a_3$
$P3d=d_3/a_3$
$P44=n_4/a_4$
$P4p=t_{4-p}/a_4$
$P4d=d_4/a4$
$Ppp=n_p/a_p$
$Ppa=a_a/a_p$

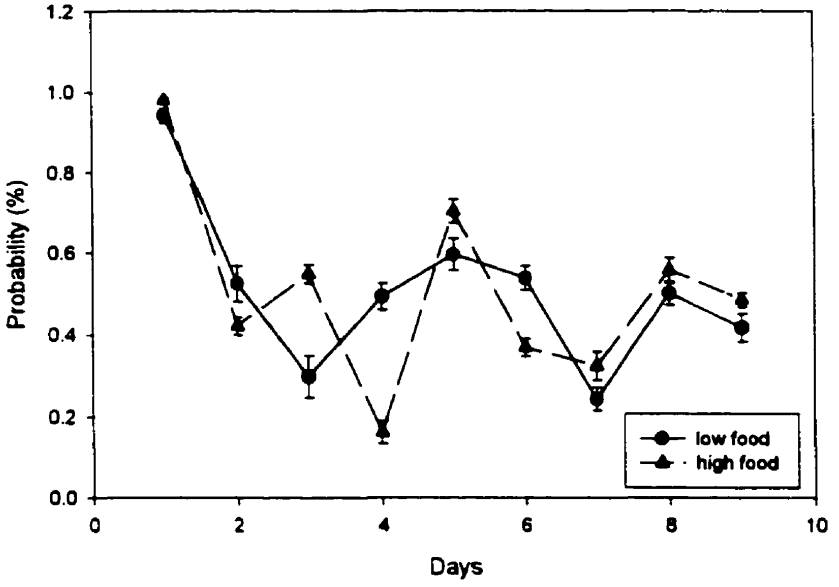
$a_1=x+m_1$
$a_2=t_{1-2}+m_2$
$a_3=t_{2-3}+m_3$
$a_4=t_{3-4}+m_4$
$a_p=t_{4-p}+m_p$
$a_a=n_a$

$t_{1-2}=(x+m_1)-n_1-d_1$
$t_{2-3}=m_2-n_2-d_2$
$t_{3-4}=m_3-n_3-d_3$
$t_{4-p}=m_4-n_4-d_4$

Figure 4.2: Time series of mean twenty-four hour transition probabilities of **A)** first instar *Aedes aegypti* to remain at that stage (P11), **B)** and to transform from the first to the second instar (P12) at low (0.04g/l yeast) and high levels (0.1g/l yeast) of food availability.

Error bars represent the standard error.

A


P11
Probability (%)
Days
low food
high food
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
2
4
6
8
10

B

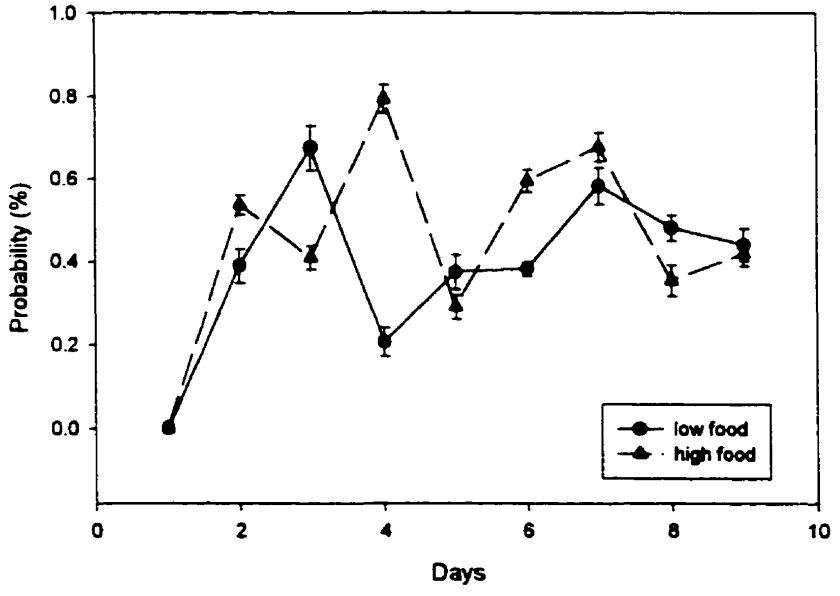
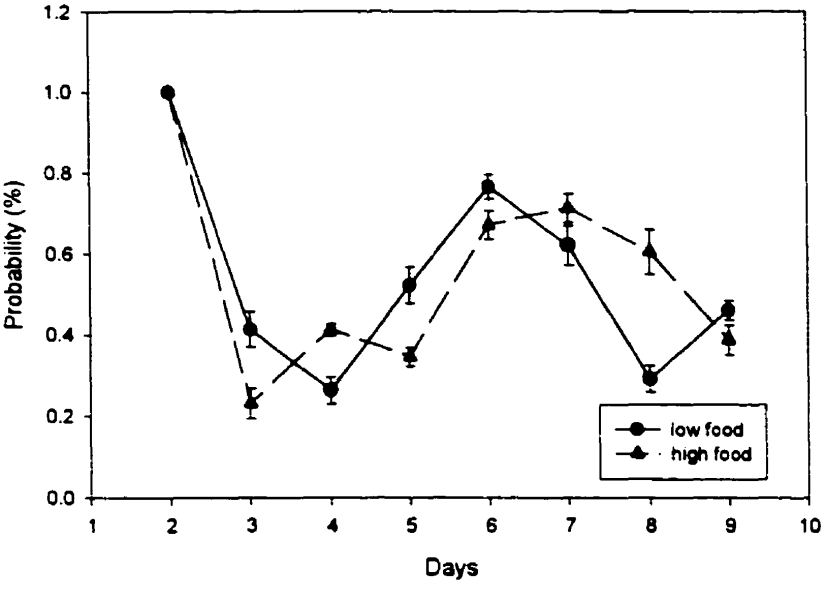

P12
Probability (%)
Days
low food
high food
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10

Figure 4.3: Time series of mean twenty-four hour transition probabilities of **A)** second instar *Aedes aegypti* to remain at that stage (P22), **B)** and to transform from the second to the third instar (P23) at low (0.04g/l yeast) and high levels (0.1g/l yeast) of food availability.

Error bars represent the standard error.

A


P22
Probability (%)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
7
8
9
10
Days
low food
high food

B

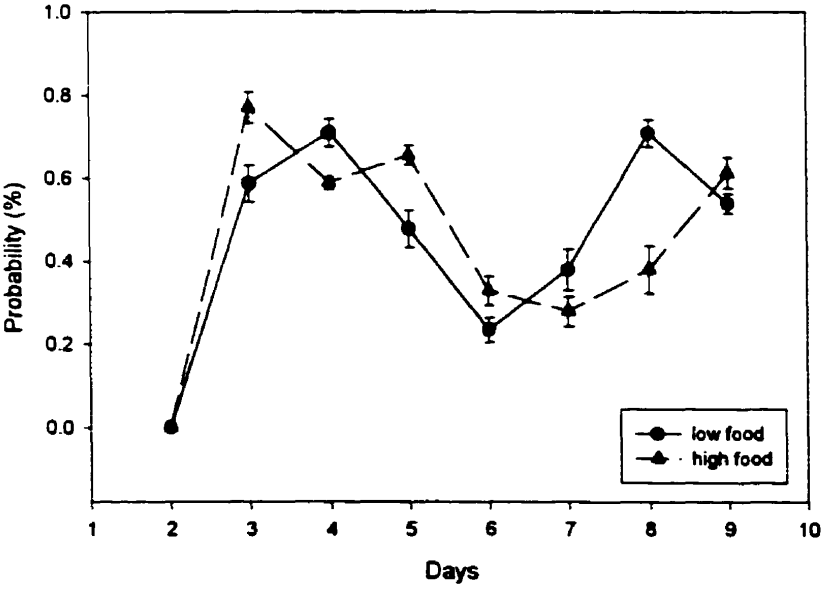
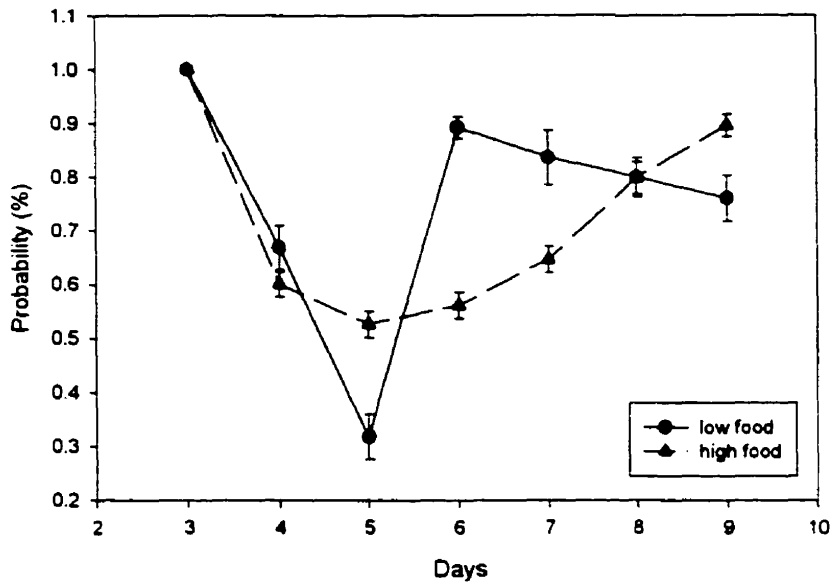

P23
Probability (%)
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
7
8
9
10
Days
low food
high food

Figure 4.4: Time series of mean twenty-four hour transition probabilities of **A)** third instar *Aedes aegypti* to remain at that stage (P33), **B)** and to transform from the third to the fourth instar (P34) at low (0.04g/l yeast) and high levels (0.1g/l yeast) of food availability.

Error bars represent the standard error.

P33

A


Probability (%)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
2
3
4
5
6
7
8
9
10
Days
low food
high food

P34

B

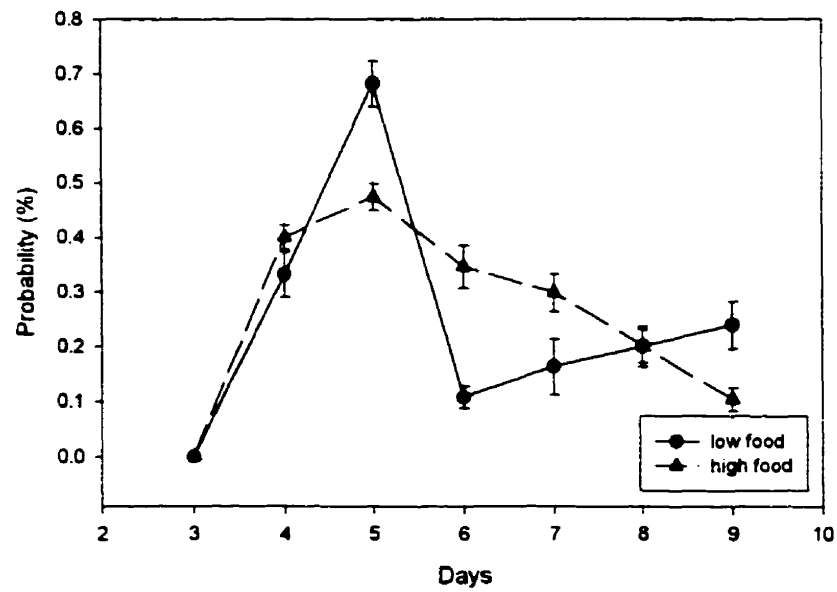
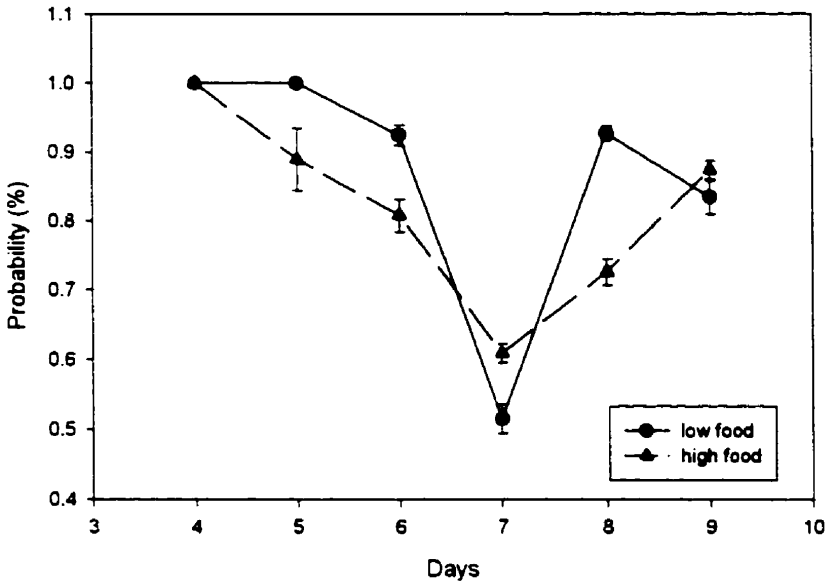

Probability (%)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
2
3
4
5
6
7
8
9
10
Days
low food
high food

Figure 4.5: Time series of mean twenty-four hour transition probabilities of **A)** fourth instar *Aedes aegypti* to remain at that stage (P44), **B)** and to transform from the fourth instar to pupa (P4p) at low (0.04g/l yeast) and high levels (0.1g/l yeast) of food availability.

Error bars represent the standard error.

A


P44
Probability (%)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
3
4
5
6
7
8
9
10
Days
low food
high food

B

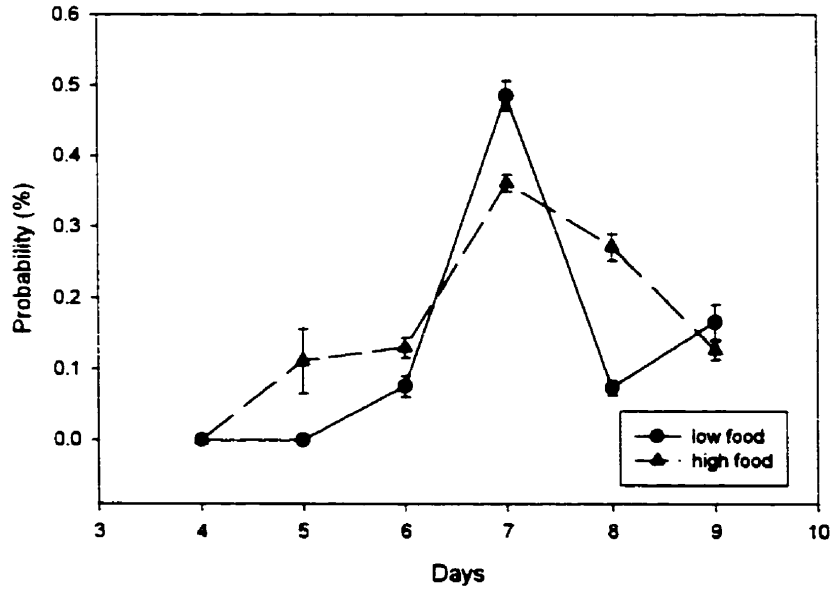
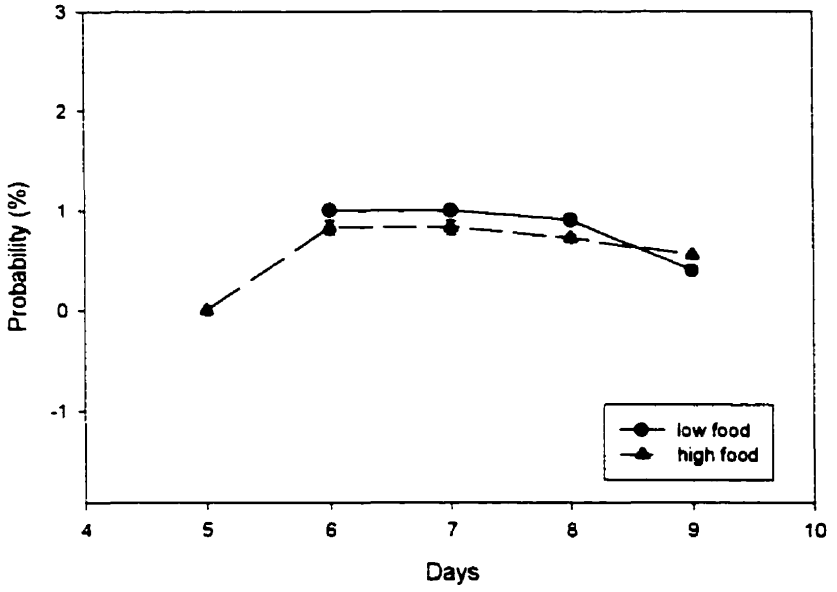

P4p
Probability (%)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
3
4
5
6
7
8
9
10
Days
low food
high food

Figure 4.6: Time series of mean twenty-four hour transition probabilities of **A)** pupal *Aedes aegypti* to remain at that stage (Ppp), and **B)** to emerge as adults (Ppa), at low (0.04g/l yeast) and high levels (0.1g/l yeast) of food availability.

Error bars represent the standard error.

A


Ppp
Probability (%)
3
2
1
0
-1
4
5
6
7
8
9
10
Days
low food
high food

B

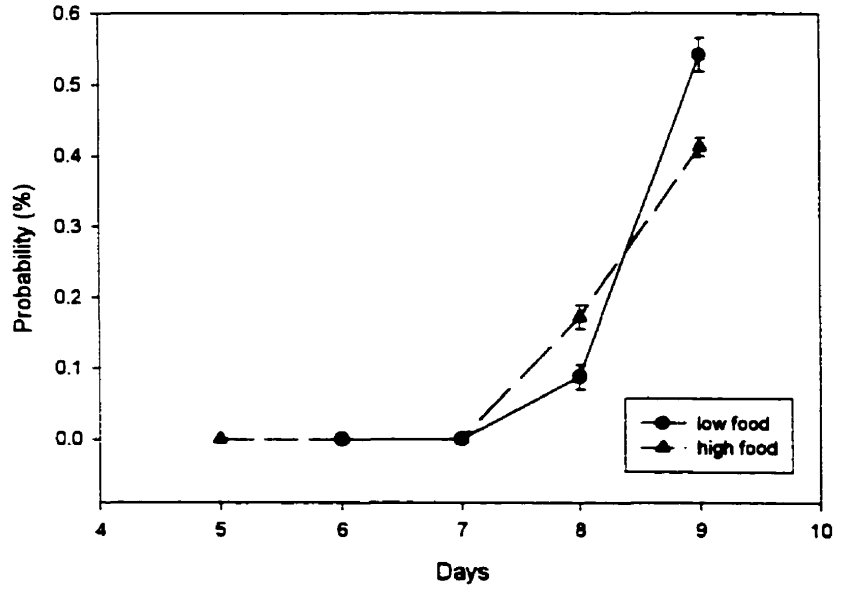

Ppa
Probability (%)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
4
5
6
7
8
9
10
Days
low food
high food

Figure 4.7: Biomass of *Aedes aegypti* larval populations in terms of fourth instars at low (0.04g/l yeast) and high levels (0.1g/l yeast) of food availability.

Error bars represent the standard error.

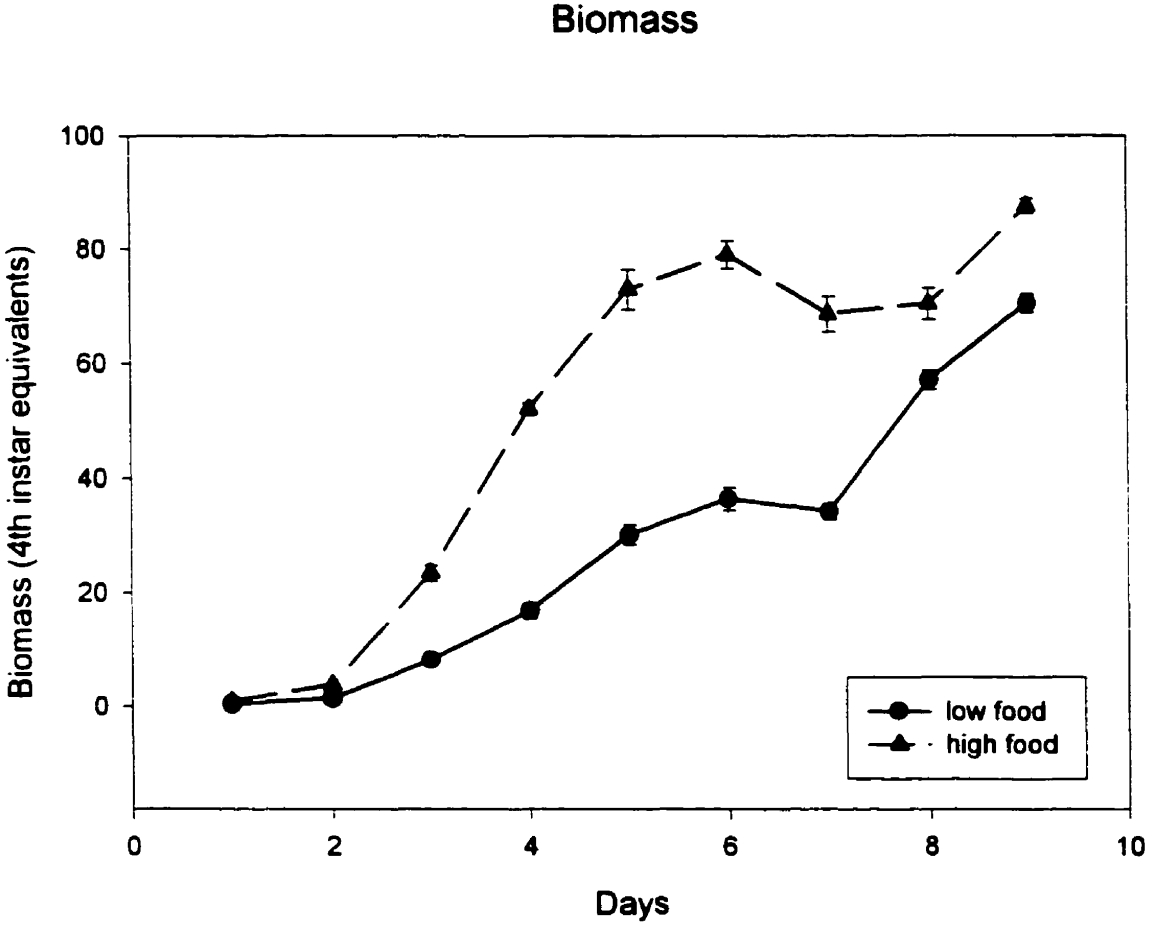

Biomass
Biomass (4th instar equivalents)
100
80
60
40
20
0
0
2
4
6
8
10
Days
low food
high food

Figure 4.8: Percentage of *Aedes aegypti* eggs hatched in waters at low (0.04g/l yeast) and high levels (0.1g/l yeast) of food availability.

Error bars represent the standard error.

Hatchability

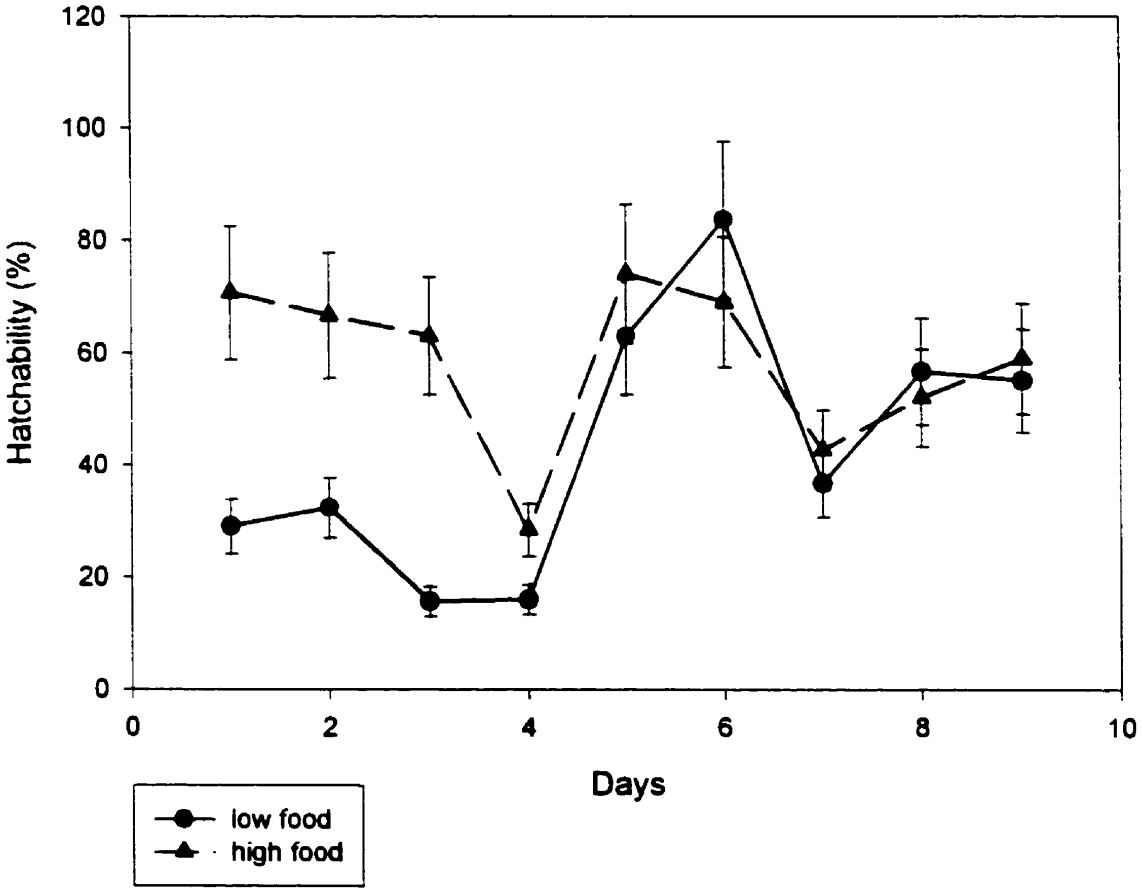
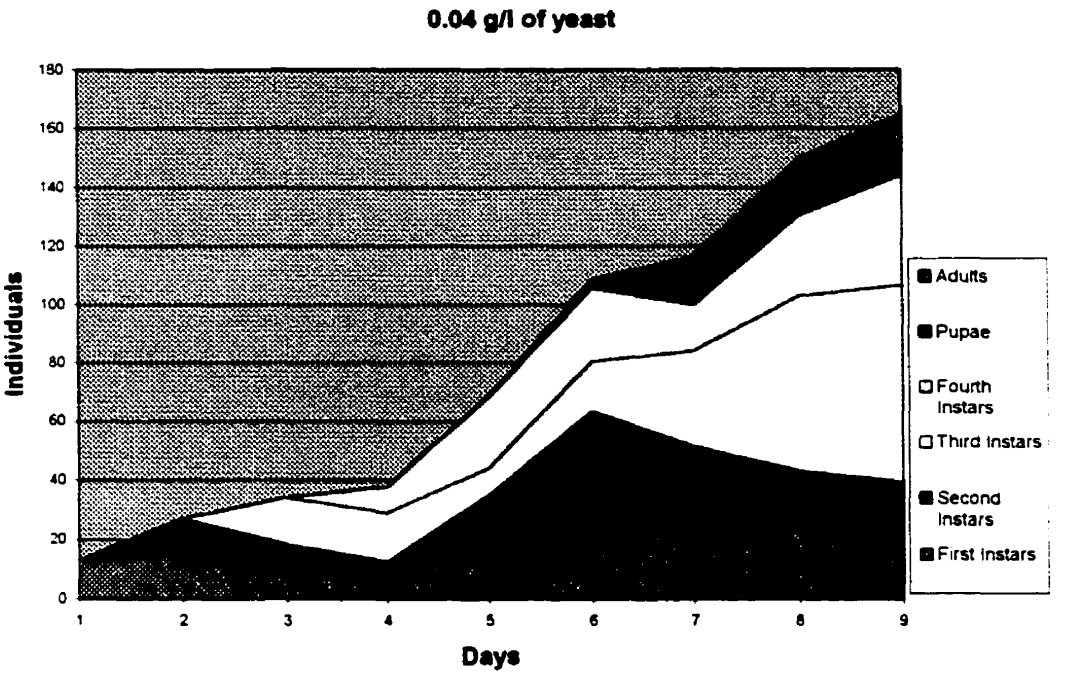


Figure 4.9: Population structure of *Aedes aegypti* larvae at **A)** at low (0.04g/l yeast) and **B)** at high levels (0.1g/l yeast) of food availability.

A


0.04 g/l of yeast
Individuals
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
Days
Adults
Pupae
Fourth Instars
Third Instars
Second Instars
First Instars

B

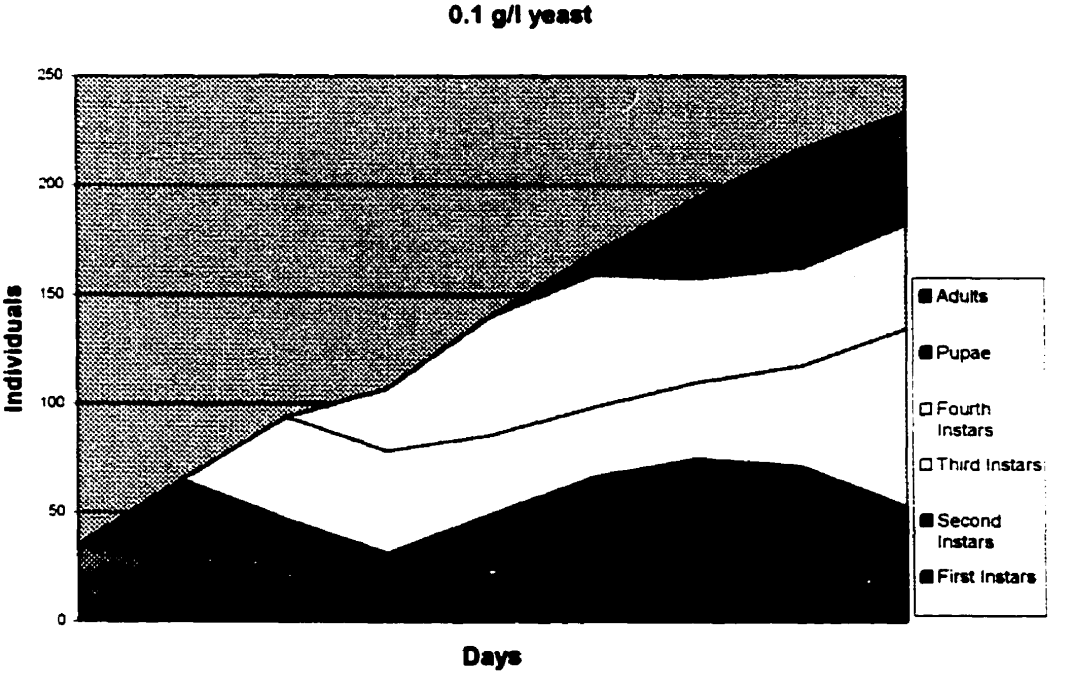

0.1 g/l yeast
Individuals
250
200
150
100
50
0
Days
Adults
Pupae
Fourth Instars
Third Instars
Second Instars
First Instars

Table 4.1*: Results of analysis of variance with repeated measures in time for time-dependent transition probabilities of *Aedes aegypti* at two levels of food availability.

Pr> F	P11	P12	P22	P23	P33	P34	P44	P4p	Ppp	Ppa
Day	0.0074	0.0005	0.0001	0.2147	0.0001	0.0083	0.0001	0.0004	0.0016	0.0001
Food	0.9403	0.0319	0.7614	0.9844	0.1985	0.5314	0.0085	0.0124	0.5545	0.6422

* Probabilities are significant if they are below 0.05

Table 4.2*: Results of analysis of variance with repeated measures in time for biomass and hatchability of *Aedes aegypti* at two levels of food availability.

Pr>F	Biomass	Hatchability
Day	0.0015	0.0557
Food	0.0001	0.5314

* Probabilities are significant if they are below 0.05

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CHAPTER 5

GENERAL DISCUSSION

Populations of *Ae. aegypti* are regulated mainly by intraspecific competition of the larval population (Southwood *et al*, 1972). It is of great importance for females to choose an appropriate oviposition site in order to ensure optimal survival and development of their offspring (Bentley and Day, 1989). Consequently females may benefit from gauging population size and the availability of nutrients in potential oviposition sites (Soman and Reuben, 1970; Allan and Kline, 1998). Furthermore, eggs of Aedes spp are able to assess habitat quality in order to determine optimal hatching time. (Livhdahl *et al*, 1984; Edgerly *et al*, 1998; Edgerly and Livdahl, 1993; Edgerly and Marvier, 1992). As newly hatched larvae are vulnerable to competition (Livdahl, 1983) and cannibalism (Frogner, 1980) in crowded or nutrient poor habitats, hatching into such environments may pose a great risk. However, since *Ae. aegypti* exploit temporary habitats, eggs must hatch and larvae must develop before the habitat dries up (Edgerly *et al*, 1998). All of the above factors interact to determine, ultimately, the number of adults that are produced. In this study, a system incorporating a number of factors, such as nutrient availability, infection with *Plagiorchis* spp, ovipositional preference and egg hatchability was created in order to observe how all these factors interact.

When food availability was optimal, exposure to *P. elegans* primarily increased pupal mortality and reduced adult emergence. There was little effect on larval development, and the larvae developed similarly in all three treatments until pupation, supporting the findings of Dempster *et al* (1986). Such minimal impact on the larval stages and a concentration of pathogenic effects on the pupae and adults is advantageous in terms of biological control (Lounibos, 1987).

When resource availability is reduced to very low levels, development is severely impaired. Under such conditions, the parasite was unable to reduce adult production further significantly, as there was a lack of late instars which are the most likely to acquire the infection (Dempster *et al*, 1986). As infected fourth instars reduce their food intake (Dempster and Rau, 1987), surviving uninfected fourth instars benefited from a higher resource availability, and were more likely to emerge successfully as adults.

No repellent effects were observed in parasite-exposed populations, allowing continued recruitment into the larval population. At high food levels, hatchling larvae have a reduced chance of surviving because of intraspecific competition and parasite exposure. Thus, habitats harbouring *P. elegans* may serve as sinks for mosquito eggs. At high food levels, attractiveness of waters to ovipositing females was positively correlated with larval biomass, as suggested by Soman and Reuben (1978). However, at low food levels, no such correlation was observed. It is conceivable that repellents produced by stressed larvae (Zahiri and Rau, 1998) may have cancelled the effects of any attractants.

As suggested by Edgerly and Marvier (1992), Livdahl *et al* (1984), Edgerly *et al* (1999), and in chapter 4, both an inhibition of hatching by the removal of resources by conspecifics, and a hatching stimulus, attributable to respiring conspecifics were

observed. Which of the two factors predominates may be largely dependent on resource availability in combination with population structure and size and is therefore difficult to predict. Nevertheless, pre-imago populations appeared to stabilize at a relatively high rate of egg hatching. Thus, reduced hatchability as described by Livdahl *et al* (1984) may be a transient phenomenon and was evident only at low population densities and low food availability. As more larvae hatched into these environments, their respiration ensured a continued, strong hatching stimulus observed in preliminary experiments in Chapters 2 and 3.

The combined effects of hatchability and ovipositional preference are difficult to predict. Presumably, if populations are high and resources are low, few females will oviposit into such sites. However, eggs will hatch readily in response to a high hatching stimulus generated by respiring larvae. Alternatively, if resources are high and larvae are abundant, both oviposition preference and hatchability would be high.

Although there was some evidence of depensatory mortality occurred in this study when *Ae. aegypti* was exposed to *P. elegans*, this only occurred under conditions highly unfavourable to adult emergence due to a lack of nutrients. When food levels were near optimal, the parasite effectively reduced the emerging adult population, and may thus prove to be of use as an adjunct in the biological control of mosquitoes. However further studies on the possibility of depensatory mortality are necessary to ensure that this will not be a problem in natural populations. The sustained attractiveness of waters harbouring *P. elegans*-infected larvae enhances the suitability of these organisms as an agent in the biological control of mosquitoes. Thus, ovipositing females continue to be drawn to waters containing the entomopathogenic cercariae and infected conspecific

larvae, instead of being deflected to other environments. As a result, there is an increased probability that they will lose their reproductive investment and will not contribute to the adult population. Finally, all these findings need to be tested in the field against a multivoltine species of mosquito.

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