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Nutrition, Competition and Mortality:The Impact of *Plagiorchis elegans* on the Development of *Aedes aegypti*

by

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Abstract

The effects of food availability and the presence of the parasite, *Plagiorchis elegans*, on the probability of *Aedes aegypti* preimagos to transform from one immature stage to the next over consecutive 24- or 48-hour periods of time were determined. Under conditions of low food availability, 24- and 48-hr transition probabilities of all larval instars to the next were reduced. Increases in food availability reversed this process, causing significant decreases in same-stage transition probabilities. However, as transition to successive stages increased among first, second, third and fourth instars, there was concomitant significant increase in first and fourth preimago mortality. Addition of *Plagiorchis elegans* cercariae caused significant decreases in the 24 and 48-hour same-state probabilities for all pre-imago stages. Successive stage transitions for first, second and third instars were significantly increased while those of fourth instars and pupae were significantly decreased following exposure to the parasites. The mortality of all preimago stages significantly increased with exposure to parasite, but was highest among fourth instars and pupae. There were strong food-by- parasite interaction effects among first, second and third instars. Differences in transition probabilities increased with increasing food levels between controlled and parasite-exposed groups. The opposite was true for fourth instars and pupae. While there was no significant interactive effect between food and the presence of the parasite on pupae regardless of food concentration, this was not true for mortality probabilities among fourth instars. Significantly more fourth instars died in response to parasite exposure and there were significant interactive effects of food and parasites on the probability of fourth instars transformation to pupae over 48 hrs.

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INTRODUCTION

In the study of insect biocontrol, many factors have been identified that influence the survival of both the pest and its control agent. The effective use of parasites as agents in biological control requires an understanding of individual and interactive relationships between the host population, the environment and the parasite population. In this context, the present study examines the impact of various levels of food availability during larval development, and exposure to infection with the entomopathogenic digenean, *Plagiorchis elegans* (Rudolphi), on the preimago population structure of *Aedes aegypti* (L.).

Competition is a modifying factor which affects birth and death in insect populations. Studies on the survival of insects in variously constrained environments have shown that competition decreases the overall fitness of the population. The intake of sufficient food at regular intervals is essential to maintain the good health and normal functioning of organisms. Inability to secure adequate food may lead to starvation. Morphological characteristics, such as adult size, and population characteristics, such as density and fecundity, tend to decrease with increasing competition for limited resources. At the same time, morbidity and mortality may rise (Ullyett, 1950; Wada, 1965; Esch et al., 1975; Anderson, 1980; Walker et al., 1987; Nasci et al., 1992).

The quality and quantity of food affect population dynamics, and translate into adult dispersal and longevity potentials. Food is one of the most important factors affecting the development of the larval stages of mosquitoes. Fourth-instars are the most active foragers. Nayar (1968) suggests that most of the energy accumulation, primarily in

the form of lipids and glycogen, occurs at this final larval stage, prior to pupation. Thus, the energy reserves and physical characteristics of the emerging adult depend on the success of fourth instars in obtaining and storing food energy. Morphological characteristics, such as femur length, thorax length, and adult wing breadth and length progressively decline as starvation of fourth instars progresses. All of the above characteristics influence mosquito flight energetics and biting vector potentials (Nayar, 1968).

Food constraints in natural communities are most often caused by increases in population densities. Among mosquitoes, high population densities in an enclosed system lead to high larval mortality, prolonged larval developmental times and a decrease in the size of emerging adults (Wada, 1965). Similar effects are seen in other insect species. Thus, Ullyett (1950) demonstrated that the developmental period of undernourished blowfly larvae is extended. Such delays can place additional nutritional constraints on an already burdened population and may also lead to increased larval predation (Ullyett, 1950; Walker et al., 1987). The high cost of survival eventually produces semi-starved, abnormal and under-sized adult flies.

The delayed development of nutritionally deprived insects allows for increased contact time with their parasites (Walker et al., 1987). At the same time, the increased rate of mortality, imposed on overcrowded host communities by their parasites, may eventually release survivors from intense competition for limited food resources and space (Ullyett, 1950; Washburn et al., 1991; Renshaw et al., 1994). Nasci et al. (1992) demonstrated that the mortality rate of nutritionally-deprived *Ae. aegypti* larvae was

extremely high (60-81%) when compared with well-fed larvae (2-16%) when both groups were infected with the same concentration of the microsporidian, *Edhazardia aedis*. Similarly, *Lambornella clarki* can influence the outcome of intraspecific competition for food among *Aedes sierrensis* larvae under conditions of fluctuating resource availability (Washburn et al., 1991). Adult *Ae. sierrensis* from parasitized, high density populations were significantly larger than those emerging from high-density, non-parasitized populations. Since mosquito adult size is correlated with the amount of food ingested during larval development, this supports the theory that the parasite reduces competition by killing larvae.

The current study established populations of *Ae. aegypti* larvae in enclosed environments, at conditions of constant recruitment, varying levels of nutrition and exposure to pathogens. Hartley (1996) suggests that such populations of larvae exhibit differential rates of development and mortality. Intraspecific competition may be severe at low levels of food availability. Moreover, large larvae may outcompete smaller conspecifics such that the latter experience reduced rates of development. With constant recruitment, early instar larvae are expected to accumulate, changing the structure of the population. The introduction of the entomopathogenic digenean, *P. elegans*, may alleviate this situation since they preferentially infect and kill late instar larvae (Dempster and Rau, 1987), thereby reducing competition with younger instars for limiting food resources.

The present study documents changes in the developmental rates of *Ae. aegypti* larvae by conducting a daily census of larval populations and calculating the probabilities

of transformation from one larval stage to the next. Such differential transition probabilities allow an analysis of variance of the developmental means of the five preimago stages over time. The study assesses the interactive effects of intraspecific competition for limited food resources and the sustained application of entomopathogenic parasites on instar-specific mortalities, developmental probabilities, population structure and adult mosquito production.

LITERATURE REVIEW

Aedes aegypti

Aedes aegypti is one of the most important and most intensely studied mosquito species. This is due, in part, to its prominence as a vector of a number of viral diseases, among them yellow fever and dengue (Harwood and James, 1977), to its ability to utilize a wide range of habitats in urban settings, and its anthropophilic nature. The insect is documented worldwide (Christophers, 1960) and is characteristically closely associated with human habitations (Barrera, 1996). The distinguishing physical features of the adult include an intricately marked body with silvery-white and yellow-white bands and stripes on a black background, and a dorsal, lyre-like pattern on the thorax. There are also bands on the legs; the tarsi of the last pair of legs are characteristically white in colour (Harwood and James, 1977).

Females of *Ae. aegypti* require a blood meal to induce oogenesis (Gjullin et al., 1961). The eggs are laid singly, close to or at the waterline and frequently in artificial containers (Harwood and James, 1977). Christophers (1960) described the egg as small (0.664 mm in length and 0.170 mm in width), rather boat-shaped with a flat upper surface and a convex lower surface perforated with fine air channels to the base of the exochorion. The female mosquito readily lays her eggs on a wet surface where the chrorionic pad can anchor its flat, ventral side to the water surface. The egg itself is fertilized in the act of oviposition and undergoes embryonation to form the primary larva. When newly deposited, the *Ae. aegypti* egg is a translucent white, it gradually darkens to a blueish hue over time and eventually reaches a black colour 4 hrs after oviposition.

Freshly laid eggs need a moist environment for a period of 24 to 72 hrs to produce fully-formed primary larva (Christophers, 1960). Such fully "conditioned" eggs are resistant to adverse environmental conditions and can withstand desiccation for up to one year (Christophers, 1960; Harwood and James, 1977).

Several stimuli must be present to induce hatching of the fully formed larvae. These include submergence of the egg in an appropriate aqueous medium (such as one which contains microorganisms, enzymes, and is low in dissolved oxygen), agitation and the presence of larvae (Christophers, 1960).

Mosquitoes undergo complete metamorphosis with four well-defined stages: the egg, the larva, the pupa and the adult (imago) (Gjullin et al., 1961). Larvae molt four times and the rate of development is influenced by environmental factors such as temperature, crowding and the availability of food (Barr, 1958). Barrera (1996) found that laboratory-reared *Ae. aegypti* require a mean of 26.6 (± 0.8) days to develop from the first instar to the adult, when raised at low-density, pure cultures and at optimum water temperatures of 24.5 - 25.5°C. Barr's 1958 study showed that mosquito larvae raised in a high-density environment are developmentally impaired. Barrera (1996) supports these conclusions by demonstrating that larvae from high-density environments are significantly smaller than those reared at low densities. The number of pupae and adults emerging from high density populations are low, suggesting a negative, intraspecific impact on developmental time and structure. Barr (1958) proposed that such crowding delays development and growth, even in the presence of abundant food, by interfering with the movements of larvae.

Larvae of *Ae. aegypti* are indiscriminant in their feeding habits. They ingest diatoms, protozoans, algae, bacteria and detritus (Barr, 1958), and may also scavenge the carcasses of conspecifics (Merritt et al., 1992). The feeding mode of the larvae is similar to the deposit-feeding of zooplankton, where they sink or dive to the substrate and re-suspend loosely-attached materials with their lateral palatal brushes. They may aggregate at food-rich sites.

Plagiorchis elegans

Plagiorchis elegans is a digenean parasite. The taxonomy of the group is based on the characteristics of their larval forms, and the configuration of their excretory system (Larue, 1957). *Plagiorchis elegans* (Rudolphi) is placed in the family Plagiorchiidae. The identification of the various species of *Plagiorchis* is based on egg and body morphometrics, as well their choice of definitive host organism.

The adult fluke is elongated and leaf-shaped, with an attenuated anterior and a pointed posterior. The well-defined external characteristics include an oral and ventral sucker. The fluke's internal anatomy is characterized by a short oesophagus and two intestinal caeca. A Y-shaped excretory vesicle is located dorsal to the uterus and empties to the outside via an excretory pore on the ventro-posterior surface of the body (Larue, 1957). The worms are dioecious: the single, globular ovary is located to the right of body axis. Posterior to the ovary lies the seminal receptacle. Testes are arranged obliquely (Olsen, 1937).

Life Cycle of *Plagiorchis elegans*

The partially embryonated eggs of *P. elegans* are passed with the droppings of the definitive hosts into water. In an aqueous environment, the eggs continue their embryonation and become infective in approximately one week. The life cycle continues when the eggs are ingested by a molluscan first intermediate host, *Stagnicola elodes* or *Lymnaea stagnalis* (Blankespoor, 1977). The miracidium hatches from the egg in the digestive system of the snail host and penetrates the host tissues to form a mother sporocyst. The mother sporocyst then gives rise to numerous daughter sporocysts, and it is from the latter that the next asexual generation of the parasite, the entomopathogenic cercaria, is produced (Blankespoor, 1977). Cercariae are formed within the sac-like daughter sporocyst in the hepatopancreas of the snail, and are first released into the aquatic environment by the thousands approximately 40 days post-infection. Cercarial production persists throughout the life of the snail host.

Cercariae are released daily from the molluscan host at dusk (Webber, 1987). The cercariae are oval and have a simple tail. The oral sucker is armed with a retractile stylet that serves to penetrate the cuticle of the second intermediate host. It is in the body of this host that the metacercarial cyst is formed. Aside from mosquito larvae several other aquatic arthropods can serve as second intermediate hosts, among them the aquatic stages of insects from the orders Odonata, Diptera, and Trichoptera. The behaviours of the cercariae and the aquatic host are coordinated to establish initial contact (Thompson, 1990). *Plagiorchis* cercariae are relatively inactive organisms that tend to remain near the

bottom of the water column. Contact between cercariae and host may occur as the latter forages near the bottom.

During the encounter, cercariae attach to the host by their suckers and penetrate the cuticle with the aid of the stylet and histolytic enzymes (Blankespoor, 1977). The ability of cercariae to penetrate their hosts declines with age and increasing water temperature. The tail is discarded during the process of penetration. Encystment may occur in 10 to 15 minutes and in any part of the host's body, but most frequently in the abdominal haemocoel (Kavelaars and Bourns, 1968).

Once encysted, metacercariae require from 4 to 6 days in the second-intermediate host to become infective to the definitive host, generally a mammal or bird (Blankespoor, 1977). The definitive host acquires the infection by ingesting infected arthropods. Metacercarial cysts are digested out of the tissue, the metacercariae excyst and grow into adult worms. Eggs are produced and passed with the droppings of the definitive host.

Host-Parasite Relationship Between *Ae. aegypti* and *P. elegans*

Cercariae of *P. elegans* are weak swimmers and do not remain suspended for long in the water column. Within 3 to 4 hrs after emergence from the snail host, most cercariae have settled to the bottom of a 20 cm water column (Lowenberger and Rau, 1994). As a result, the infection process depends largely on the motility of the mosquito larvae. Since larvae of *Ae. aegypti* are primarily bottom feeders, contact with the parasite is most likely to occur at that time.

Environmental factors that influence the behaviour of the larvae also affect parasite acquisition. Thus, larvae aggregate in the daytime and disperse at night. Such dispersal promotes parasite acquisition (Webber, 1987). Age of the cercariae also influences the infection process. Cercariae remain active for approximately 10 hrs post-emergence. Thereafter, activity declines precipitously, and infectivity reaches zero 24 hrs post-emergence (Lowenberger and Rau, 1994).

The size of the mosquito larva influences its chances of parasite acquisition (Dempster and Rau, 1987). Infection increases progressively with larval size, and decreases abruptly at pupation. Fourth instar larvae are, by far, the stage most susceptible to infection. The small first instar larvae effectively avoid the relatively large cercariae, whereas the relatively large fourth instars cannot. Nevertheless, small larvae, if infected, are more likely to die as a result of infection. The thick cuticle and the behaviour of the pupae may deter penetration of cercariae.

Mosquito larvae respond behaviourally to the presence of cercariae. Gilchrist (1994) demonstrated that *Ae. aegypti* larvae exhibit avoidance reactions in response to cercariae during daylight hours, spending less time foraging at the bottom of the water column. In darkness, however, the presence of the cercariae elicits diving responses that enhance contact between host and parasite.

Resource Limitation and Competition

Under natural conditions, the growth of animal populations assumes an intrinsic rate that is governed by factors such as birth, death, immigration and emigration rates. These rates underlie the dynamics of the population and influence its structure and

density. Population growth is influenced by exogenous factors such as climate and resources, and by endogenous factors such as intraspecific competition. Both inter- and intraspecific competition play a major role in the regulation of population growth of insects. Thus, Ullyett (1950) demonstrated that competition for food effectively limits the size of natural populations of three *Chrysomyia* species. Furthermore, populations surviving under conditions of resource restraint suffer declines in overall fitness. Morphological characteristics such as adult size, and population characteristics such as density and fecundity tend to decrease with increasing competition for limited resources, whereas mortality and morbidity rise (Ullyett, 1950; Wada, 1965; Walker et al., 1987; Nasci et al., 1992).

Ullyett (1950), in a study of competitive interaction between blowflies, demonstrated a tendency for most individuals in a nutritionally restricted population to survive. However, since the developmental period of undernourished larvae is extended, increasingly severe nutritional constraints are placed on an already burdened population which may contribute to higher risks of larval predation (Ullyett, 1950; Walker et al., 1987). The high cost of survival eventually produces semi-starved, abnormal and under-sized adult flies. This may be an adaptation to allow more individuals to survive on a limited food resource than would be possible otherwise (Ullyett, 1950). Since adult body size strongly influences female reproductive success, Ullyett (1950) and Washburn et al. (1991) underscored the greater need of females to feed during larval development.

Nasci (1986) reported that larger females of *Ae. aegypti* tend to be more successful in locating hosts for bloodmeals and to survive longer. Female size is strongly

related with the magnitude of energy reserves accumulated during larval development (Nayar, 1968; Nasci, 1986). A similar relationship has been established for *Ae. cantans*. Larger females are stronger fliers than their smaller counterparts and locate hosts sooner. They also take larger bloodmeals and produce correspondingly larger egg clutches (Renshaw et al., 1994).

Food shortage in natural communities is most often caused by an increase in population density. The effects of high density may be expressed in conjunction or separately from the effects of food shortage. High density of mosquito larvae in an enclosed system leads to high larval mortality, prolonged larval developmental time and a decrease in the size of emerging adults, even when a constant amount of food per larva was maintained. This suggests that overcrowding and starvation have similar effects but may act independently (Wada, 1965).

There appears to be an optimum density at which the *Ae. aegypti* larvae are stimulated to grow rapidly. Larvae growing below this optimum density are delayed in their development and exhibit higher rates of mortality. Furthermore, emerging adults may be smaller than those emerging under conditions of optimum density. It was also revealed that in a highly unfavourable growing environment, males tend to have a higher probability of survival to pupation than females. Adult size of *Ae. aegypti* is affected by both population density and food quality and quantity. Again, the size of emerging female mosquitoes reflects their higher sensitivity to the growing conditions (Wada, 1965). Conditions of overcrowding generate both inter- and intraspecific conflict as organisms compete for living space. Such conflict may be expressed as physical or chemical

aggression. Thus, under overcrowded conditions, older instars of *Ae. aegypti* may produce toxic factors which impair the development and survival of younger conspecific and heterospecific instars (Ikeshoji and Mulla, 1970). Furthermore, current research in the production of chemical factors by *Ae. aegypti* larvae and pupae showed that nutritionally deprived preimagos can release chemical signals into their aquatic environments to induce repellency of such sites to ovipositing conspecific females (Zahiri et al., 1997a). Apparently, the length of the duration of starvation also determines the attractiveness of the environment, as gravid females tend to increasingly avoid waters containing larvae starved over longer periods.

Combined Effects of Parasitism and Competition on Host Population Dynamics

Parasitism may play a dual role in its effect on host population dynamics (Ullyett, 1950; Petersen, 1973; Walker et al., 1987; Washburn et al., 1991; Nasci et al., 1992; Renshaw et al., 1994). The increased rate of mortality superimposed on overcrowded host communities by their parasites may eventually release the host population from intense competition for limited food resources and space (Ullyett, 1950; Washburn et al., 1991, Renshaw et al., 1994). Parasitism, as well as predation, may serve to adjust the equilibrium between competing species of adult blowflies and the number of eggs that are laid by each species, thus reducing interspecific competition (Ullyett, 1950). Nasci et al. (1992) demonstrated that the mortality rate of nutritionally-deprived *Ae. aegypti* larvae was very much higher than that of well-fed larvae when both groups were infected with the same intensity of the microsporidian *Edhazardia aedis*. Similarly, the ciliate

Lambornella clarki can influence the outcome of intraspecific larval competition for fluctuating food resources. Emerging adults of *Ae. sierrensis* from parasitized, high density populations were significantly larger than those emerging from high-density, non-parasitized populations. Since adult mosquito size is related to the amount of food ingested during larval development, this supports the theory that the parasite reduces competition by its effect on larval survival. *Lambornella clarki* removes early instars before food resources become restricted. The survivors from a previously dense population are able to develop into adults of similar size as those from low-density populations (Washburn et al., 1991). However, several studies suggest that host population responses are controlled more effectively by food constraints than the presence of the parasite in the community (Walker et al., 1987; Nasci et al., 1992).

Zahiri et al. (1997b) showed that the presence of parasite-infected *Ae. aegypti* larvae can also inhibit gravid conspecific females from laying their eggs within the same environment. The ability of the resident larvae to communicate an adverse, parasite-ridden environment via chemical signals and the ability of the female mosquito to receive these communications may represent adaptive behaviours to ensure the successful survival of hatching larvae, and the continued existence of the species.

MATERIALS AND METHODS

Maintenance of *Aedes aegypti*

Populations of adult *Ae. aegypti* were maintained at 28°C (± 1) and a photoperiod of 14 hrs light and 10 hrs darkness in 56 cm (length) x 21 cm (width) x 23 cm (height) flight cages. The adults were fed 10% sucrose solution *ad lib*: females were provided with a weekly bloodmeal to induce oviposition. Females laid eggs in round, plastic containers (11.5 cm diameter x 7.5 cm height) lined with filter paper and containing 250 ml of distilled water. Filter papers and adhering eggs were removed, eggs were conditioned for 5 days before dry storage at 28°C (± 1) until needed. Eggs were used within 3 months. Eggs were hatched at 28°C in 400 ml distilled water that had been deoxygenated by boiling for approximately 20 minutes. Only first instars hatching within the first 10 minutes were used in the experiment.

Maintenance of *P. elegans* Cercariae

Snails, *Stagnicola elodes* (Say), naturally infected with *P. elegans*, were maintained in the laboratory as described by Webber (1987). In order to obtain freshly emerged cercariae, 25-30 snails were placed in 100 ml of aerated well water at 20°C for 2 hrs in total darkness just prior to the regular scotophase (12 PM) when cercariae usually emerge from the snail host. The number of cercariae shed was estimated volumetrically on the basis of the mean number of cercariae in ten 0.1 ml samples counted at 20 X magnification under a dissecting microscope.

Experimental Design

The experimental design was a 2 x 5 factorial with 3 replications for each of the 10 treatment combinations. There were two levels of parasitism and five levels of food availability. The effects of food availability and parasitism on the population were assessed in terms of development of preimagos, mortality and emergence of adults, using repeated measures analysis of variance.

Experimental microcosms were established in shallow, rectangular pans (30 x 14 x 6.8 cm^3), filled to a depth of 0.8 cm with 450 ml of distilled water containing 0.001, 0.002, 0.003, 0.005 or 0.008 g of Tetramin fish food (TetraWerke, Melle, Germany), with or without 900 cercariae of *P. elegans* and seeded with 10 first instars of *Ae. aegypti*. The range of food availability levels was determined in preliminary experiments. All allowed at least some adult emergence, whereas the highest level approached optimal food availability.

Every 24 hours for 31 consecutive days, all living larvae were identified as to instar and transferred to a corresponding, fresh microcosm. Living pupae were counted and transferred to individual glass vials containing 10 ml of aerated tap water for the recuperation of emerging adult mosquitoes. As a final step, 10 newly-hatched, first instars were added to simulate recruitment. The microcosms were returned to an incubator (28°C).

Data Processing and Calculation of Time-Dependent Transition Probabilities (TDTP)

Daily censuses of all mosquito life stages, as well as the number of deaths at different preimago stages, were tabulated on a day-to-day basis over a period of 31 days. Population structure graphs were generated by averaging daily values from the three replicates for each food level by parasite level combination. Data pertaining to the number of adults emerging were presented cumulatively, while those belonging to the preimago stages were not. Time-dependent transition probabilities were calculated based on the count values. These represented the probabilities that a particular preimago stage had been rendered incapable of transforming to a successive stage due to a reduced developmental rate, the probabilities of a molt and successful transformation of one immature mosquito stage to the next successive stage, and the probabilities of death at a preimago stage resulting from a variety of causes including insufficient food, parasitic infection or a combination of both.

Even under optimum environmental conditions, in the absence of parasitic infection or food restriction, larval development is still subject to time constraints. First and second, and to some extent, third instars may transform to a successive larval stage within 24 hrs, whereas fourth instars and pupae require a minimum of 48 hrs (Christophers, 1960). Consequently, the 24-hr transition probabilities obtained for fourth instars and pupae are not admissible and are not reported in this initial experiment. Under optimal conditions, first instars can reach third instar stage in 48 hrs. This also applies to the transition of second instar stage to the fourth. Therefore, 48-hr transition probabilities

of first instar to third, second to fourth and third to pupal stages were calculated from the raw data at 48-hr intervals.

For 24-hr transitions, the calculations of transition probabilities from one stage to the next depended on the number of living and dead individuals of a particular stage at time t-1 and the number of individuals at the successive stage at time t. The 48-hr transition probabilities were obtained by taking into consideration the number of living and dead individuals at particular life stages during times t-2 and t-1. Therefore, in order to account for individuals from time t-2, it was necessary to incorporate some of the 24-hr transition probabilities into the equations to obtain transition probabilities for a 48-hr time span. A listing of the mathematical equations used to obtain 24- and 48-hr transition probabilities is provided below.

Declaration of Variables for Time-Dependent Transition Probability Equations

Time-dependent transition probabilities are estimated based on the raw censused data. The equation variables are defined as followed:

P11: The probability that a first (1) instar larva remains first (1) instar for the first 24-hr period within a 48-hr period window.

P12: The probability that a first (1) instar larva successfully develops into second (2) instar for the first 24-hr period within a 48-hr period window.

P1d: The probability for a first (1) instar larva to die in the first 24-hr period within a 48-hr period window.

Pxy: The probability that a mosquito at developmental stage x transforms into stage y in the first 24-hr period within a 48-hr period window.

The remaining 24-hr probabilities include: P2d, P33, P34, P3d, P44, P4P, P4d, PPP, PPA, and PPd. These follow the same principles as explained above, where P and A denote the pupal and adult stages.

q11: The probability that a first (1) instar larva remains first (1) instar for the second 24-hr period within a 48-hr period window.

qxy: The probability that a mosquito at developmental stage x transforms into stage y in the second 24-hr period within a 48-hr period window.

m1: Number of first instars at time t-1.

n1: Number of first instars at time t.

m2: Number of second instars at time t-1.

n2: Number of second instars at time t.

mx: Number of living x-stage mosquitoes at time t-1.

nx: Number of living x-stage mosquitoes at time t.

m1d: Number of dead first instars at time t-1.

n1d: Number of dead first instars at time t.

m2d: Number of dead second instars at time t-1.

n2d: Number of dead second instars at time t.

mxd: Number of mosquitoes at x-stage that are dead at time t-1.

nxd: Number of mosquitoes at x-stage that are dead at time t.

R11: The probability that a first instar larva remains at the first instar stage in 48 hrs.

R12: The probability that a first instar larva successfully develops into second instar stage in 48 hrs.

R13: The probability that a first instar larva successfully develops into third instar stage in 48 hrs.

R1d: The probability that a first instar larva dies in 48 hrs.

Rxy: The probability that a mosquito at developmental stage x on one day of census is at stage y two days later.

The remaining 48-hr probabilities include: R24, R2d, R33, R34, R3d, R3P, R44, R4P, R4A, R4d, RPP, RPA, and RPd. These follow the same principles as explained above.

The following equations were used to calculate time-dependent transition probabilities:

Twenty-Four-Hour Transition Time

PPA = (nA-mA)/mP

PPd = (nPd-mPd)/mP

PPP = 1-PPd-PPA

P34 = [n4-(P44)(m4)]/m3

P3d = (n3d-m3d)/m3

P33 = 1-P3d-P34

P12 = [n2-(P22)(m2)]/m1

P1d = (n1d-m1d)/m1

P11 = 1-P1d-P12

P4P =[nP - (PPP)(mP)]/m4

P4d = (n4d-m4d)/m4

P44 = 1-P4P-P4d

P23 = [n3-(P33)(m3)]/m2

P2d = (n2d-m2d)/m2

P22 = 1-P2d-P23

Forty-Eight-Hour Transition Time

R11 = P11 x q11 R22 = P22 x q22

R12 = P11 x q12 + P12 x q22 R23 = P22 x q23 + P23 x q33

R13 = P12 x q23 R24 = P23 x q34

R1d = P1d+P11 x q1d R2d = P2d+P22 x q2d

R33 = P33 x q33 R44 = P44 x q44

R34 = P33 x q34+P34 x q44 R4P = P44*q4P+P4P*qPP

R3P = P34 x q4P R4A = P4P x qPA

R3d = P3d+P33 x q3d R4d = P4d+P44 x q4d

RPP = PPP x qPP

RPA = PPP x qPA+PPA x qAA

RPd = PPd+PPP x qPd

A computer program written in Turbo Pascal - Version 6.0 was used to incorporate the raw data and perform the calculations. The equations above are also implemented in a SAS code called Mosquito5.sas.

Statistical Analyses

Time series of mean transition probabilities were computed for each of the five food levels, the two parasite levels and the 10 food by parasite combinations, using the MEANS procedure of SAS (SAS Institute Inc., 1988). A univariate repeated measures analysis of variance (Crowder and Hand, 1990) was performed on the daily time-dependent transition probabilities, with the day as a repetition factor (i.e., the factor

whose levels correspond to the profile vector of repeated measures) and the food and parasitism levels as treatment factors. The GLM procedure of SAS (SAS Institute Inc., 1988) and its statement REPEATED were used for this repeated measures analysis of variance. An adjustment of the probabilities of significance of the ANOVA F tests for effects involving the time factor (main effects and interactions) was required due to the lack of independence and the heterogeneity of variance of the TDTP data. The Greenhouse-Geisser adjustment was used here in a conservative approach (Crowder and Hand, 1990).

All three factors (time, food, parasite) were crossed, so that seven effects were tested: the time, food and parasite main effects, the time-by-food, time-by-parasite and food-by-parasite two-way interactions, and the three-way interaction.

Due to missing data (> 10 days), the lowest food level (0.001 g of Tetramin/450 ml) was deleted from the analyses of 24-hr TDTP for third and fourth instars and pupae, as well as from the analyses of 48-hr TDTP for all preimago stages. To perform analyses on the 48-hr TDTP of fourth instars and pupae, the missing data were replaced by the predicted values provided by the GLM procedure carried out in a preliminary stage. As there were over ten days of missing data, to provide for credible predictions, it was also necessary to remove the 0.003 g of Tetramin/450 ml food level from statistical analyses of fourth instar and pupal TDTP.

RESULTS

Main Effects of Time on Preimago Development

The repeated measures analysis for the 24-hr time interval indicated that over the 31-day experimental period, there was highly significant ($p < 0.001$, Table 1) variation in the transition probabilities of mosquito individuals for all life stages, but not for mortality ($p \geq 0.05$, Table 1).

With the 48-hr time interval, time had a significant effect on the transition probabilities of mainly the first and second instar life stages ($p < 0.01$ for most first and second instar TDTPs, Table 2). The mortality probabilities for all instars and pupae were not affected over time ($p \geq 0.05$).

Effects of Time-by-Food, Time-by-Parasite and Three-Factor Interaction Effects on Preimago Development

Twenty-Four-Hour Transition

Whereas the time-by-food interaction was not significant for the TDTP of dying for first, second and third instars, that interaction was significant for several first, second and third instar TDTPs ($p = 0.0494$ for P12, $p = 0.0428$ for P22, $p = 0.0225$ for P23, $p = 0.0156$ for P33).

The time-by-parasite interaction was significant only for the transition probability of first instars remaining at the same stage ($p = 0.0461$ for P11), of first instars dying ($p = 0.0486$ for P1d) and of second instars transforming into third instars ($p = 0.0435$ for P23). All the other TDTPs were not significantly affected by the time-by-parasite interaction (Table 1).

The time-by-food-by-parasite interaction was significant only for the TDTPs of second instars to remain second instars ($p = 0.0392$ for P22) and of second instars to become third instars ($p = 0.0449$ for P23).

Forty-Eight-Hour Transition

The repeated measures analysis of variance yielded the following results for the time-by-food interaction. For first instars, the time-by-food interaction significantly affected the probability of a first instar advancing to the second instar ($p = 0.0344$ for R12, Table 2). The time-by-food interaction was non-significant ($p \geq 0.05$) for all transition probabilities involving second instars. For third instars, only their probability of remaining third instars was significantly affected by the time-by-food interaction ($p = 0.0222$ for R33). With the exception of R4A, all TDTPs for fourth instars showed non-significant time-by-food interaction ($p \geq 0.05$). In contrast, all TDTPs for pupae showed significant ($p < 0.05$, RPP) or highly significant ($p < 0.01$, RPA and RPd) time-by-food interaction.

The two-way interaction between time and parasite was significant ($p < 0.05$) for R11, R12, R1d, R22, R23, R4A, RPP, and RPA. Finally, the three-way interaction among food, parasite and time, was non-significant ($p \geq 0.05$) for most 48-hr TDTPs (i.e., R13, R22, R23, R24, R2d, R33, R34, R3P, R3d, R44, R4P, R4A, and R4d).

Main Effects of Food on Preimago Development

First Instars

The results indicated that after both 24 hrs and 48 hrs, the first instars provided with extremely low amounts of food did not develop as rapidly as first instars in an environment rich in food. For both 24- and 48-hr time intervals, the TDTPs P11 and R11 decreased in values with increasing food concentrations, while correspondingly P12, R12 and R13 rose steadily at higher food levels (Figures 1 and 2). The analysis of variance showed that food had a significant effect on the TDTP of first instars and this was verified in that the TDTPs P11 and R11 were highest at the lowest food levels and steadily declined with higher food concentrations ($p = 0.0001$). This pattern reversed for successive state transitions ($p = 0.0001$ for P12, R12 and R13) yielding very highly significant main effects of food. The transition probabilities to death P1d and R1d also increased at higher food levels ($p = 0.0001$).

Second Instars

For both 24 hrs and 48 hrs, similar results were obtained for the development of second instars under the five food regimes. Food was shown to have a significant effect on the mean TDTP of second instars. Development was retarded at low food levels and most second instars remained at the same stage for 24 and 48 hrs, resulting in high P22 and R22 and lower P23, R23 and R24 probabilities (Figures 3 and 4). Higher food levels significantly decreased the mortality of second instars after 24 and 48 hrs ($p = 0.0011$ and $p = 0.002$, respectively).

Third Instars

For either time interval length (24 or 48 hrs), third instars were affected significantly by low food concentrations. In fact, the lowest food concentration, 0.001 g of Tetramin, was insufficient to support larval development to the third stage. Second instars either did not transform in 24 hrs, as indicated by high P22 probabilities, or if they did, third stage larvae were incapable of developing further. The large number of zero counts generated made it inappropriate to calculate the TDTPs for the third instars. As a consequence, larvae at 0.001 g of Tetramin were deleted from the repeated measures analysis of variance.

The four remaining food levels did not support high developmental probabilities for third instars within 24 and 48 hrs. This can be seen for significantly higher P33 and R33, and the much lower corresponding P34, R34 and R3P (Figures 5 and 6). This frequent inability to transform from third to fourth instars and to pupae was somewhat alleviated by increasing food levels for both time interval lengths, reflecting the highly significant ($p < 0.001$) effect of food on third instar larval development. The 24-hr probability of mortality, P3d, was significantly decreased by increasing the amount of food available ($p = 0.0019$, Figure 5c), but the food main effects on the 48-hr probability of mortality, R3d, narrowly escaped significance ($p = 0.055$, Figure 6d).

Fourth Instars

Food main effects played a significant role in the development of fourth instars to pupae ($p = 0.0001$) and adulthood ($p = 0.0005$). Food also significantly influenced the probability of mortality in this group ($p = 0.0112$). With increasing food availability, the

probability of remaining at the same stage for 48 hrs (R44) was significantly decreased (p = 0.0001), but the probability of dying was increased (Figure 7). Although the calculated TDTPs showed that some fourth instars reached the adult stage in 48 hrs, the increase in food availability primarily allowed an increase in transition probability from fourth instar to pupa (R4P, Figure 7b), rather than to adult (R4A, Figure 7c). Food had no significant effect on the TDTP of *Ae. aegypti* pupae ($p \geq 0.05$, Tables 5a, 5c and 5e, Figure 8).

Main Effects of the Parasite on Preimago Development

First Instars

With the addition of *P. elegans* cercariae, the youngest larval stage showed a significant increase in the mean developmental probability to the second (P12, p = 0.0001 and R12, p = 0.04) and to the third instar (R13, p = 0.0001) at both 24- and 48-hr intervals (Figures 9 and 10). At the same time, mortality among first instar larvae increased significantly (p = 0.001, Figures 9c and 10d).

Second Instars

Similar effects were documented for second instars (Figures 11 and 12). Transformation to the third (P23 at 24 hrs and R23 at 48 hrs) and to the fourth instar (R24 at 48 hrs) was significantly higher in experimental sets exposed to the parasite than in unexposed controls (p =0.0001 for P22, P23, and R23, and p = 0.0002 for R24). However, at the 24-hr interval, *P. elegans* did not significantly affect the mortality of second instars ($p \geq 0.05$, Figure 11c). In contrast, the 48-hr TDTP of mortality among

second instars (R2d) increased significantly with exposure to *P. elegans* ($p = 0.0137$, Figure 12d).

Third Instars

Exposure to *P. elegans* affected the development of third instars in much the same manner as it did for first and second instars (Figures 13 and 14). The addition of *P. elegans* caused a significant increase in the transition probability of third instars to transform to fourth ($p = 0.0001$ for P34, and $p = 0.0007$ for R34). Transformation of third instars to pupae (R3P) was not significantly affected by the parasite. However, the results showed that the parasite significantly increased the probability of death among third instars (P3d and R3d, $p = 0.0001$) for both time interval lengths (Figures 13c and 14d).

Fourth Instars

With the exception of the death probability (R4d), the presence of *P. elegans* caused a significant decrease in all 48-hr TDTPs for fourth instars ($p = 0.0001$). Although the probability of remaining at the same stage (R44) was decreased, the fourth instars did not manifest a higher probability of transformation to successive stages (R4P and R4A, Figures 15b and 15c). Instead, there was a higher probability of mortality as evidenced by the pronounced increase of R4d in parasite-exposed groups ($p = 0.0001$, Figure 15d).

Pupae

Among pupae from experimental sets exposed to cercariae of *P. elegans*, mortality was high and successive stage transitions were not frequent (Figure 16). The presence of

the parasite significantly reduced the probabilities of existing pupae to remain at the same stage (p = 0.0007 for RPP), while simultaneously decreasing their probability of becoming adults (p = 0.0001, Figures 16a and b). Compared to non-parasitized experimental sets, pupae exposed to parasites experienced significantly higher mortality (p = 0.0001 for RPd, Figure 16c).

Food-by-Parasite Interaction Effect on Preimago Development

First Instars

Analysis of variance revealed a strong effect of food-by-parasite interaction on the development of first instars. At 24 hrs, the presence of *P. elegans*, coupled with increased food, caused a decrease in P11 (p = 0.0001, Figure 17) and an increase in P12 (p = 0.0001, Figure 18). Similarly, at 48 hrs, there was a significant interaction for R11 (p = 0.02, Figure 20) and R13 (p = 0.0003, Figure 22). Again, in parasite-exposed experimental sets, the probability to remain a first instar was decreased while those of transition to subsequent stages were increased. Although there was an increase in the probability of transformation from first to second instars after 48 hrs (Figure 21), this increase was not influenced by the interaction between food and parasite ($p \geq 0.05$).

Mortality among first instars was also affected significantly by the interaction (p = 0.0001 for P1d at 24 hrs, and p = 0.0003 for R1d at 48 hrs). Differences in mortality between control and parasite-exposed sets increased with increasing food availability (Figures 19 and 23).

Second Instars

There were strong interactive effects between food and parasite on P22 and R22 (24 and 48 hrs, $p = 0.0002$), P23 and R23 (24 hrs, $p = 0.0002$ and 48 hrs, $p = 0.0049$) and R24 (48 hrs, $p = 0.0031$). With the exception of the mortality probability, i.e., P2d and R2d, the differences between control and parasite-exposed experimental sets for all TDTPs increased significantly with increasing food levels. There was no significant food-by-parasite interaction on mortality among the second instars during 24- and 48-hr intervals ($p \geq 0.05$, Figures 26 and 30).

For the probability of remaining a second instar, the mean values of P22 and R22 were higher in controls than in parasite-exposed sets (Figures 24 and 27). However, in view of P23, R23 and R24 transformation of second instars to successive stages was higher in parasite-exposed systems (Figures 25, 28 and 29).

Third Instars

Effects similar to those on first instars were observed for third instar larvae. Significant differences between control and parasite-exposed experimental sets were revealed in the overall mean of P33 and R33 (24 hrs, $p = 0.0001$ and 48 hrs, $p = 0.0003$), P34 and R34 (24 hrs, $p = 0.0005$ and 48 hrs, $p = 0.02$) and P3d and R3d (24 and 48 hrs, $p = 0.0001$). There was no significant interactive effect on the transformation of third instars to pupae (48 hrs, $p = 0.50$, Figure 36).

The differences in overall mean transition probabilities increased with increasing food levels for P33 and R33 (Figures 31 and 34), P34 and R34 (Figures 32 and 35) and P3d and R3d (Figures 33 and 37).

Fourth Instars

The mean value of the probability of fourth instars remaining at the same stage after a 48-hr period (R44) was not significantly affected by the interaction between food and parasite ($p \geq 0.05$, Figure 38). This was also true for R4A ($p \geq 0.05$, Figure 40). However, the transition from fourth instars to pupae and the mortality among fourth instars were highly influenced by interactive effects between food and parasite ($p = 0.02$ for R4P and $p = 0.004$ for R4d). The differences in transition probabilities between control and parasite-exposed systems were highest at the 0.005 g food level for R4P (Figure 39). Fourth instars also exhibited higher probabilities of transforming to the pupal stage in non-parasitized systems (Figure 39a). Differences in the mean value of the probability of fourth instars dying over 48 hrs increased with increasing food levels. Fourth instars experienced a higher probability of dying when exposed to parasites (Figure 41). There was no significant interaction between food and parasite for any of the TDTPs for the pupal stage ($p \geq 0.05$ for RPP, RPA and RPd, Tables 5a, 5d and 5f, Figures 42, 43 and 44).

Effects of Food and *P. elegans* on the Population Structure of *Ae. aegypti* and the Emergence of Adults

At the end of the 31-day experimental period, all non-parasitized experimental sets sustained a higher population density than any of the parasite-exposed sets. Within the lowest food level, 0.001 g of Tetramin, both the control and the parasite-exposed systems reduced the production of adults.

There was an accumulation of first instars at all food levels among control sets, which was inversely related to food availability. In non-parasitized systems, the decline in accumulation of first instars with increasing food levels produced more fourth instars and eventually, more adults (Figures 48a and 49a). However, among parasite-exposed experimental sets, the reduced accumulation of first instars was not accompanied by any corresponding increase in the production of fourth instars, pupae and adults (Figures 48b and 49b).

The appearance of third and fourth instars was delayed at low food levels (Figures 45, 46 and 47). By contrast, at all food levels, the appearance of third and fourth instars occurred earlier when *P. elegans* cercariae were added. With the exception of the lowest food level, 0.001 g of Tetramin, the number of fourth instars and emerging adults was consistently higher in the absence of parasites. The number of emerging adults was highest in control sets at the two highest levels of food availability (0.005 and 0.008 g of Tetramin, Figures 48a and 49a). The presence of *P. elegans* resulted in a decline in population density over time and reduced the number of emerging adults (Figures 45b, 46b, 47b, 48b and 49b).

DISCUSSION

The data confirmed earlier studies that the availability of food plays an important role in the development of all larval instars (Barbosa et al., 1972; Barbosa and Peters, 1973; van Handel, 1988; Chambers and Klowden, 1990; Pumpuni et al., 1992; Novak et al., 1993; Barrera, 1996; Hartley, 1996). All of the above studies demonstrated that starved mosquito larvae are subject to a variety of detrimental effects, among them, retarded developmental, production of growth-retarding factor (GRF), decreased metabolic activity, reduced body size and weight, increased mortality and reduction in the number of adults produced. Suboptimal larval diets may give rise to small adults which have impaired host-finding abilities and lay fewer eggs.

Although food restriction affected all larval stages, the effect was most pronounced among the first and fourth instars. At the two lowest food levels, there was a large increase in the cummulative number of first instar larvae over time (Figures 45 and 46). Hartley (1996) demonstrated that the number of first instars was increased by a factor of 7 when subjected to a suboptimal diet. At the same time, the number of fourth instars remained relatively low. The scarcity of food in such controlled systems created competition between larvae and resulted in the delayed development of first, second and third instars. As judged by their accumulation over time, first instar larvae were the most susceptible and fourth instars the least. This supports the findings that fourth instar larvae will outcompete earlier instars under conditions of low food availability. In the present

study, first instars appeared incapable of acquiring sufficient food to allow a timely transformation to the second instar, as suggested by low transition probabilities.

Chambers and Klowden (1990) suggest that *Ae. aegypti* larvae must attain a critical weight before they will begin to metamorphose. Furthermore, this weight and the timing of the metamorphosis are determined by the availability of food sufficient for the acquisition of a minimum level of nutrient reserves. In the current study, the high probabilities of remaining at the first instar and the correspondingly low probabilities of transition to the second and third instar suggested such nutritional insufficiencies.

As progressively more food was added to the system (up to a level of 0.008 g), the accumulation of first instars decreased concomitantly (Figures 47, 48 and 49). The population structure suggested a more steady flow-through of first instar larvae, particularly at the highest level (Figure 49a). More fourth instars and more pupae appeared as food levels rose. However, the observed progressive increase in the number of adults produced also suggested that the levels of nutrition provided were still less than adequate for normal development, a common feature in field populations (Washburn, 1995). Thus, at the lowest concentration, no adults were produced, and only 4 per day at the highest. Indeed, Hartley (1996) suggested that 0.05 g of food per 200 ml were required to sustain optimal development of *Ae. aegypti* preimagos.

Increases in food availability not only caused a rise in transformation probabilities of all preimago stadia, but also a rise in mortality. Because all food concentrations in the current study were less than optimal, this increase in mortality may be linked to Chambers and Klowden's (1990) findings. As starved larvae eventually acquired sufficient food to

attain the minimum critical weight, they may have initiated the process of moulting. However, this minimum weight may not be sufficient to allow these instars, perhaps weakened by prolonged starvation, to complete their moult successfully.

According to Christophers (1960), Barbosa and Peters (1973) and van Handel (1988), *Ae. aegypti* can reach the fourth instar in as little as four days. Pupation, however, requires a minimum of 2 days. This suggests that under optimal conditions first, second and third instars may all molt at 24-hr intervals to reach the successive developmental phase. In the present study, only a few first instars and second instars reached the third and fourth larval stage, respectively. Development from the fourth instar to the pupa required 48 hrs. However, because of generally insufficient nutrition, the probabilities of these events were minimal. Instead, malnourished larvae had high probabilities of dying within 48 hrs. In fact, an increase in the availability of food precipitated significantly higher mortality in first and fourth instar population over 48 hrs. Third instar mortality escaped significance by a slim margin (R3d, $p = 0.055$).

The data confirmed the impact of resource availability on mosquito populations. Specifically, malnourishment and starvation will delay larval and pupal development. The larvae's inability to develop to successive stages translated into low numbers of pupae and consequently a reduction in the number of emerging adults. One of the most intriguing aspects noted in this study is the gradual accumulation of first instar larvae, particularly at low food availability. In their 1972 and 1973 studies, Babosa et al. referred to the possibility of the production of growth-retarding factors by later instars which may inhibit the development of younger larvae. Such factors produced by *Ae. aegypti* fourth instars

may impede the development of earlier instars and add to the direct effects of starvation thus causing their accumulation over time.

Exposure of mosquito larvae to cercariae of *P. elegans* caused significant increases in the transition probabilities of first, second and third instars. For all preimago stadia, the presence of the parasites reduced the probabilities of remaining at the same stage within 24 and 48 hrs. Whereas the first, second and third instars transformed more readily to successive stages, this was not true for fourth instars and pupae. With the exception of second instars (24 hrs only), the mortality among all preimago groups increased significantly in parasite-exposed sets, but was most pronounced among fourth instars and pupae. Thus, fourth instars and pupae experienced a near 18-fold and 8-fold increase in mortality, respectively.

According to Dempster and Rau (1987), infection with *P. elegans* cercariae is most prevalent among the fourth instars. Parasite acquisition is a function of host body size, and therefore tends to increase with successive instars. The present study confirms these findings. Fourth instars experienced the highest mortality in response to infection. Pupae and first instars were similarly affected, with a significant higher mortality in the presence of *P. elegans*. Dempster and Rau (1987) reported that first instars generally failed to acquire *P. elegans*, whereas fourth instars were highly susceptible. It is unlikely then, that first instars died as a direct consequence of parasitic acquisition. Conceivably, as fourth instars died of their infection, intraspecific competition for food among the younger larvae was reduced. The absence of competition for food and possibly the opportunity to scavenge the carcasses of dead fourth instars (Barbosa and Peters, 1973),

may allow surviving younger instars to gain the necessary critical weight to begin metamorphosis. Since the risk of dying is increased with higher transformation probabilities, the mortality of first instars may be due indirectly to the presence of *P. elegans*. In contrast, most fourth instars probably died as a direct result of infection: a few managed to pupate but succumbed during the process or soon thereafter. Necropsy of pupae revealed numerous metacercariae. Few adults were produced.

The addition of cercariae did not have a significant effect on the survival of second instars within 24 hrs. However, within 48 hrs, there was a significant increase in mortality among this group. Again, the parasites may have reduced host density, not through direct mortality, but indirectly by relaxing intraspecific competition for restricted food resources and enhancing mortality during metamorphosis. Along with higher probabilities of transformation to the fourth instars, third instars also suffered increased mortality probabilities at both 24 and 48 hrs in parasite-exposed experimental sets. Infection with *P. elegans* did not affect the probability of third instars to become pupae within 48 hrs. This confirms Christophers' (1960) and van Handel's (1988) findings that pupation requires at least 48 hrs beyond the fourth instar. The fact that *P. elegans* can exert high mortality on its insect intermediate hosts, regardless of food availability, may render it attractive as a biocontrol agent. Other studies on the effects of parasitism (Service, 1995; Copeland and Craig, 1992; Washburn et al., 1991, Ullyett, 1950) suggest that the use of parasites as agents in the biological control of insects requires careful consideration. In theory, parasites may reduce the host population, but they may also serve to increase it under certain environmental conditions. In a resource-limited environment, parasites may

relieve intraspecific competition by killing primary competitors. Surviving hosts may acquire sufficient food to complete their development, frequently yielding large females that quickly replenish the population. Because *P. elegans* cercariae have been shown to cause high mortality, particularly among fourth instars, the use of such an entomopathogenic digenean may potentially resolve this problem. Although infected fourth instar competitors are killed, leaving younger instars enough food to sustain development, the latter are killed once they reach the final, highly susceptible larval stage. The insignificant interactive effects between food availability and exposure to parasites among fourth instars and pupae reinforce the potential of *P. elegans* as a control agent of mosquitoes. In natural environments, where resource availability may be highly variable, the cercariae may cause high fourth instar mortality.

The particularly high susceptibility of late instars to *P. elegans* infection further enhances its potential as a biological control agent. Service (1995) cautions against using parasites that target young instars only, as this may reduce intraspecific competition for food and allow older larvae to pupate and subsequently emerge as adults. Rather, he advocates the use of parasites that are capable of controlling older mosquito larvae and pupae.

In this respect, *P. elegans* cercariae may be suitable as agents in the biocontrol of mosquitoes. Not only does *P. elegans* primarily infect and kill late in the mosquito's aquatic life cycle, but the cercariae are released by the snail host for the duration of the

snail host's life. Although the parasite releases the early instars from competition for limited food resources, it also intercepts and kills these larvae before they transform into adults.

CONCLUSION

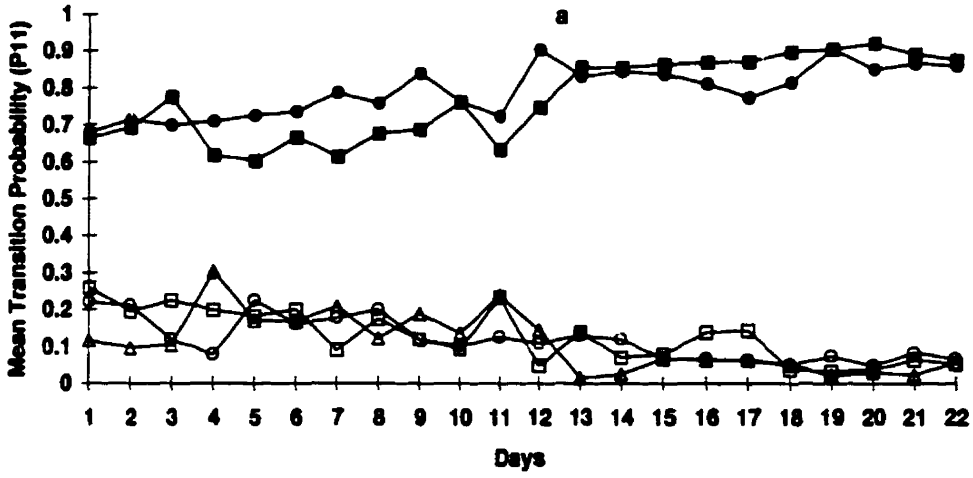
Potential of *P. elegans* as a Biocontrol Agent

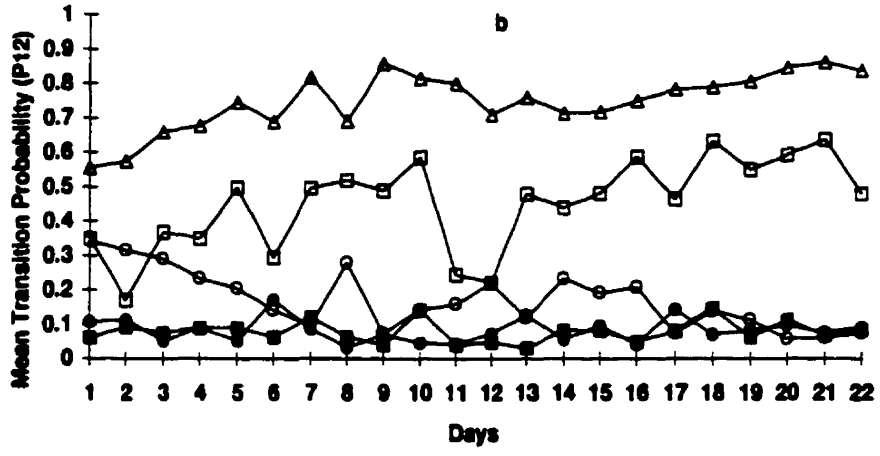
The fact that *P. elegans* can exert high mortality upon its hosts, regardless of food availability, renders it highly attractive as a biocontrol agent. Other studies on the effects of parasitisms (Service, 1995; Copeland and Craig, 1992; Washburn et al., 1991, Ullyett, 1950) caution that using parasites as a means of biocontrol requires careful considerations. Theoretically, parasitism may not only result in the reduction of host population, but may also serve to increase it under certain conditions. Within a resource-limited environment, the use of parasites that relieve intraspecific competition for food by killing competitors may work against the biocontrol strategy. The surviving hosts may acquire sufficient food and complete their development, yielding larger adult pests that will quickly restore population density. Because *P. elegans* cercariae cause high mortality mainly among fourth instars, use of these parasites potentially resolves this problem. Although the infected fourth instar competitors are killed, leaving the younger instars enough food to sustain development, these, in turn, are killed as they reached the final larval stage. The insignificant interactive effect (food x parasites) on fourth instars and pupae, in the present study, reinforces *P. elegans'* potential. In natural environments, with fluctuating resource availability, one can expect the cercariae to have a significant impact on the mortality of fourth instars.

The tendency of *P. elegans* to infect older instars further supports its practicability. Service (1995) effectively sums up biological control strategies by stating that it may be easier to reduce the pest mosquitoes to an acceptable level rather than attempting to control vectors whose populations need to be reduced tremendously to reach critical threshold values. In the same paper, Service also cautions against using parasites that target younger instars, as this would reduce intraspecific competition for food and allow older larvae to pupate and give rise to adults more readily. As such, he stresses the importance of using parasites that are capable of controlling older mosquito larvae and pupae, instead. This further favours the use of *P. elegans* cercariae. A further advantage is that by delaying host mortality until the final stages, the parasites does not impact highly on the natural aquatic food chain, where mosquito larvae comprise important resources for various aquatic fauna (Service, 1995).

Finally, the deployment of *P. elegans* cercariae in the field is facilitated by its asexual reproduction within the first molluscan intermediate host. Several species of *Lymnaeidae* and *Stagnicola* snails can be infected with the eggs of *P. elegans*. Where these snails share their environment with mosquito larvae, the deployment of *P. elegans* eggs will ensure infections that will provide sustained, inundative releases of entomopathogenic cercariae.

Figure 1: **Food effects transition probabilities of first instar *Aedes aegypti* larvae (a) to remain first instars, (b) to become second instars, (c) to die within 24 hours**


a
Mean Transition Probability (P11)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days


b
Mean Transition Probability (P12)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days

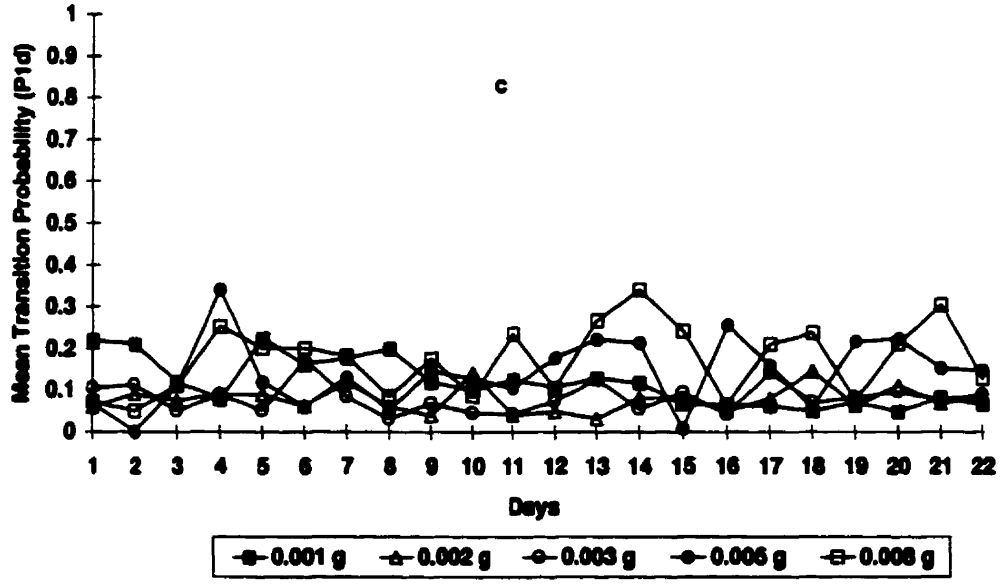
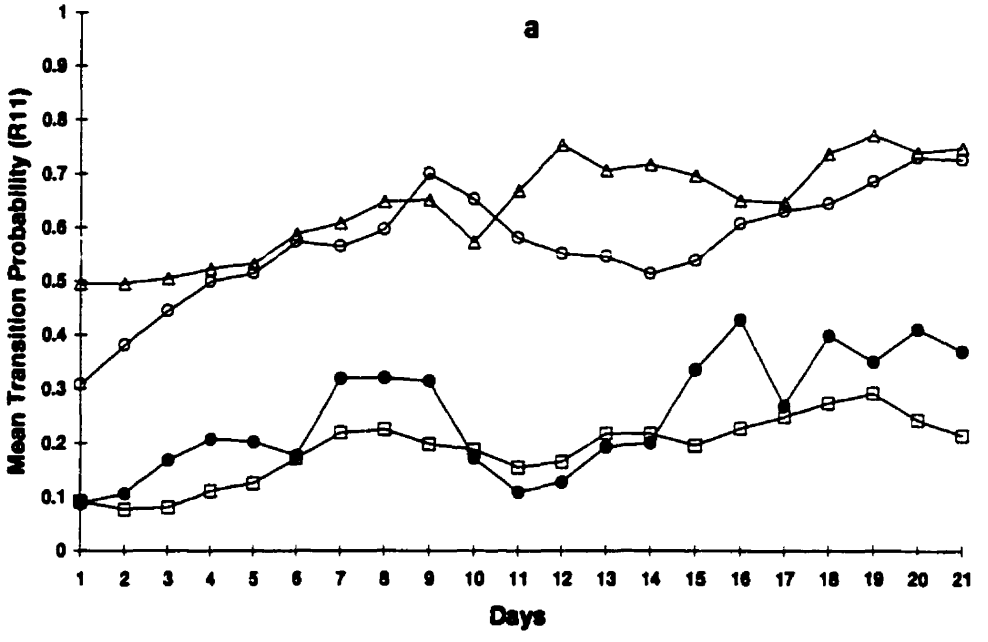

c
Mean Transition Probability (P1d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
0.001 g
0.002 g
0.003 g
0.006 g
0.008 g

Figure 2: **Food effects transition probabilities of first instar *Aedes aegypti* larvae (a) to remain first instars, (b) to become second instars**


a
Mean Transition Probability (R11)
Days

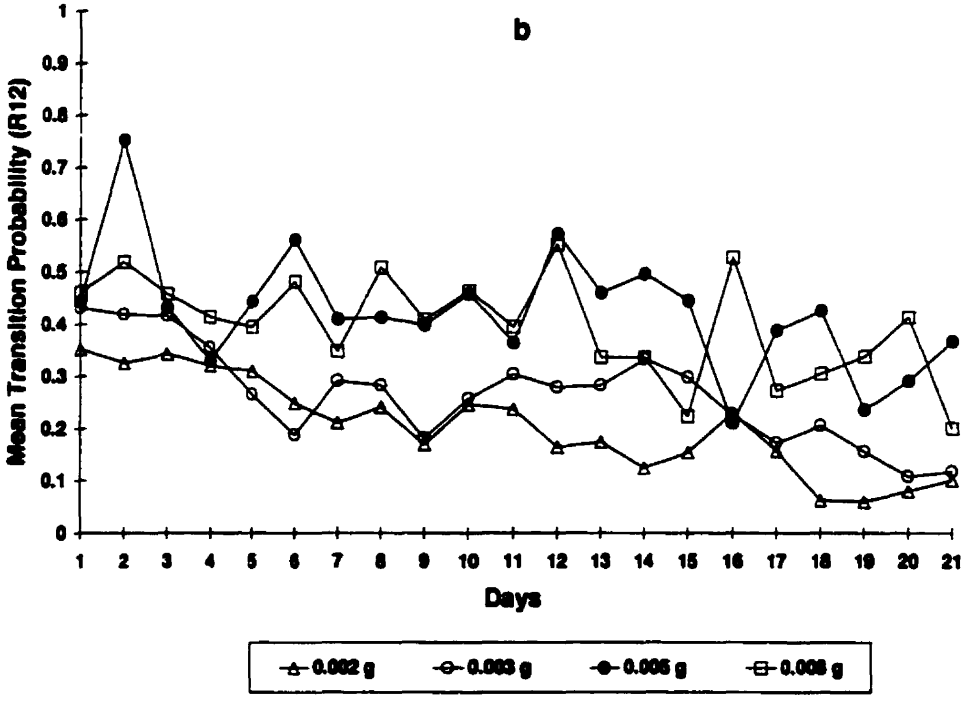
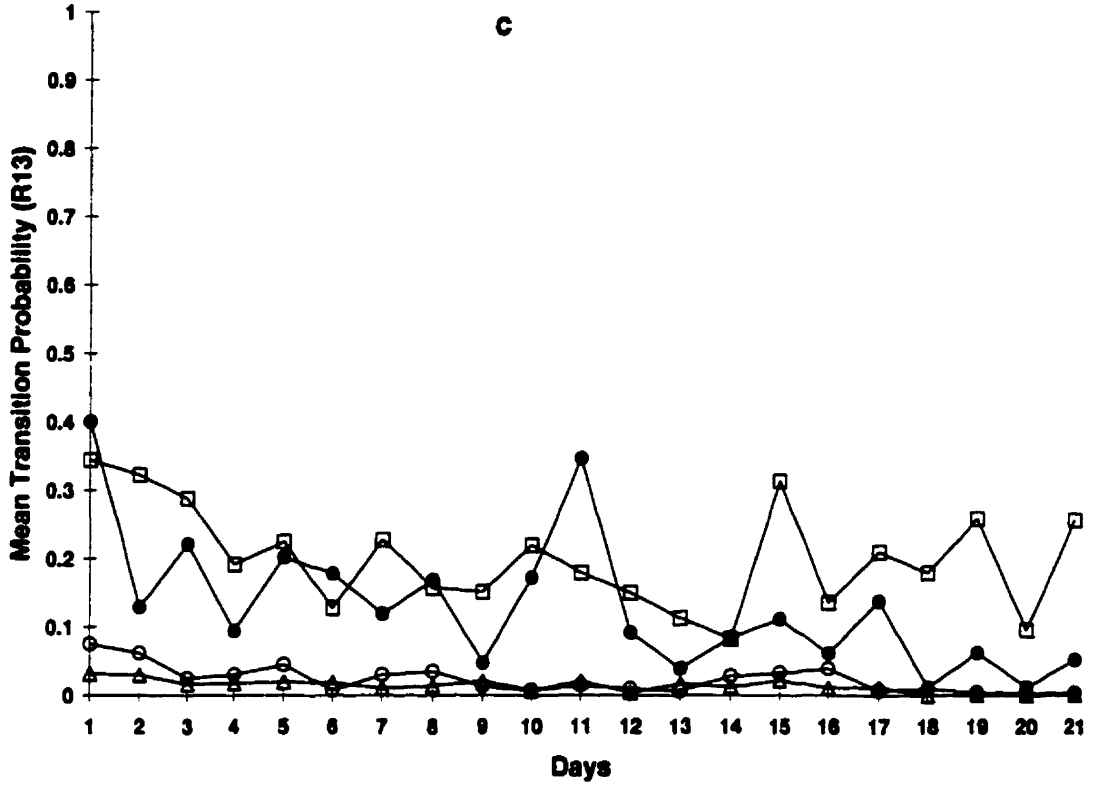

b
Mean Transition Probability (R12)
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 2: **Food effects transition probabilities of first instar *Aedes aegypti* larvae (c) to become third instars, (d) to die within 48 hours**


c
Mean Transition Probability (R13)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

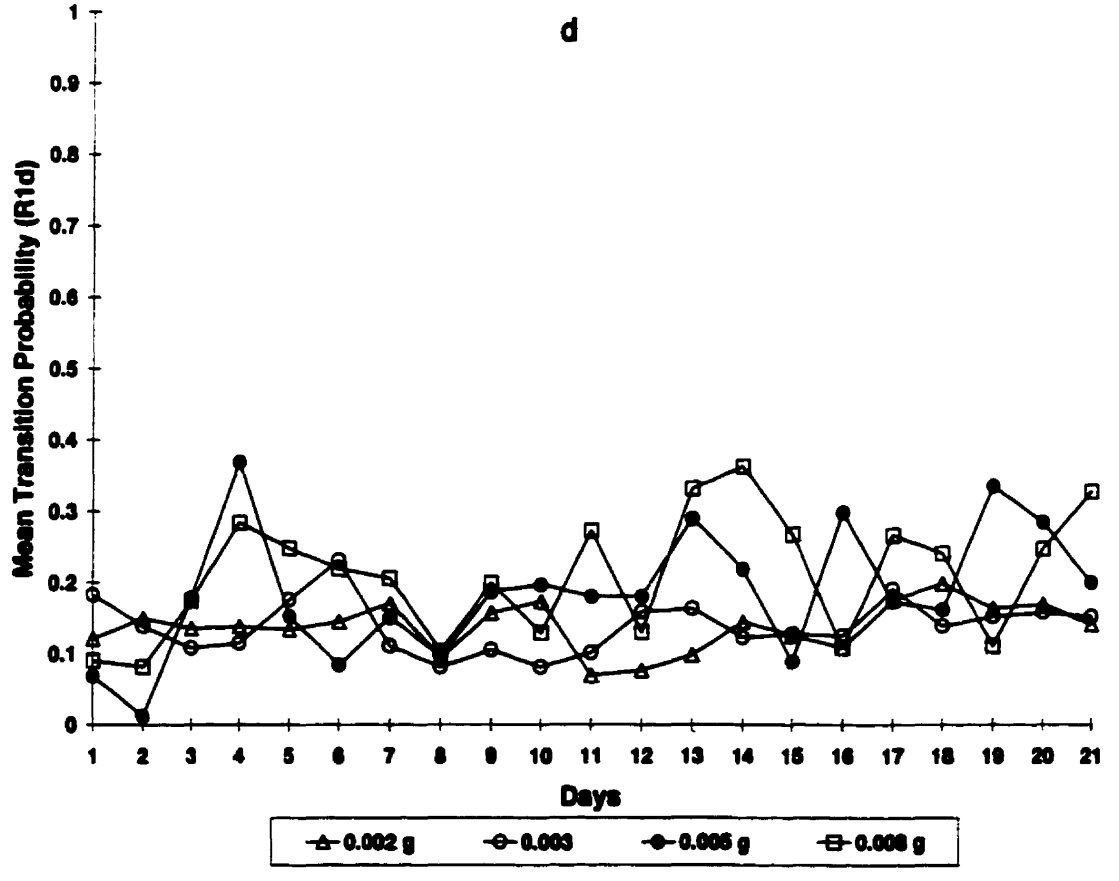

d
Mean Transition Probability (R1d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
0.002 g
0.003
0.005 g
0.008 g

Figure 3: **Food effects transition probabilities of second instar *Aedes aegypti* larvae second instars (a) to remain second instars, (b) to become third instars, (c) to die within 24 hours**

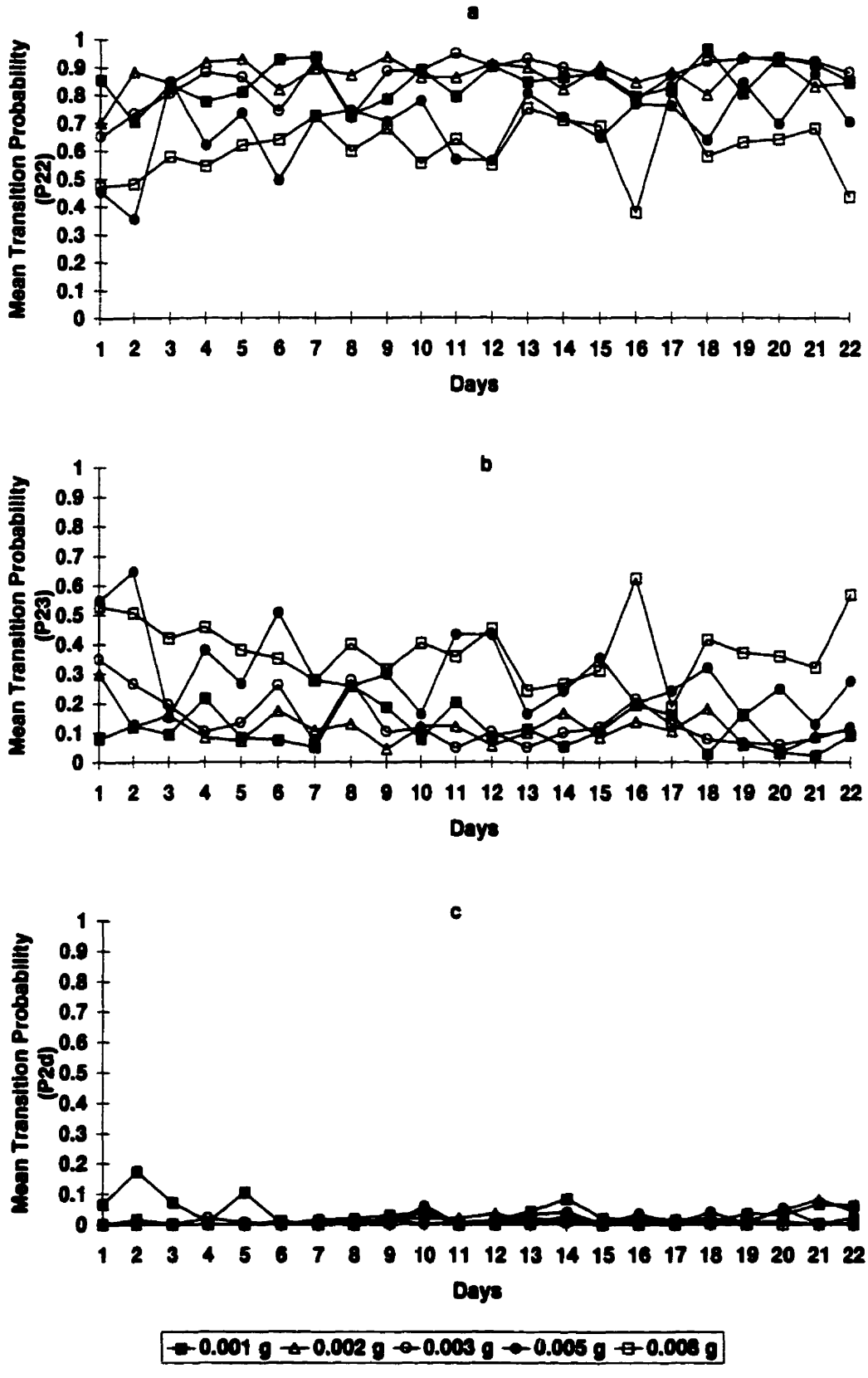
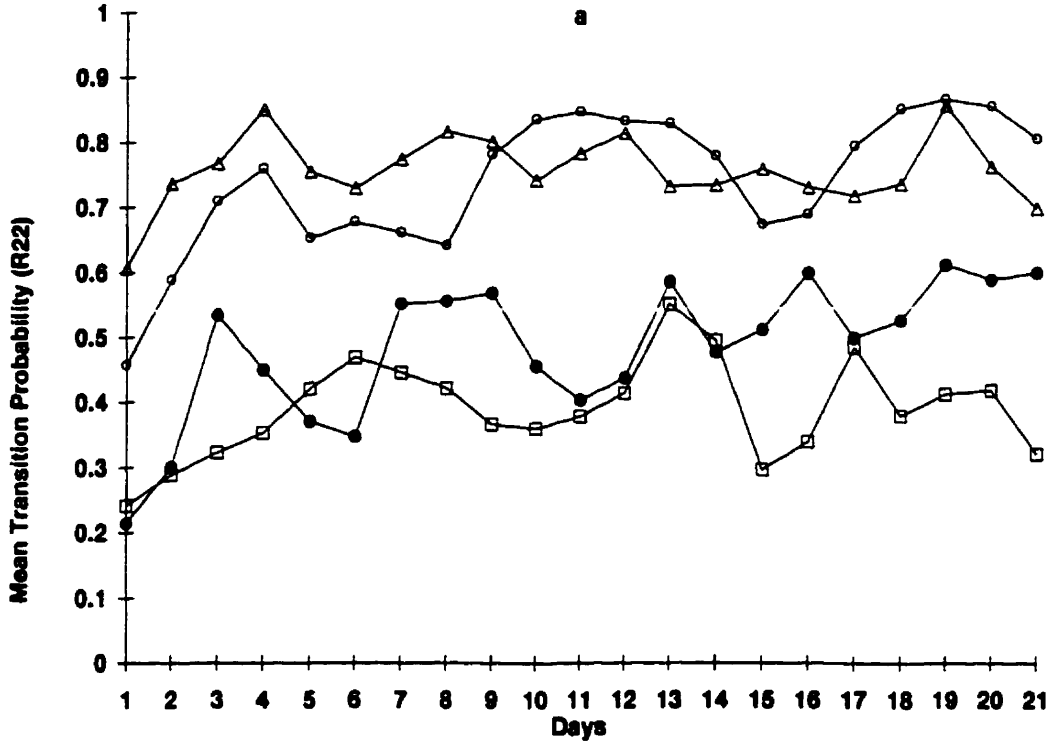

a
Mean Transition Probability (P22)
Days
b
Mean Transition Probability (P23)
Days
c
Mean Transition Probability (P2d)
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 4: **Food effects transition probabilities of second instar *Aedes aegypti* larvae (a) to remain second instars, (b) to become third instars**


a
Mean Transition Probability (R22)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

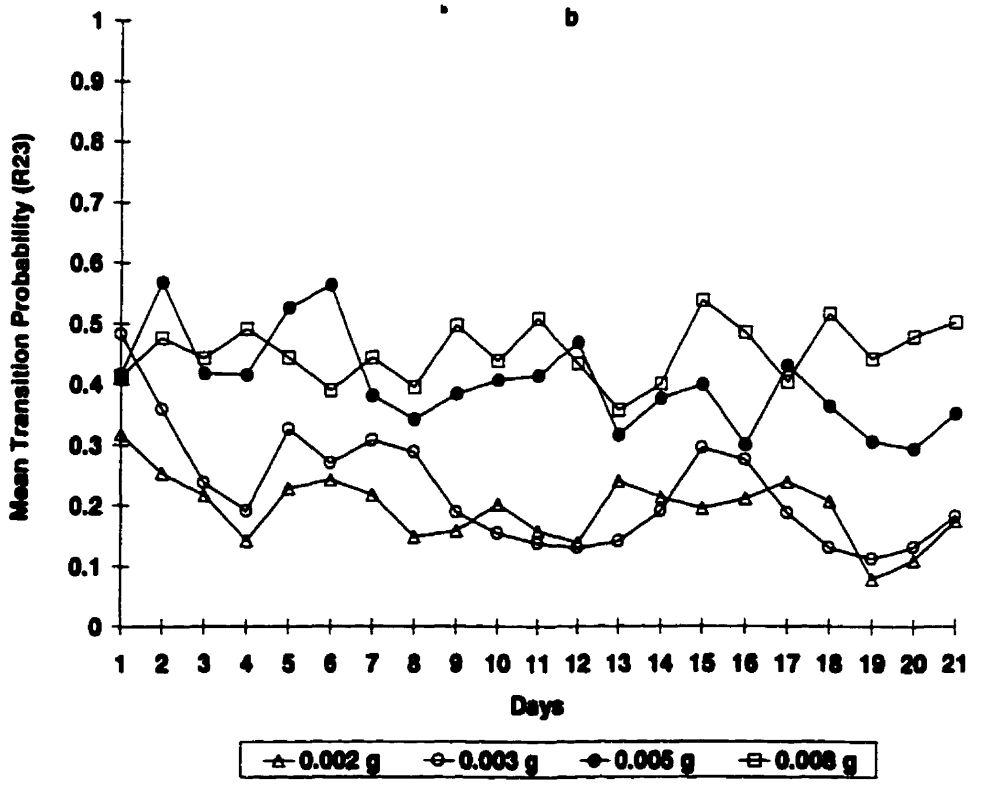
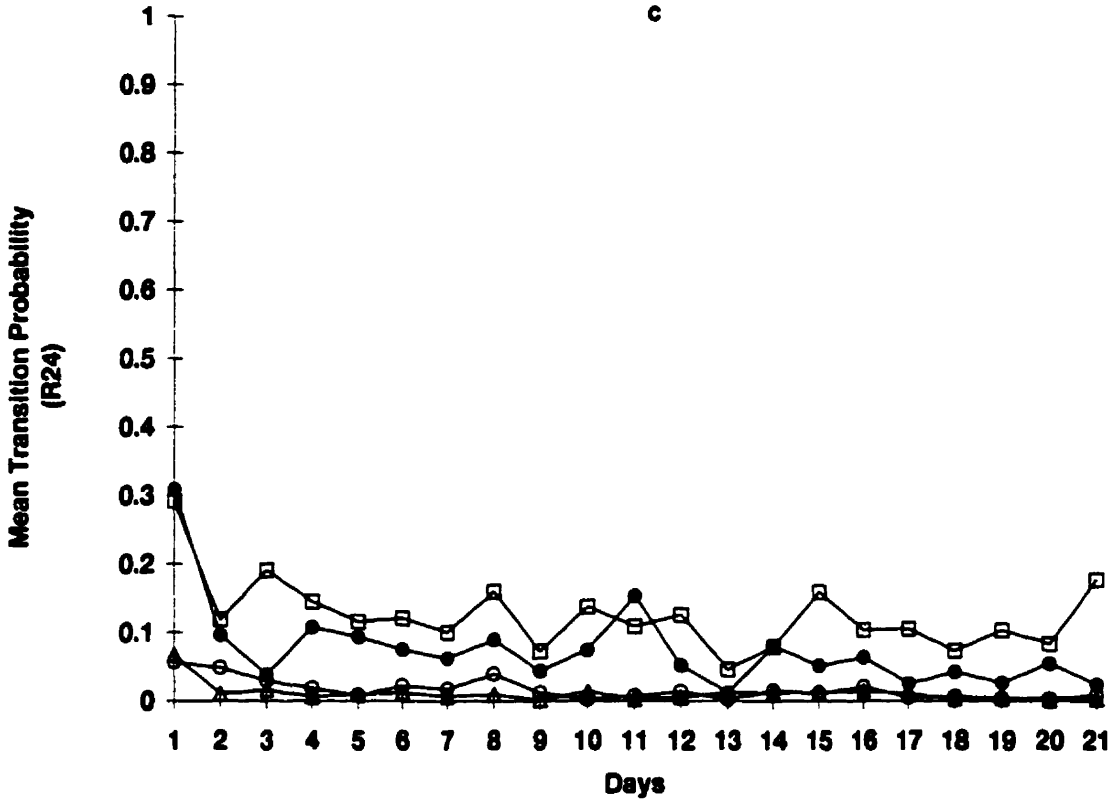

b
Mean Transition Probability (R23)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 4: **Food effects transition probabilities of second instar *Aedes aegypti* larvae (c) to become fourth instars, (d) to die within 48 hours.**


c
Mean Transition Probability (R24)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

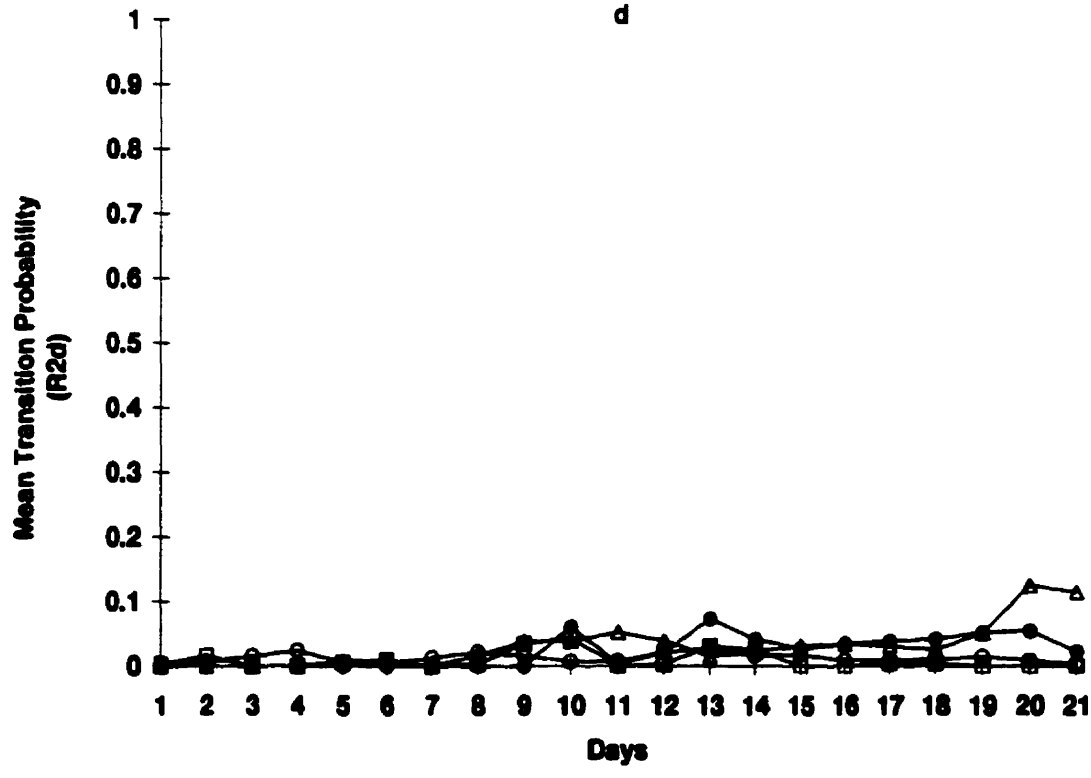

d
Mean Transition Probability (R2d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
0.002 g
0.003
0.005 g
0.008 g

Figure 5: **Food effects transition probabilities of third instar *Aedes aegypti* larvae (a) to remain third instars, (b) to become fourth instars, (c) to die within 24 hours.**

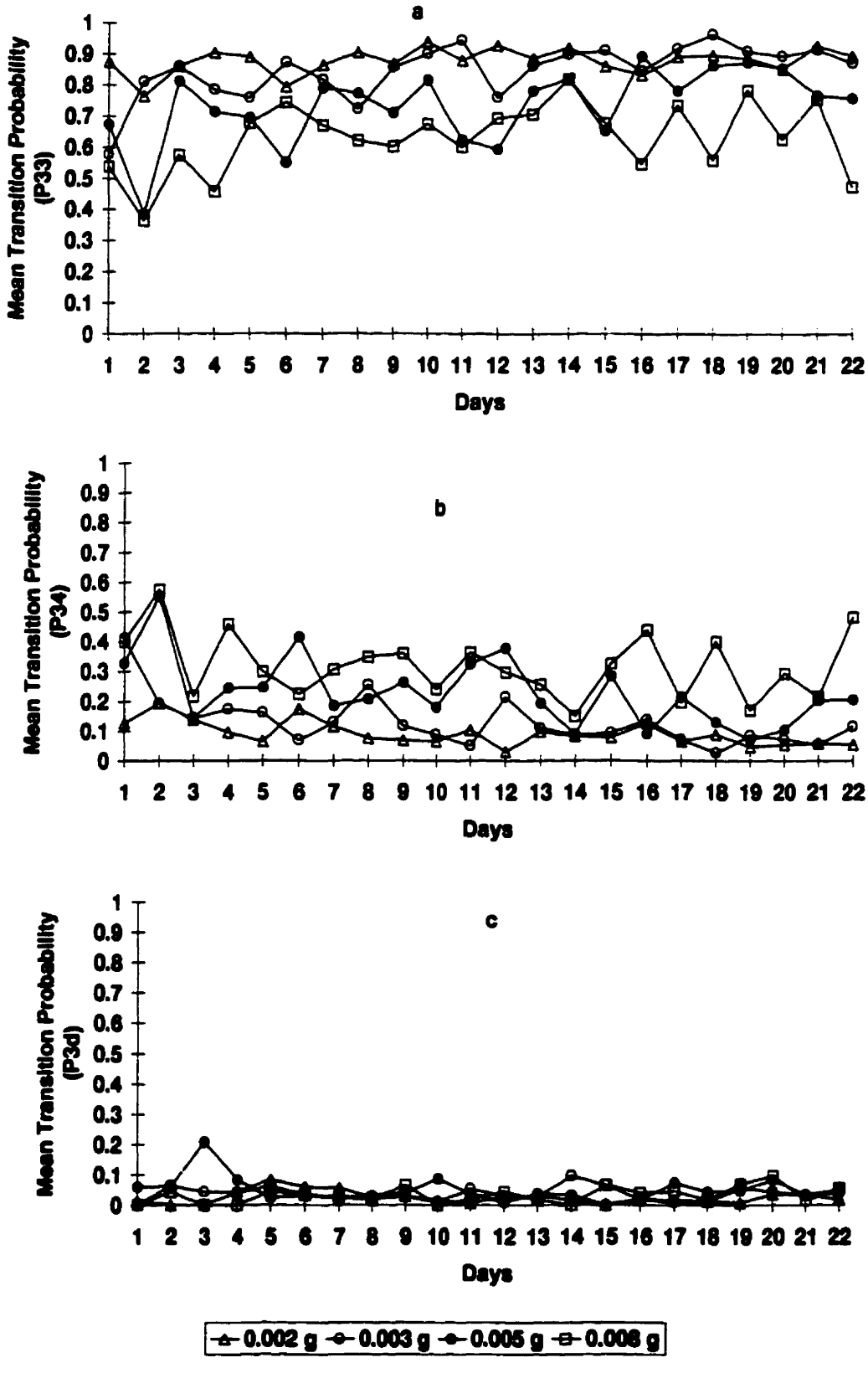
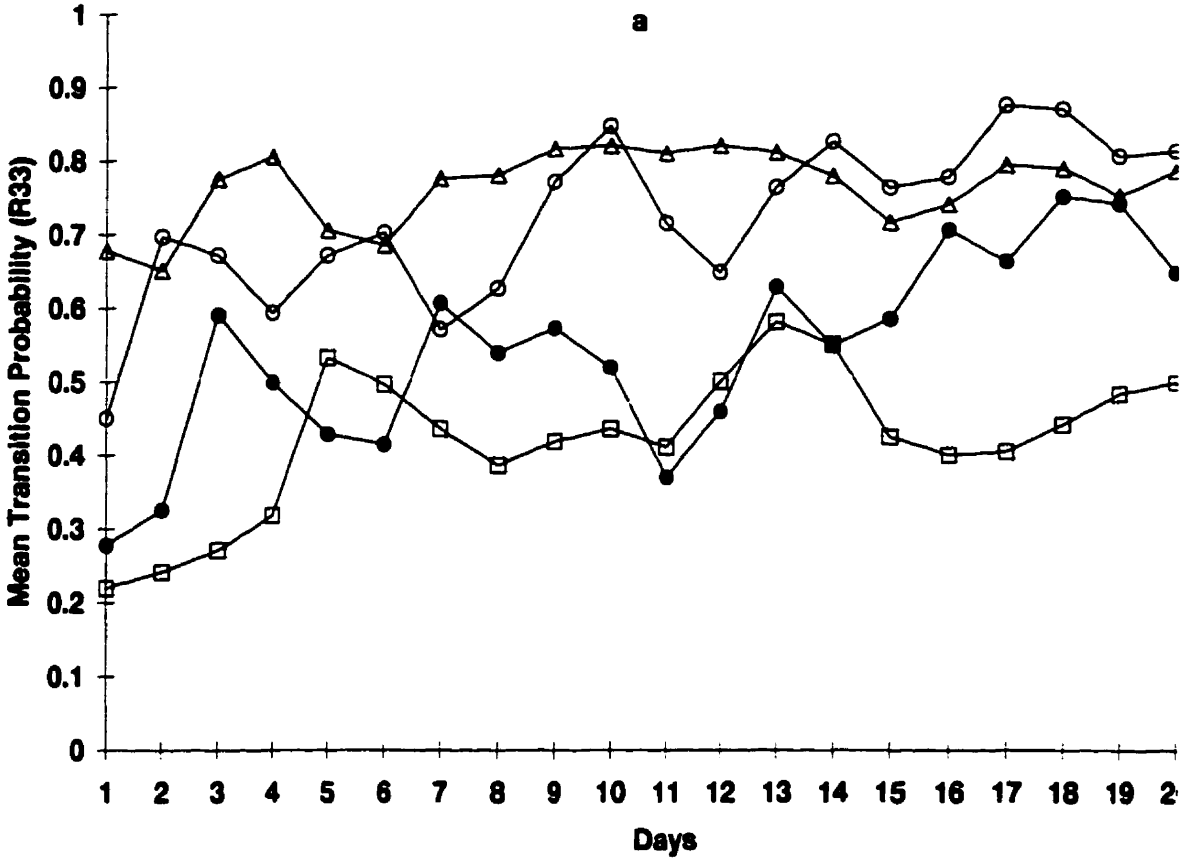

a
Mean Transition Probability (P33)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
b
Mean Transition Probability (P34)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
c
Mean Transition Probability (P3d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
0.002 g
0.003 g
0.005 g
0.006 g

Figure 6: **Food effects transition probabilities of third instar *Aedes aegypti* larvae (a) to remain third instars, (b) to become fourth instars**


a
Mean Transition Probability (R33)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 2
Days

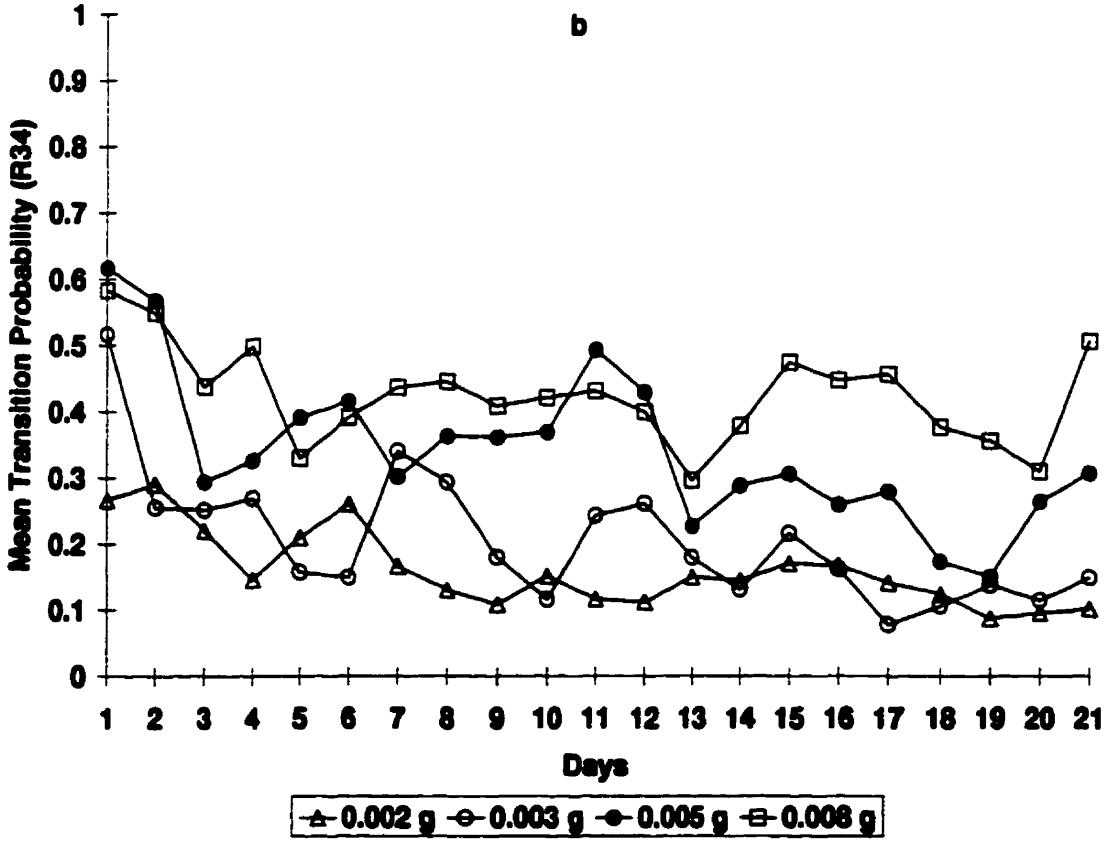
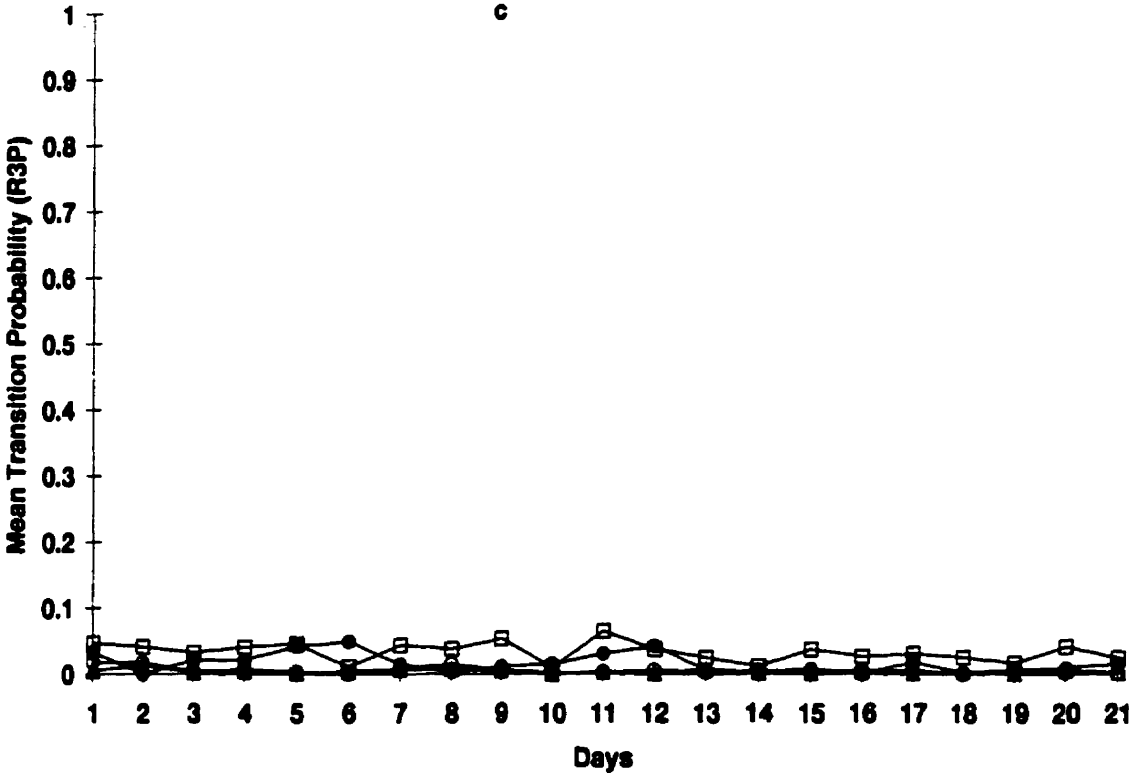

b
Mean Transition Probability (R34)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 6: **Food effects transition probabilities of third instar *Aedes aegypti* larvae (c) to become pupae, (d) to die within 48 hours.**

c


Mean Transition Probability (R3P)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

d

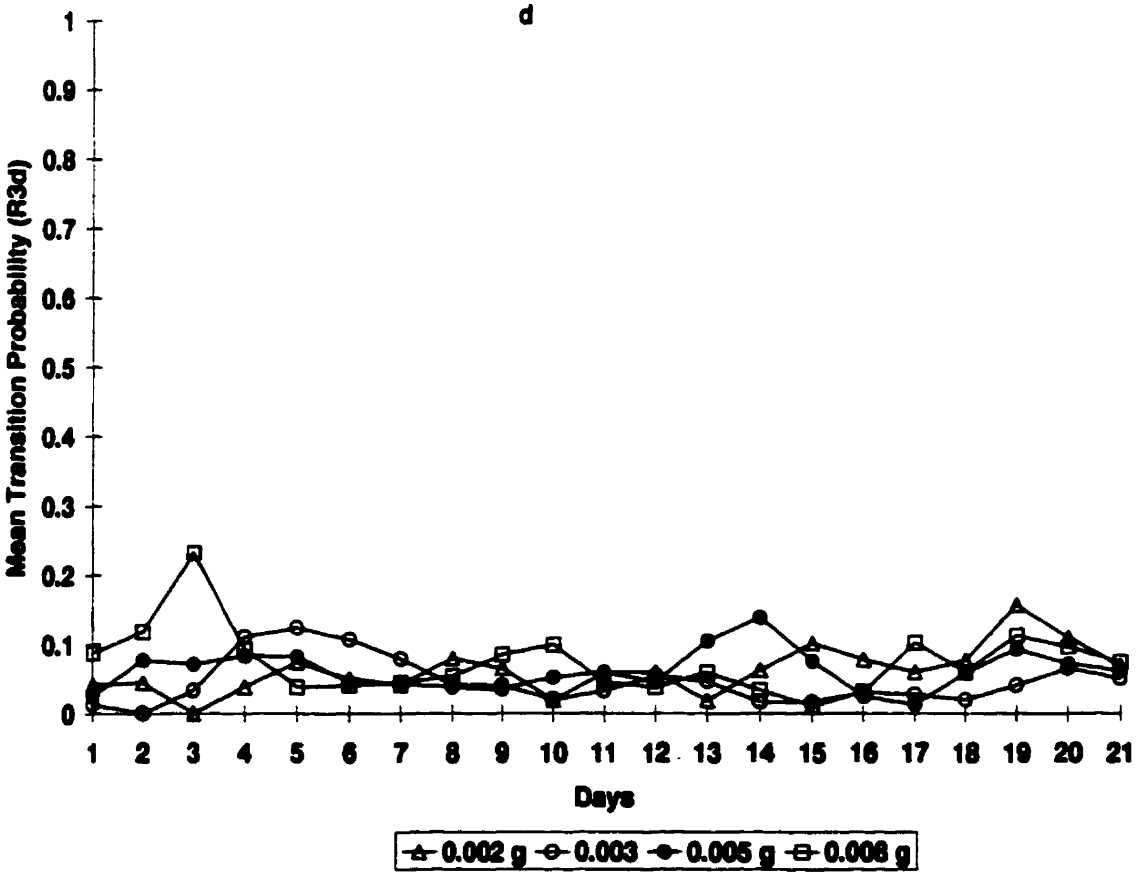
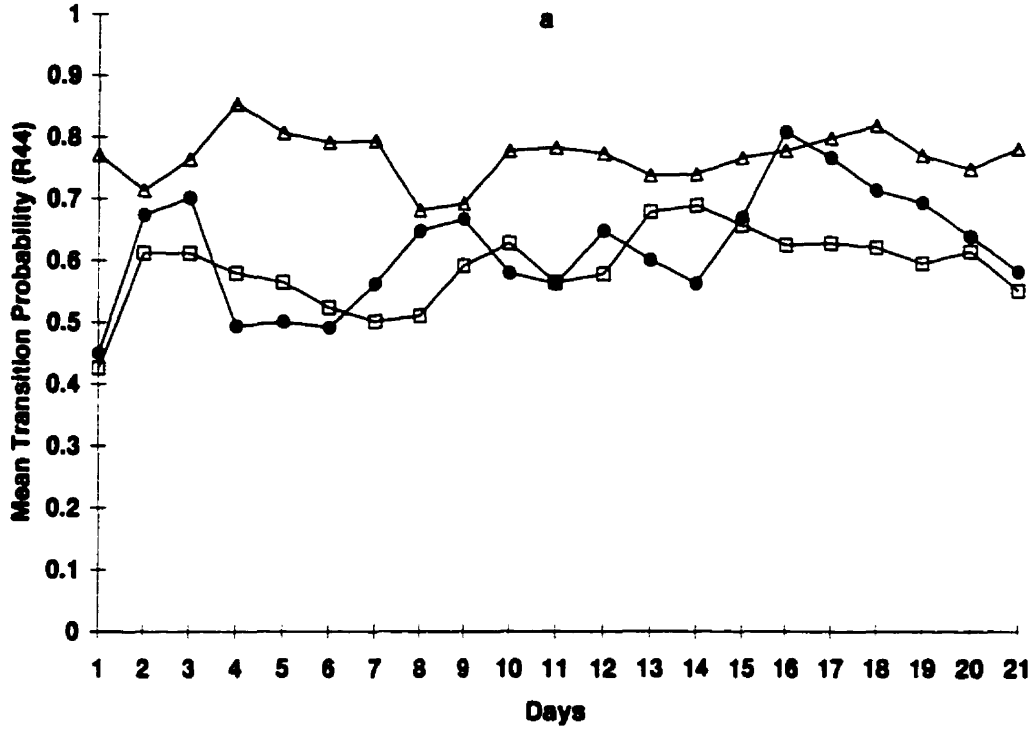

Mean Transition Probability (R3d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
0.002 g
0.003
0.005 g
0.008 g

Figure 7: **Food effects transition probabilities of fourth instar *Aedes aegypti* larvae (a) to remain fourth instars, (b) to become pupae**


a
Mean Transition Probability (R44)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

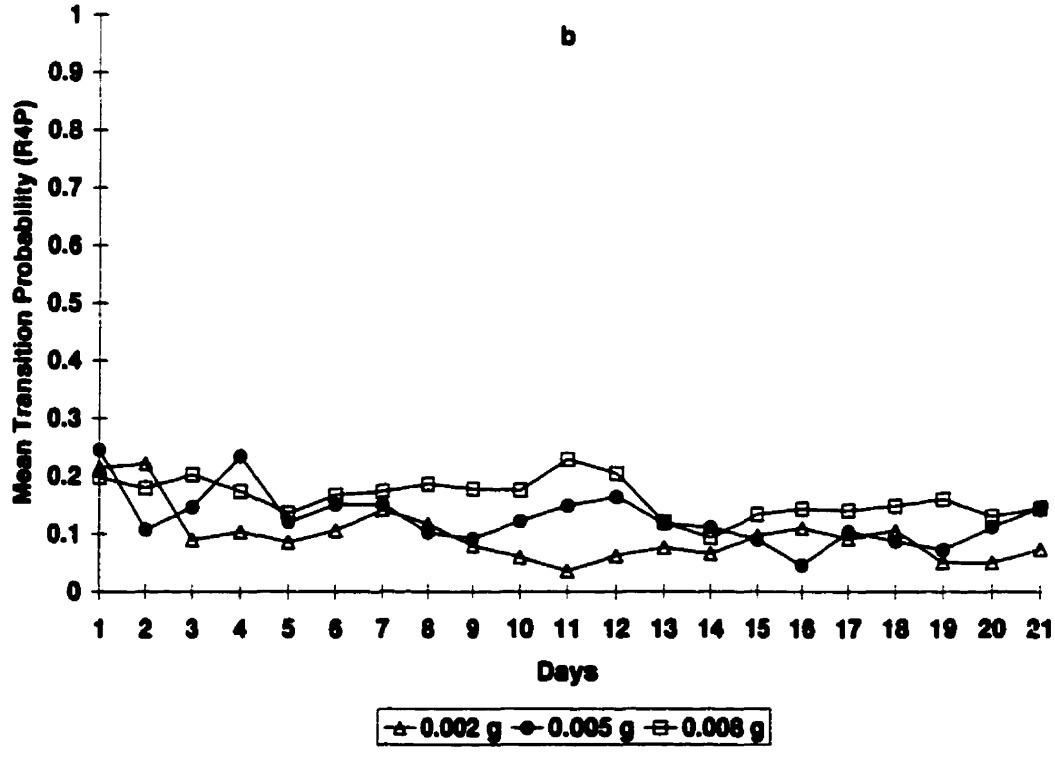
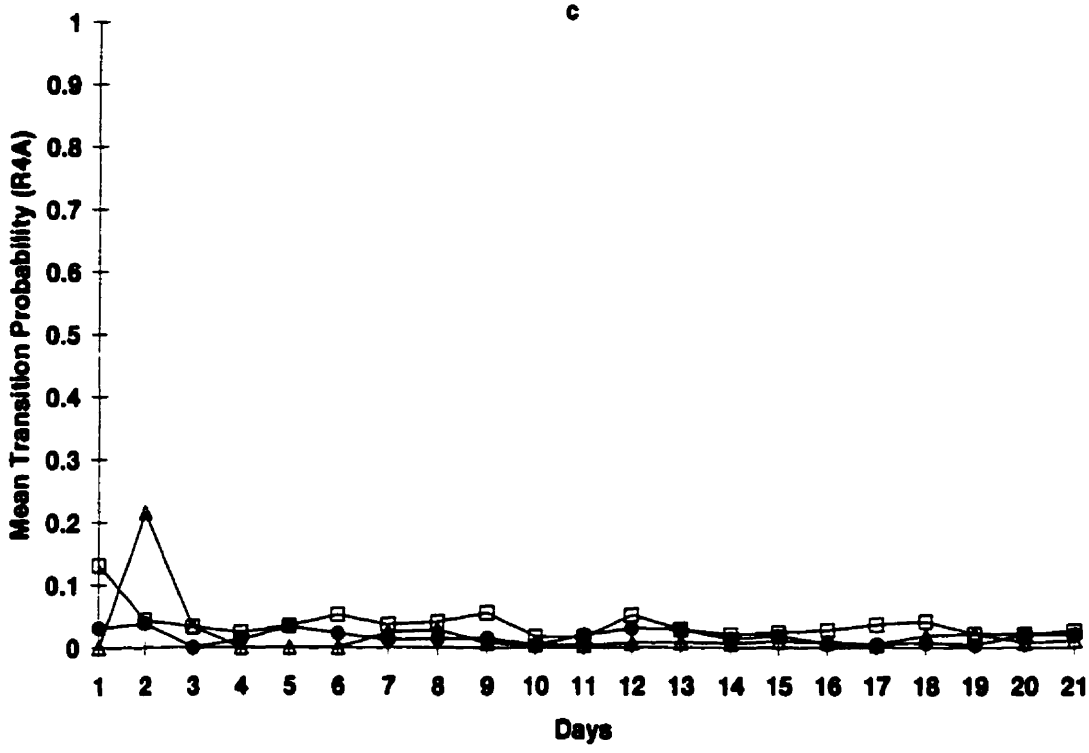

b
Mean Transition Probability (R4P)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
0.002 g
0.005 g
0.008 g

Figure 7: **Food effects transition probabilities of fourth instar *Aedes aegypti* larvae (c) to become adults, (d) to die within 48 hours.**

c


Mean Transition Probability (R4A)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

d

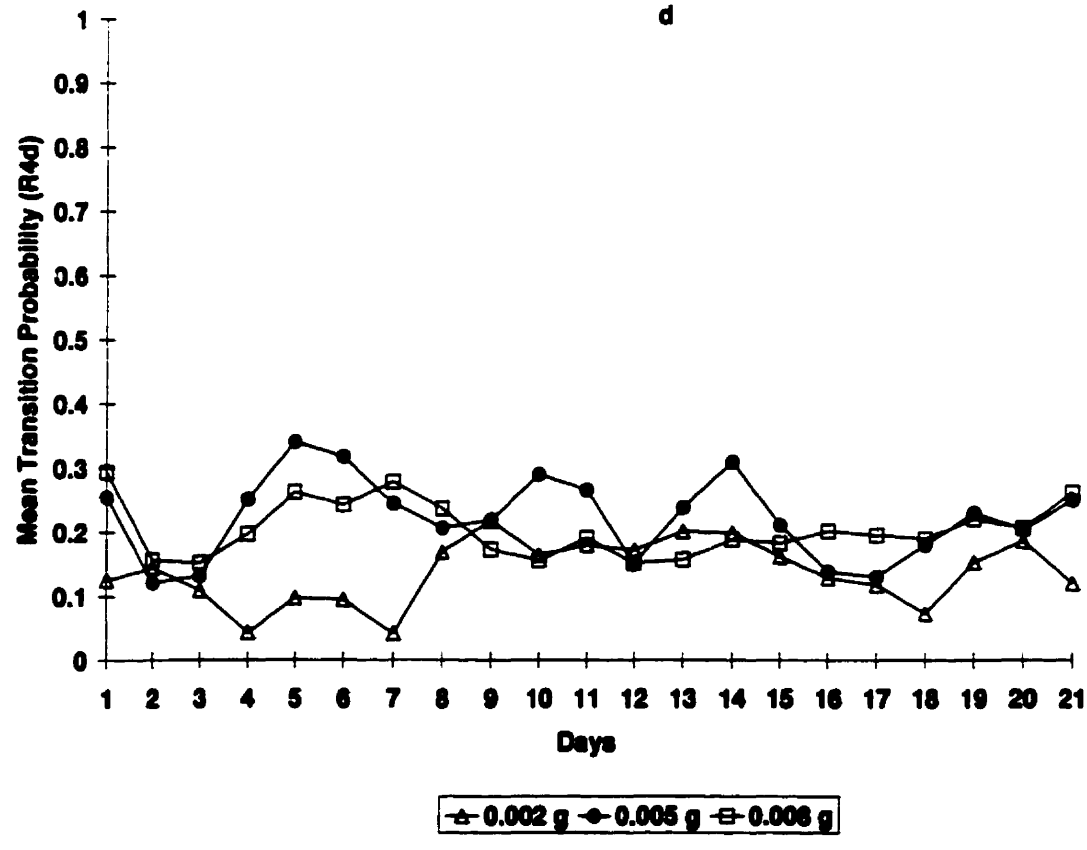
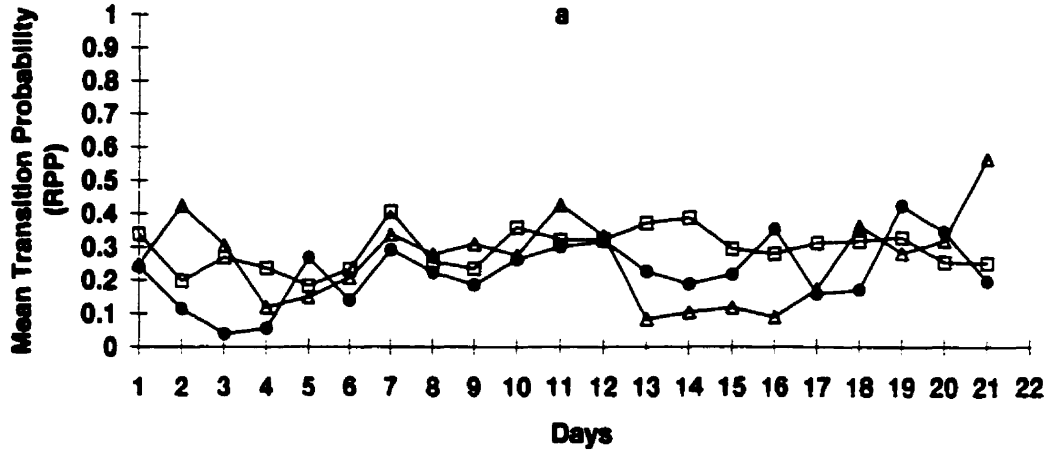
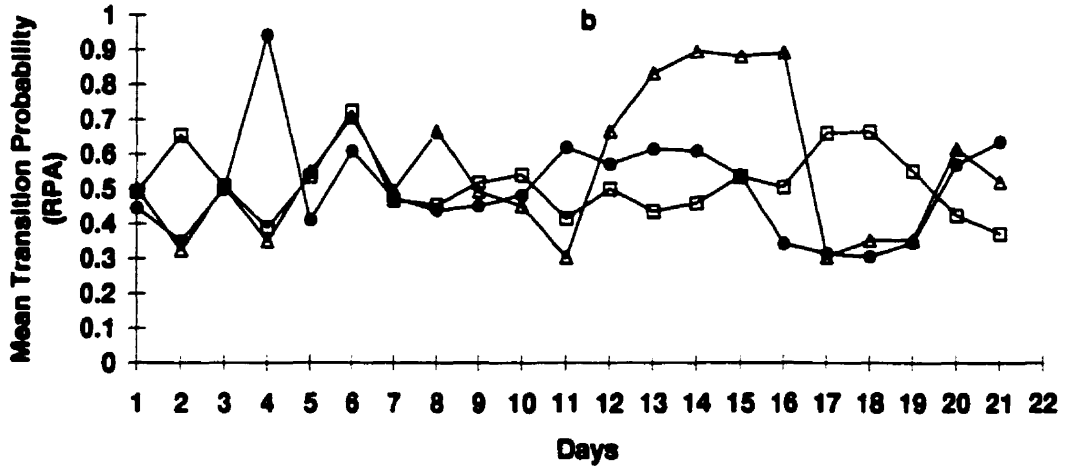

Mean Transition Probability (R4d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
0.002 g
0.005 g
0.008 g

Figure 8: **Food effects transition probability of *Aedes aegypti* pupae (a) to remain pupae, (b) to become adults, (c) to die within 48 hours.**


a
Mean Transition Probability (RPP)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days


b
Mean Transition Probability (RPA)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days

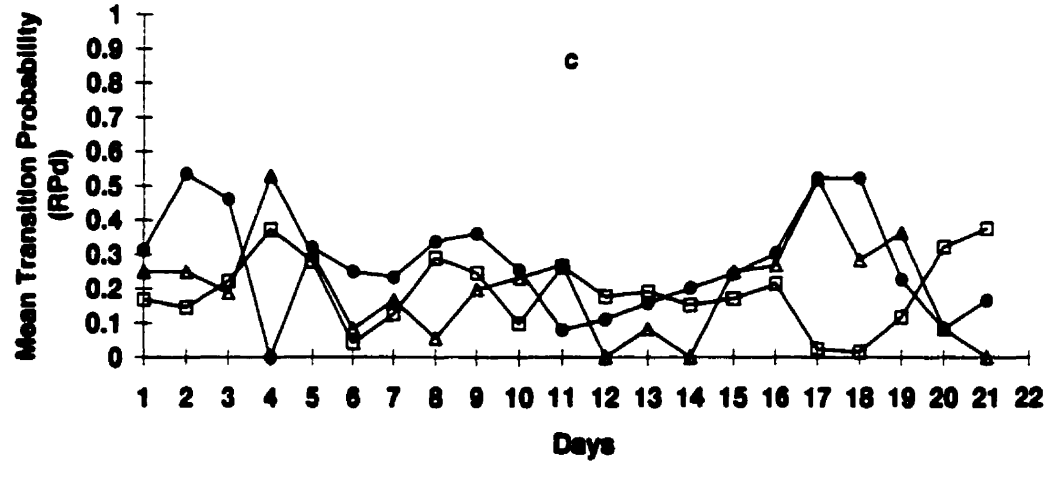

c
Mean Transition Probability (RPd)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
0.002 g
0.005 g
0.008 g

Figure 9: **Parasite effects transition probabilities of first instar *Aedes aegypti* larvae (a) to remain first instars, (b) to become second instars, (c) to die within 24 hours.**

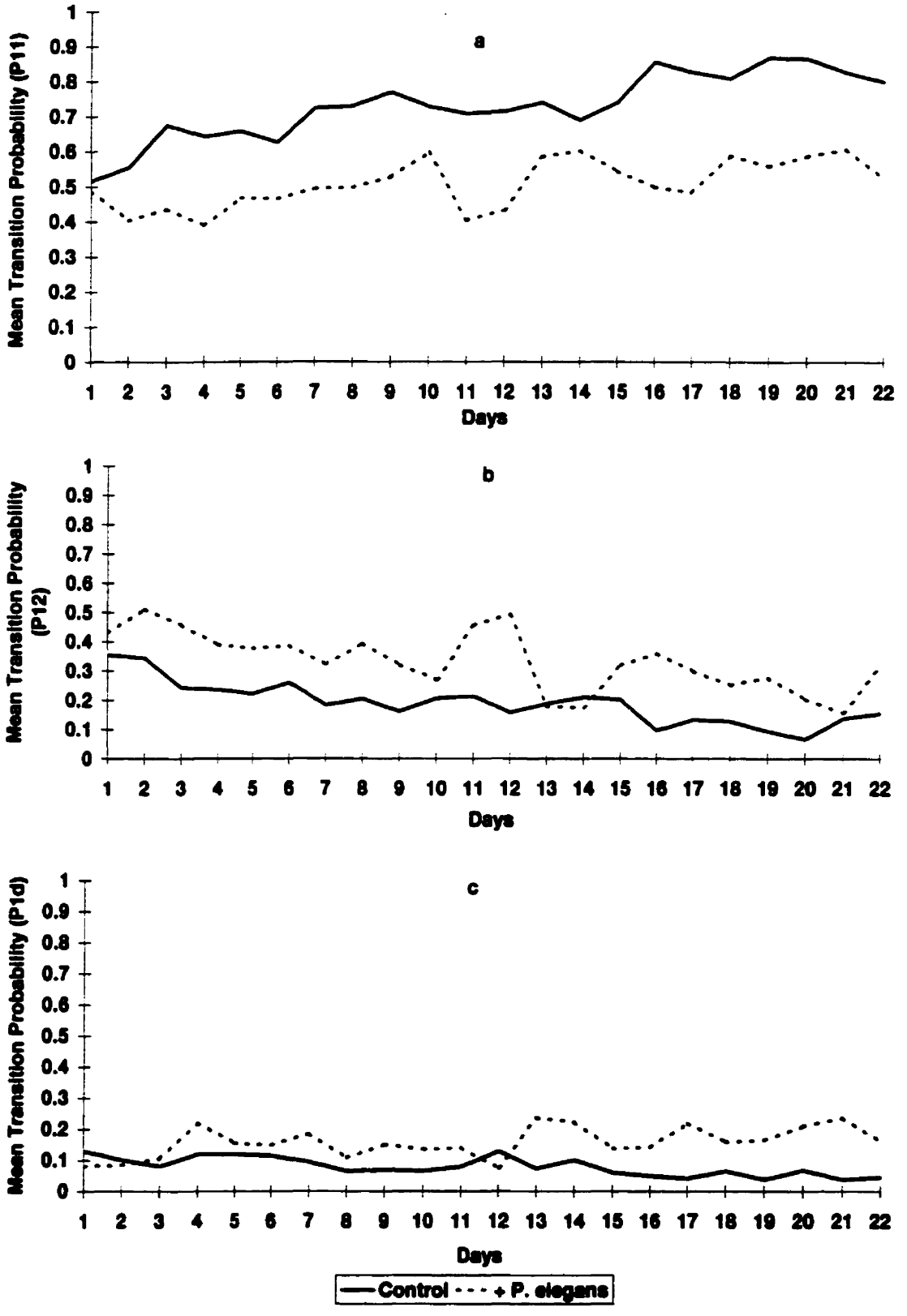

a
Mean Transition Probability (P11)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
b
Mean Transition Probability (P12)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
c
Mean Transition Probability (P1d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
Control
+ P. elegans

Figure 10: **Parasite effects transition probabilities of first instar *Aedes aegypti* larvae (a) to remain first instars, (b) to become second instars**

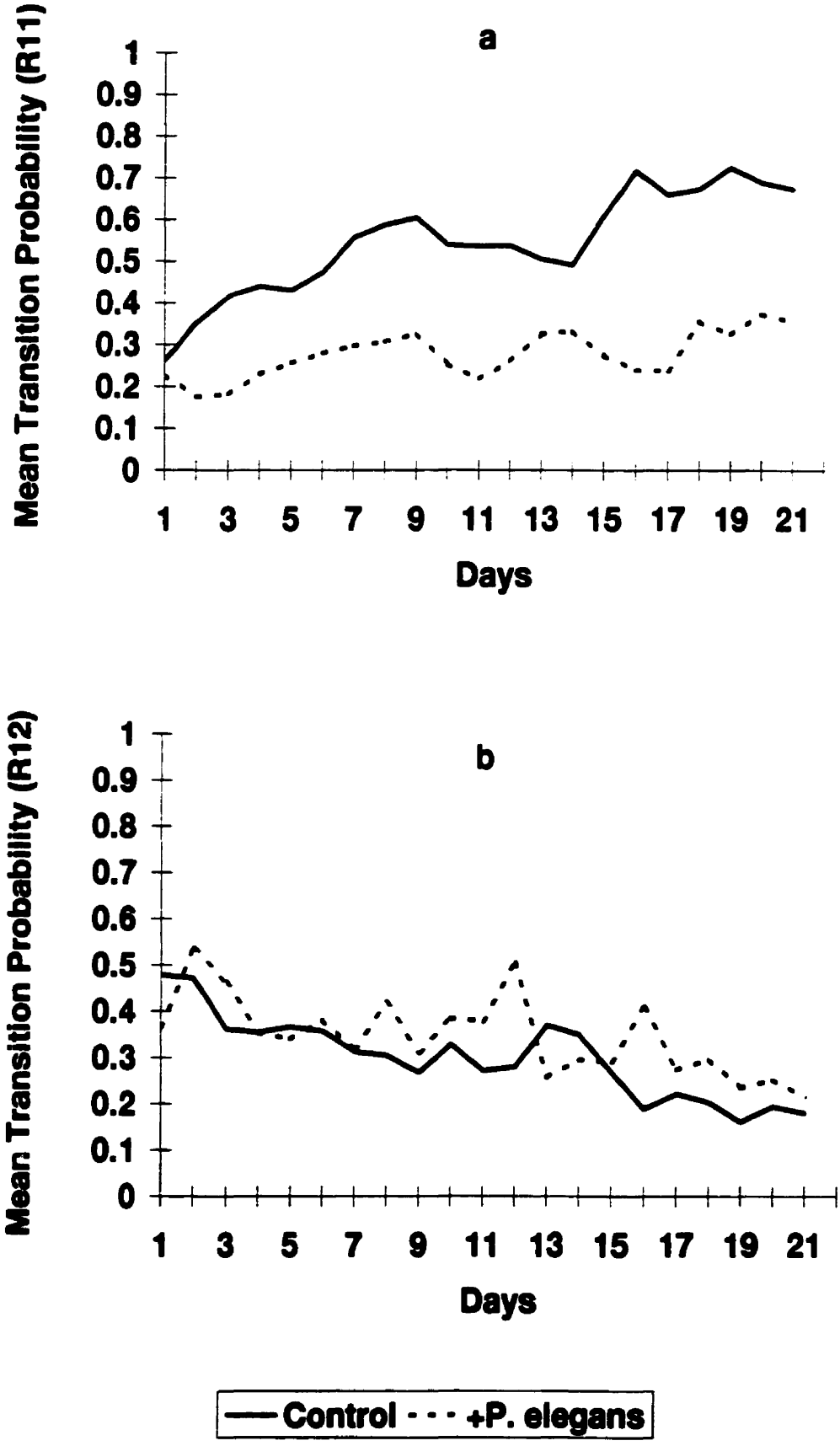
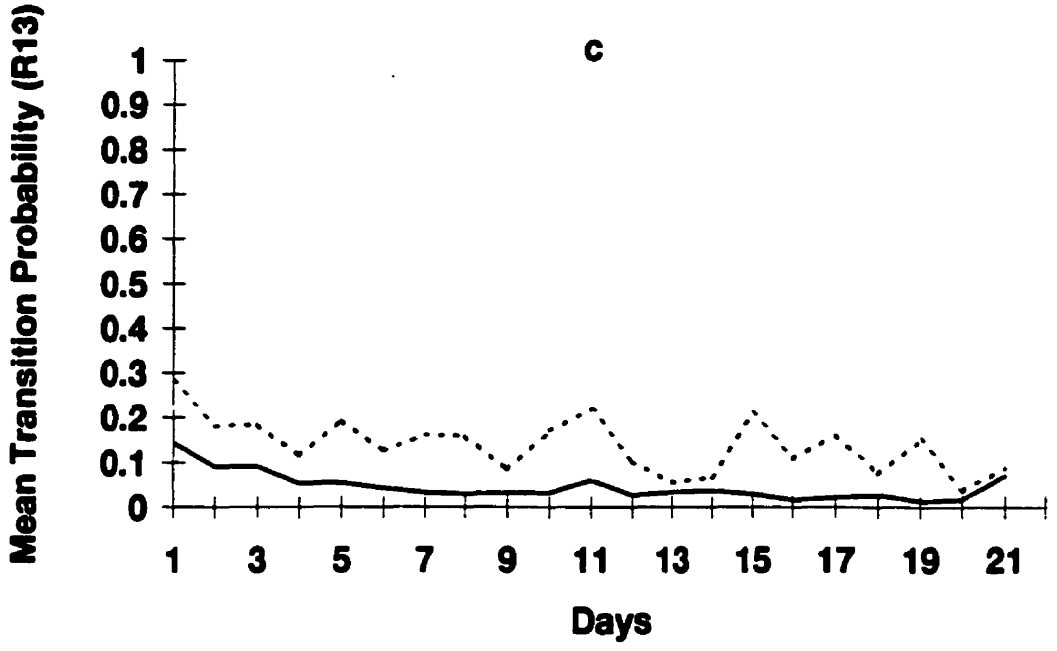
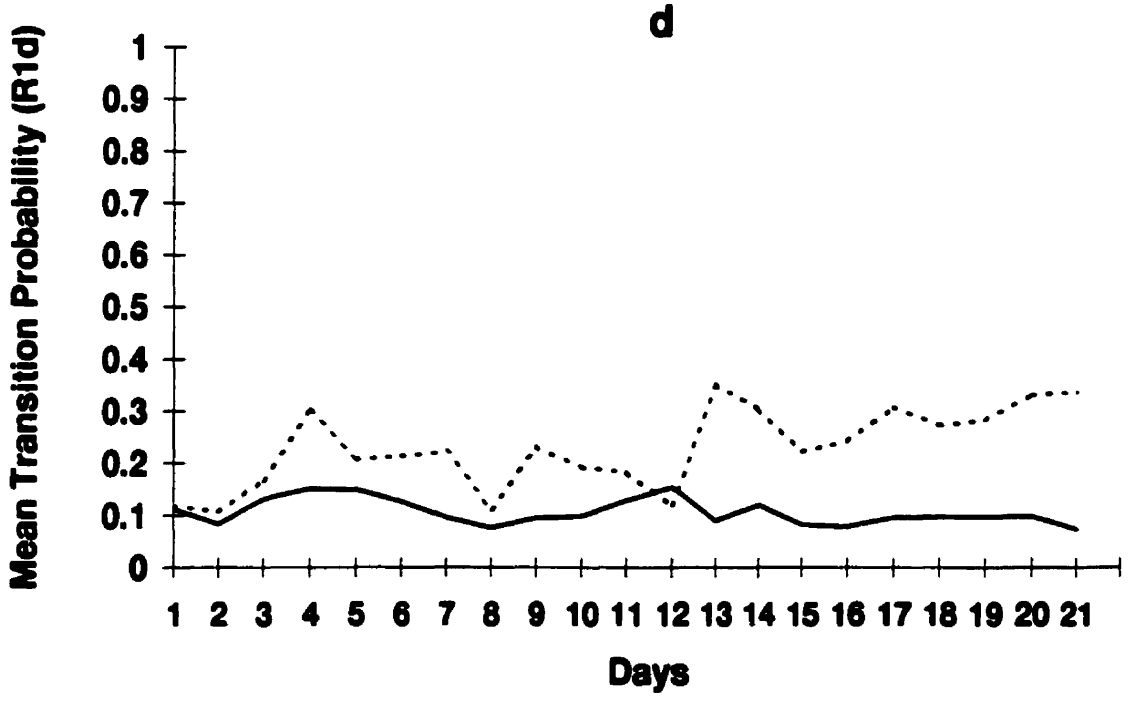

a
Mean Transition Probability (R11)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (R12)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
Control
+P. elegans

Figure 10: **Parasite effects transition probabilities of first instar *Aedes aegypti* larvae (c) to become third instars, (d) to die within 48 hours.**


c
Mean Transition Probability (R13)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days


d
Mean Transition Probability (R1d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

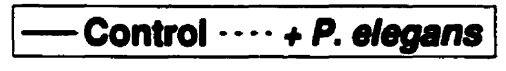
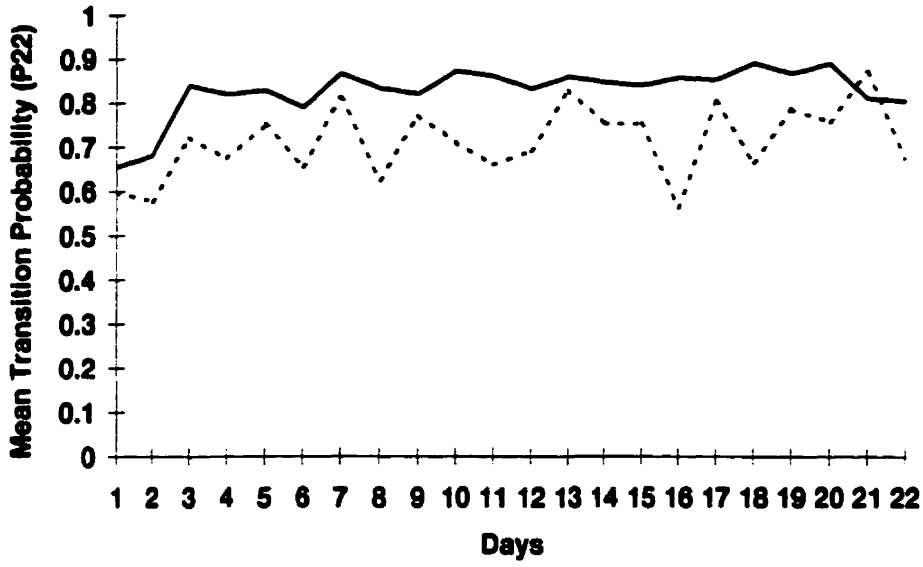
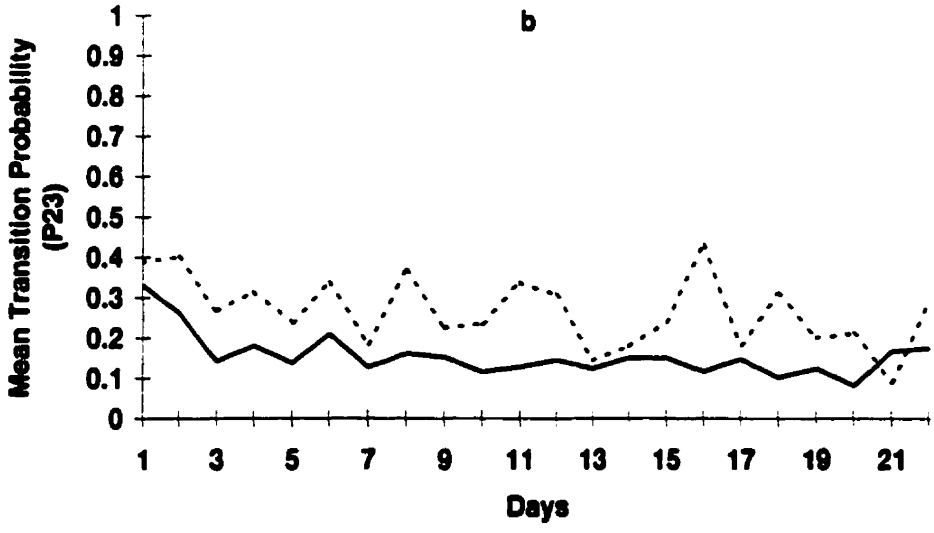

Control
+ P. elegans

Figure 11: **Parasite effects transition probabilities of second instar *Aedes aegypti* larvae (a) to remain second instars, (b) to become third instars, (c) to die within 24 hours.**

a


Mean Transition Probability (P22)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days

b


Mean Transition Probability (P23)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

c

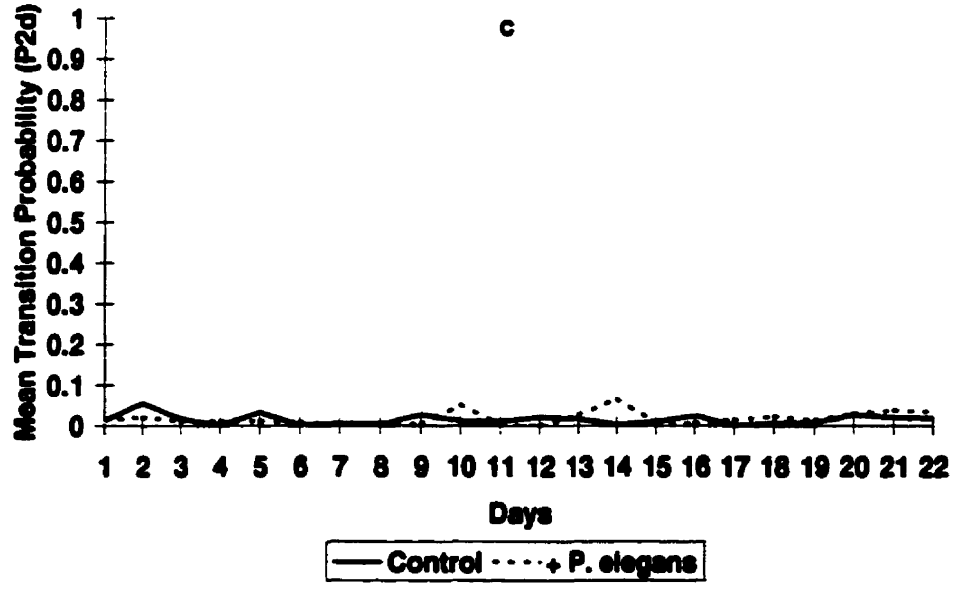
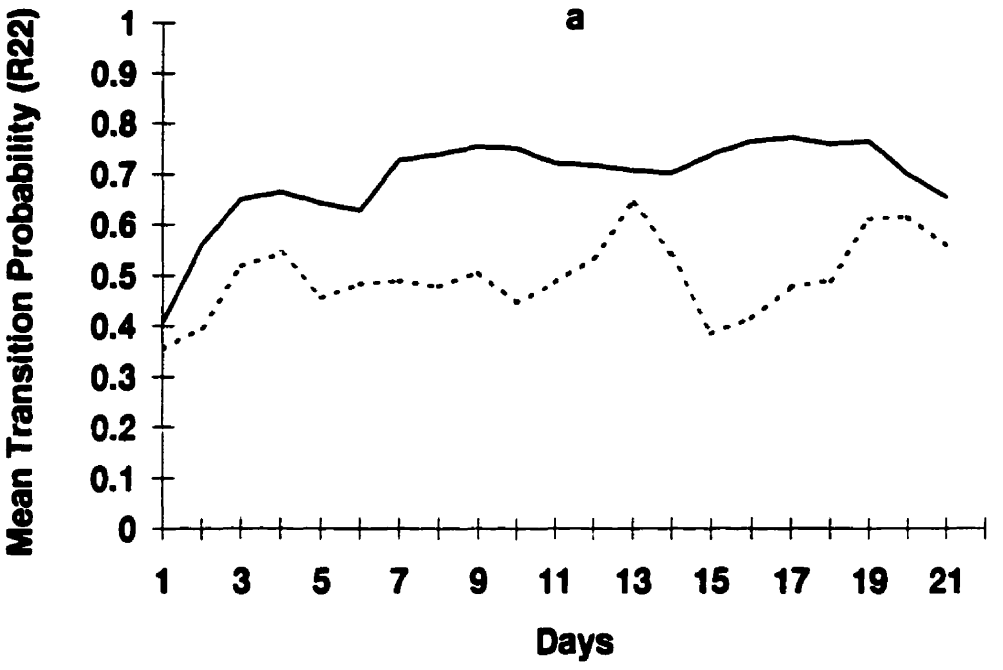
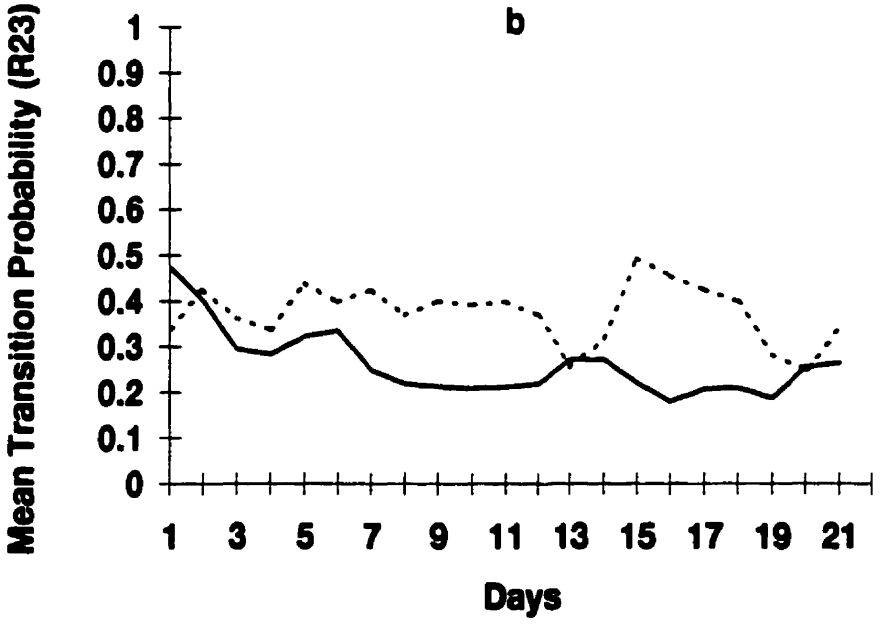

Mean Transition Probability (P2d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
Control
P. elegans

Figure 12: **Parasite effects transition probabilities of second instar *Aedes aegypti* larvae (a) to remain second instars, (b) to become third instars**


a
Mean Transition Probability (R22)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days


b
Mean Transition Probability (R23)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

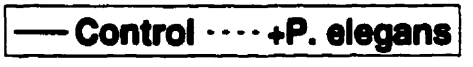
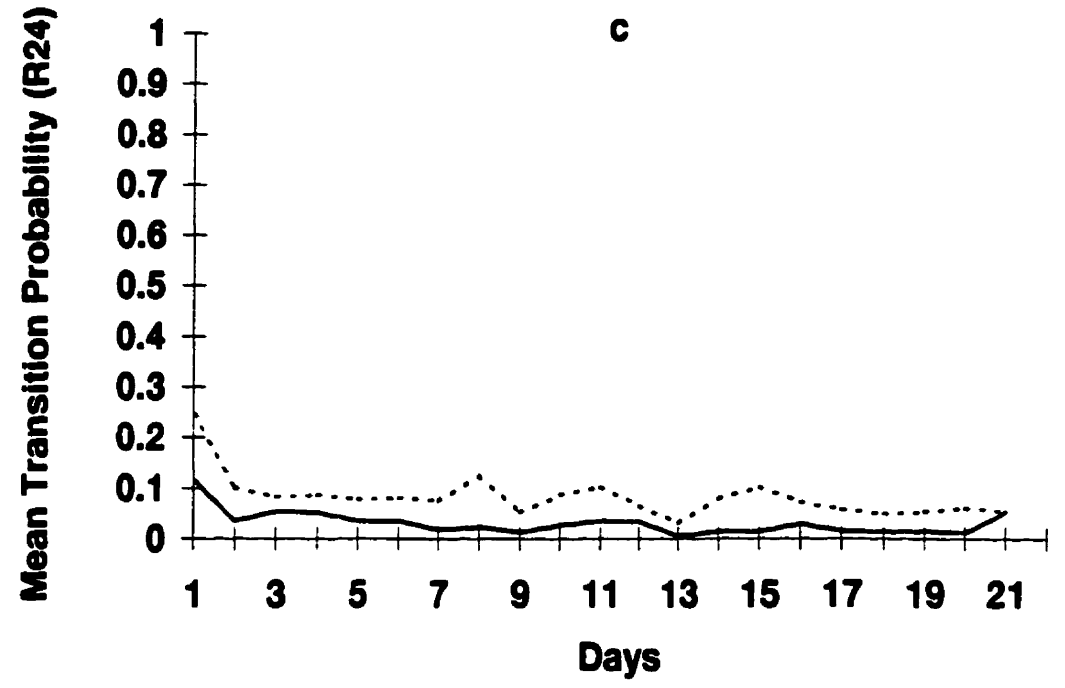
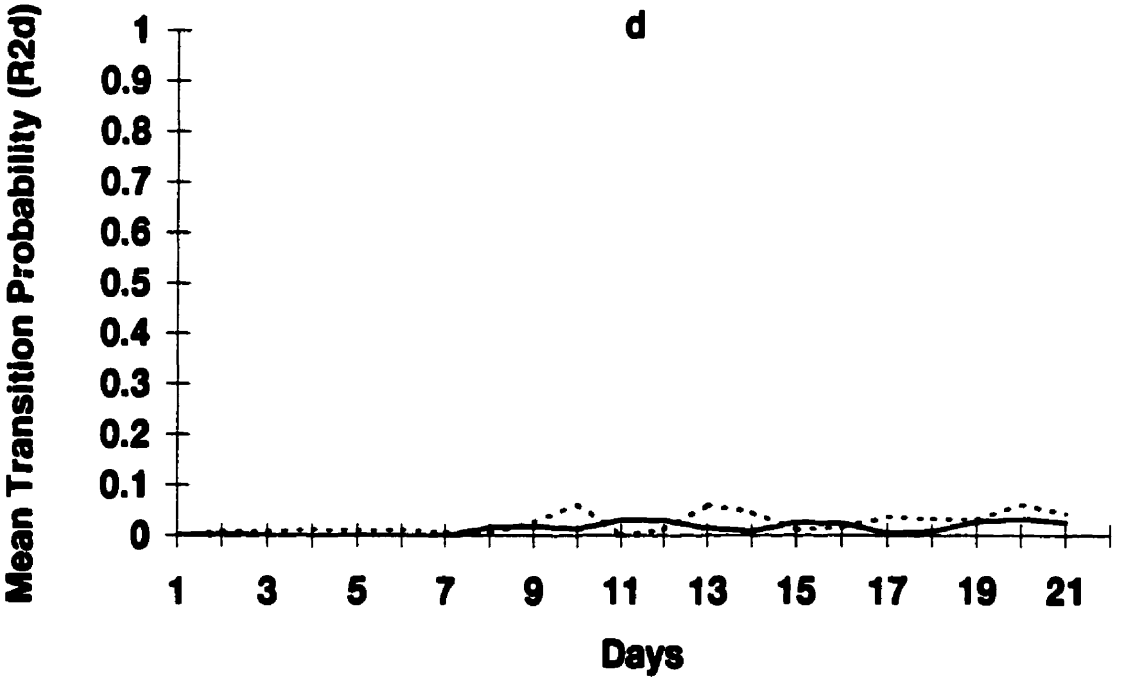

Control
+P. elegans

Figure 12: **Parasite effects transition probabilities of second instar *Aedes aegypti* larvae (c) to become fourth instars, (d) to die within 48 hours.**


c
Mean Transition Probability (R24)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days


d
Mean Transition Probability (R2d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

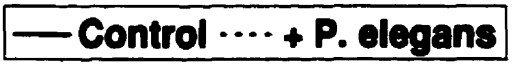

Control
+ P. elegans

Figure 13: **Parasite effects transition probabilities of third instar *Aedes aegypti* larvae (a) to remain third instars, (b) to become fourth instars, (c) to die within 24 hours.**

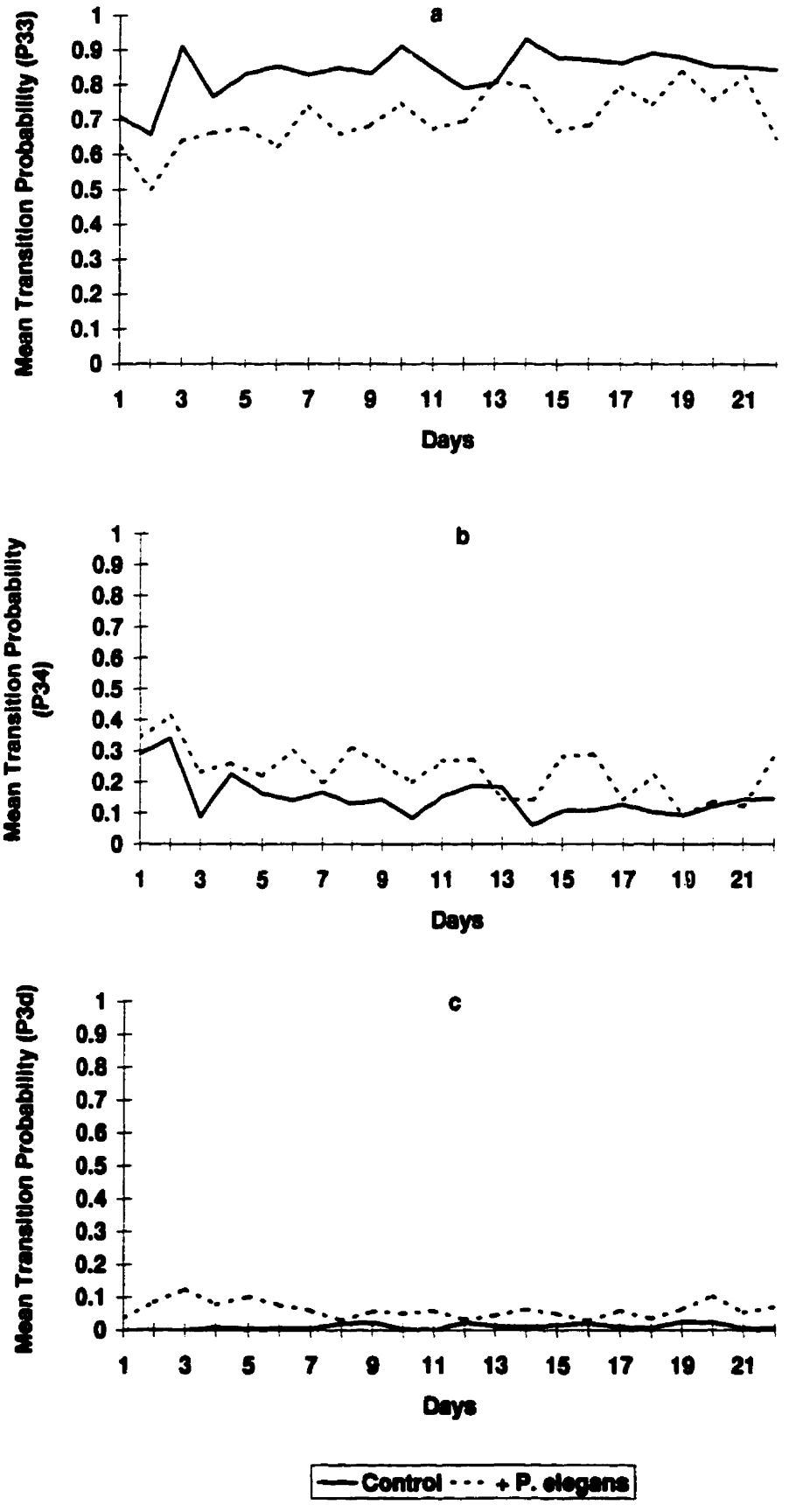
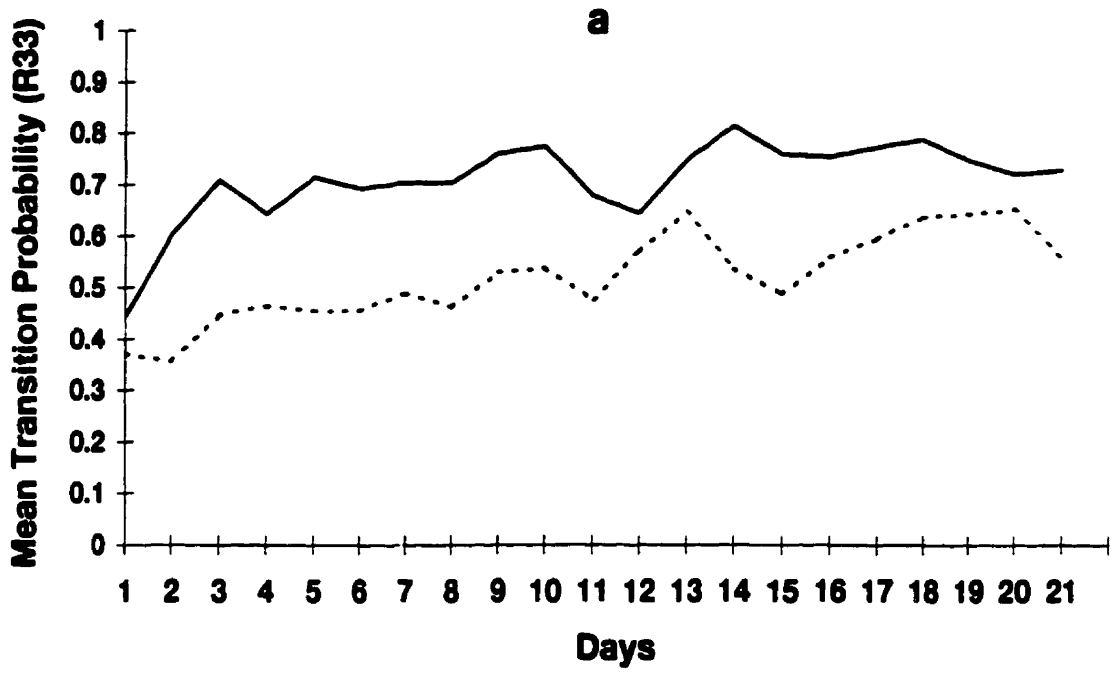

a
Mean Transition Probability (P33)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (P34)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
c
Mean Transition Probability (P3d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
Control
+ P. elegans

Figure 14: **Parasite effects transition probabilities of third instar *Aedes aegypti* larvae (a) to remain third instars, (b) to become fourth instars**


a
Mean Transition Probability (R33)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

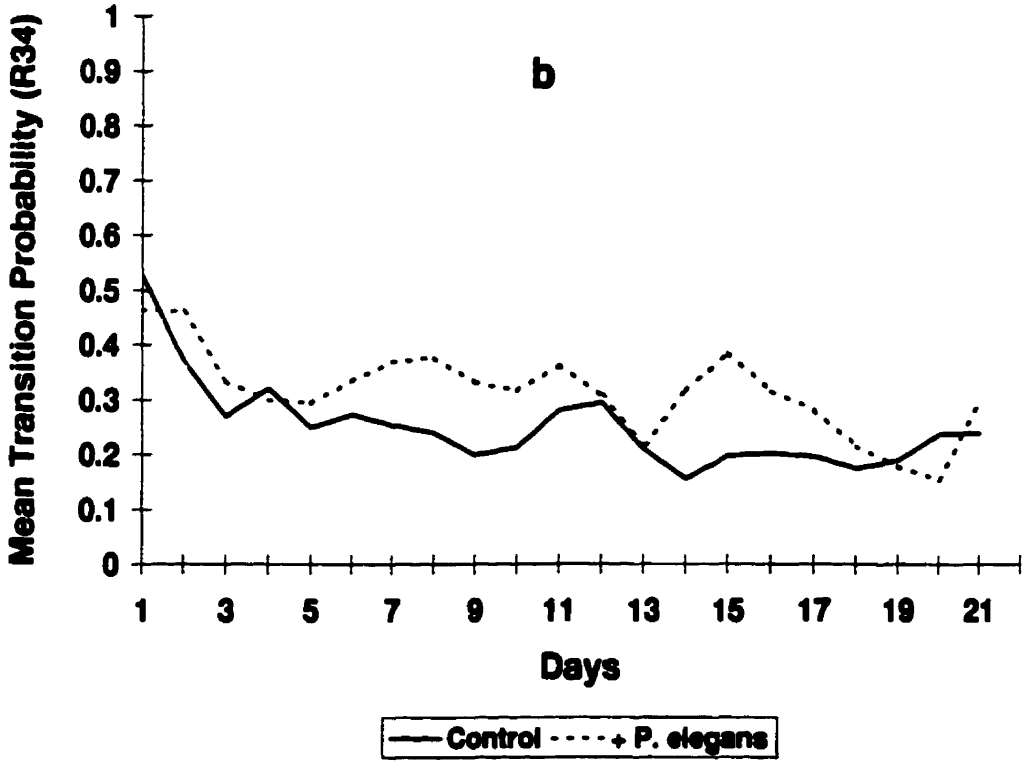
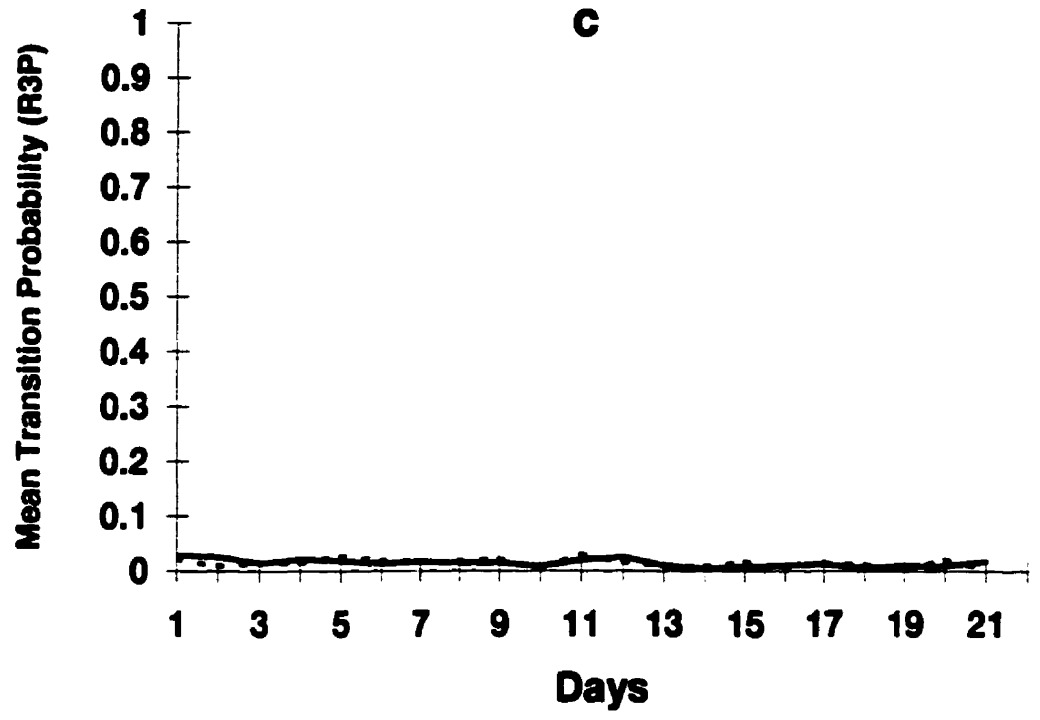
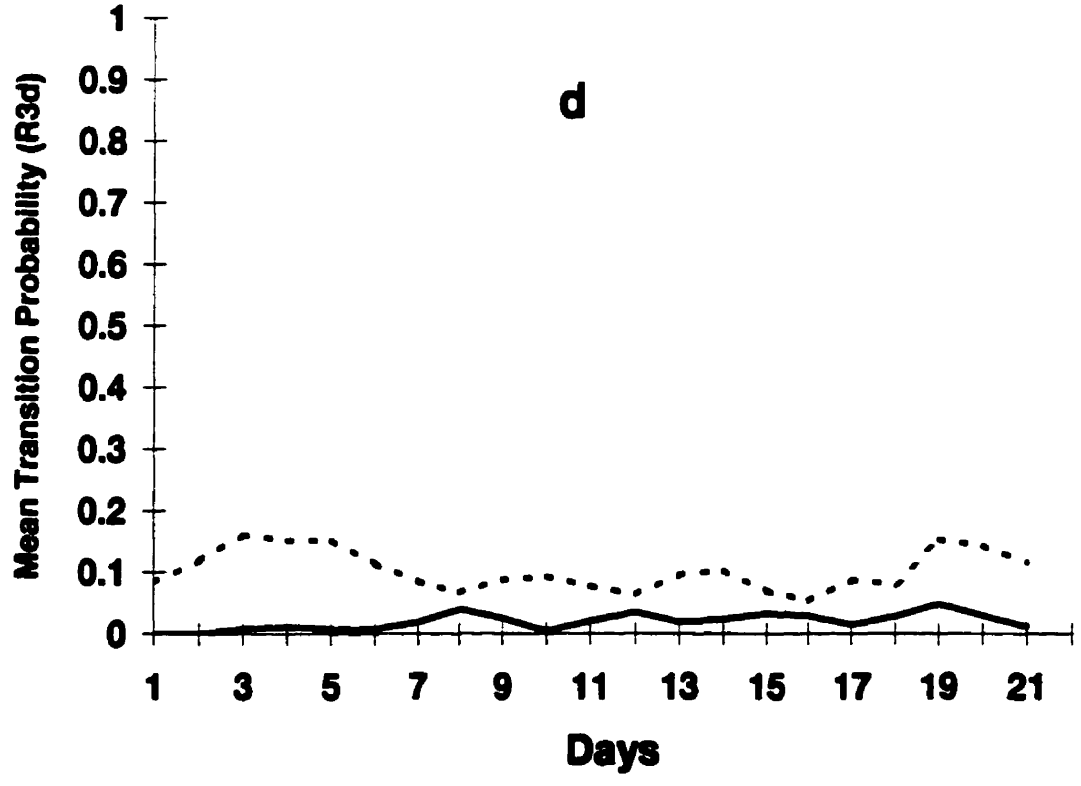

b
Mean Transition Probability (R34)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
Control
+ P. elegans

Figure 14: **Parasite effects transition probabilities of third instar *Aedes aegypti* larvae (c) to become pupae, (d) to die within 48 hours.**


c
Mean Transition Probability (R3P)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days


d
Mean Transition Probability (R3d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days


Control
+ P. elegans

Figure 15: **Parasite effects transition probabilities of fourth instar *Aedes aegypti* larvae (a) to remain fourth instars, (b) to become pupae**

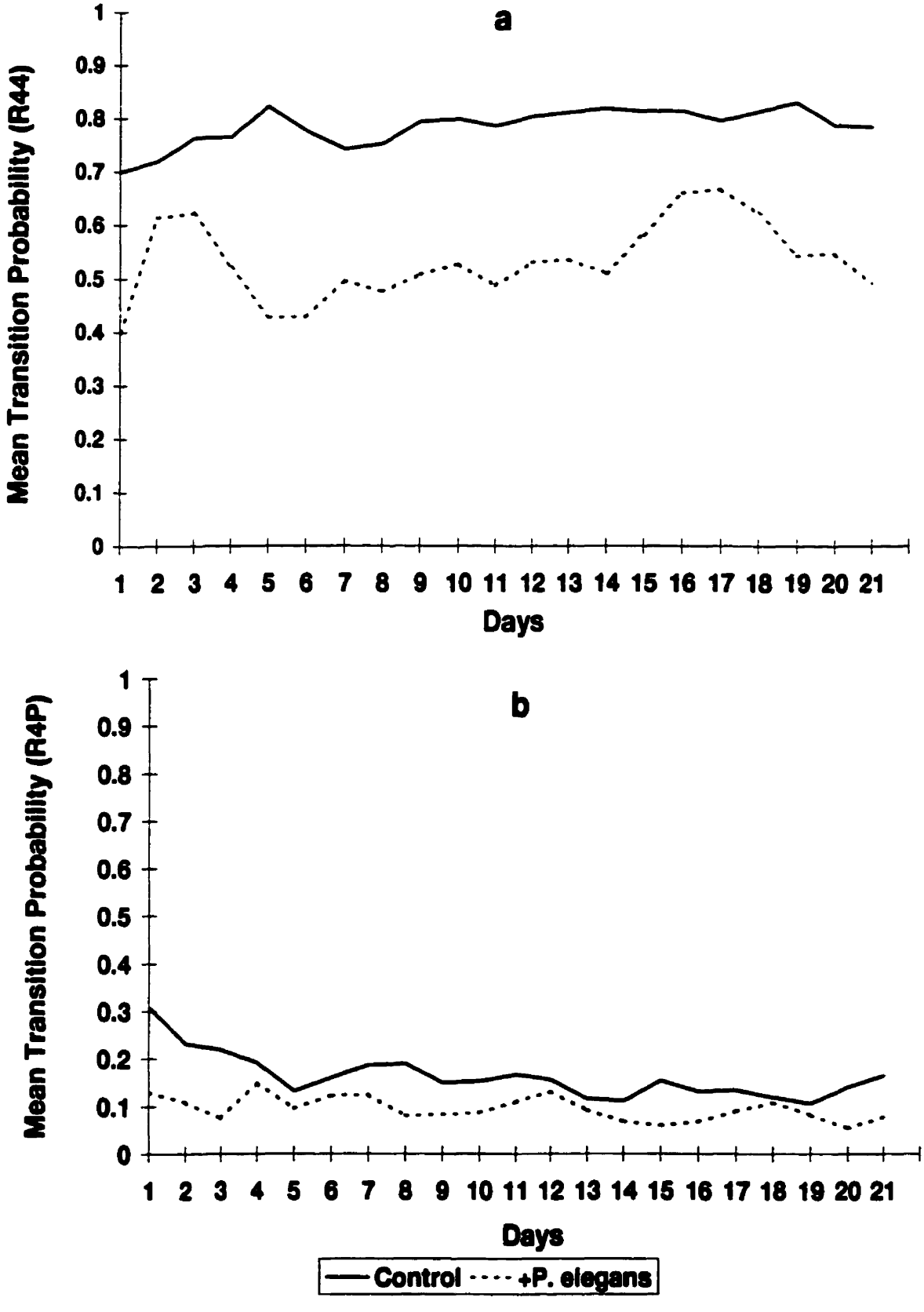
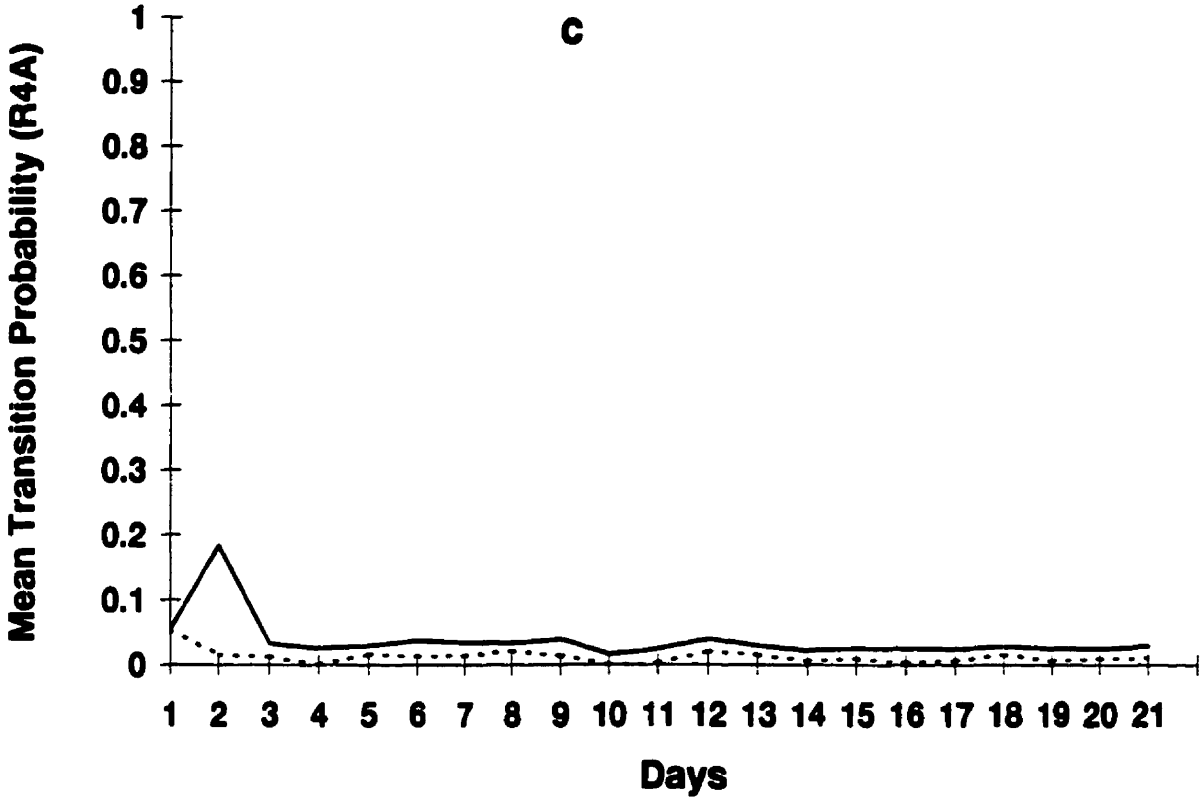

a
Mean Transition Probability (R44)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
b
Mean Transition Probability (R4P)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
Control
+P. elegans

Figure 15: **Parasite effects transition probabilities of fourth instar *Aedes aegypti* larvae (c) to become adults, (d) to die within 48 hours.**


c
Mean Transition Probability (R4A)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

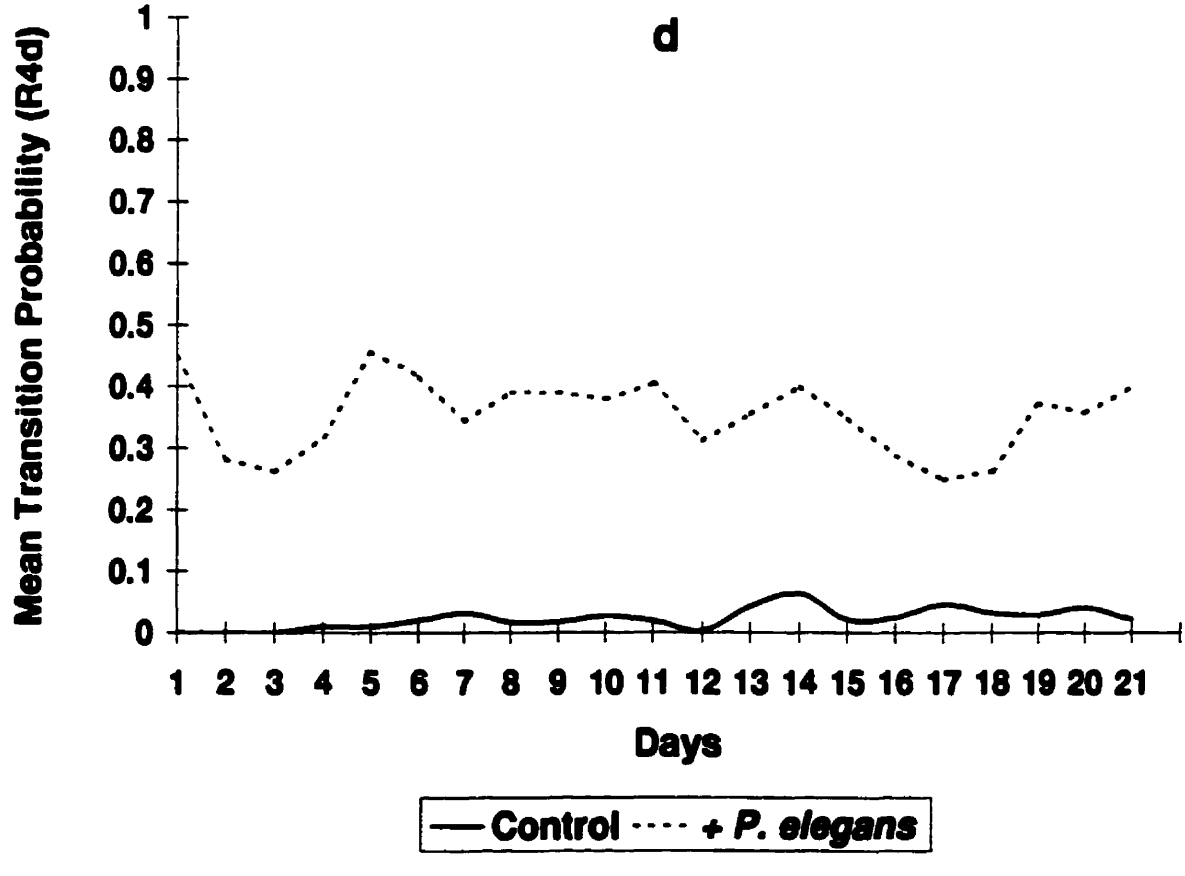

d
Mean Transition Probability (R4d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
Control
+ P. elegans

Figure 16: **Parasite effects transition probability of *Aedes aegypti* pupae (a) to remain pupae, (b) to become adults, (c) to die within 48 hours.**

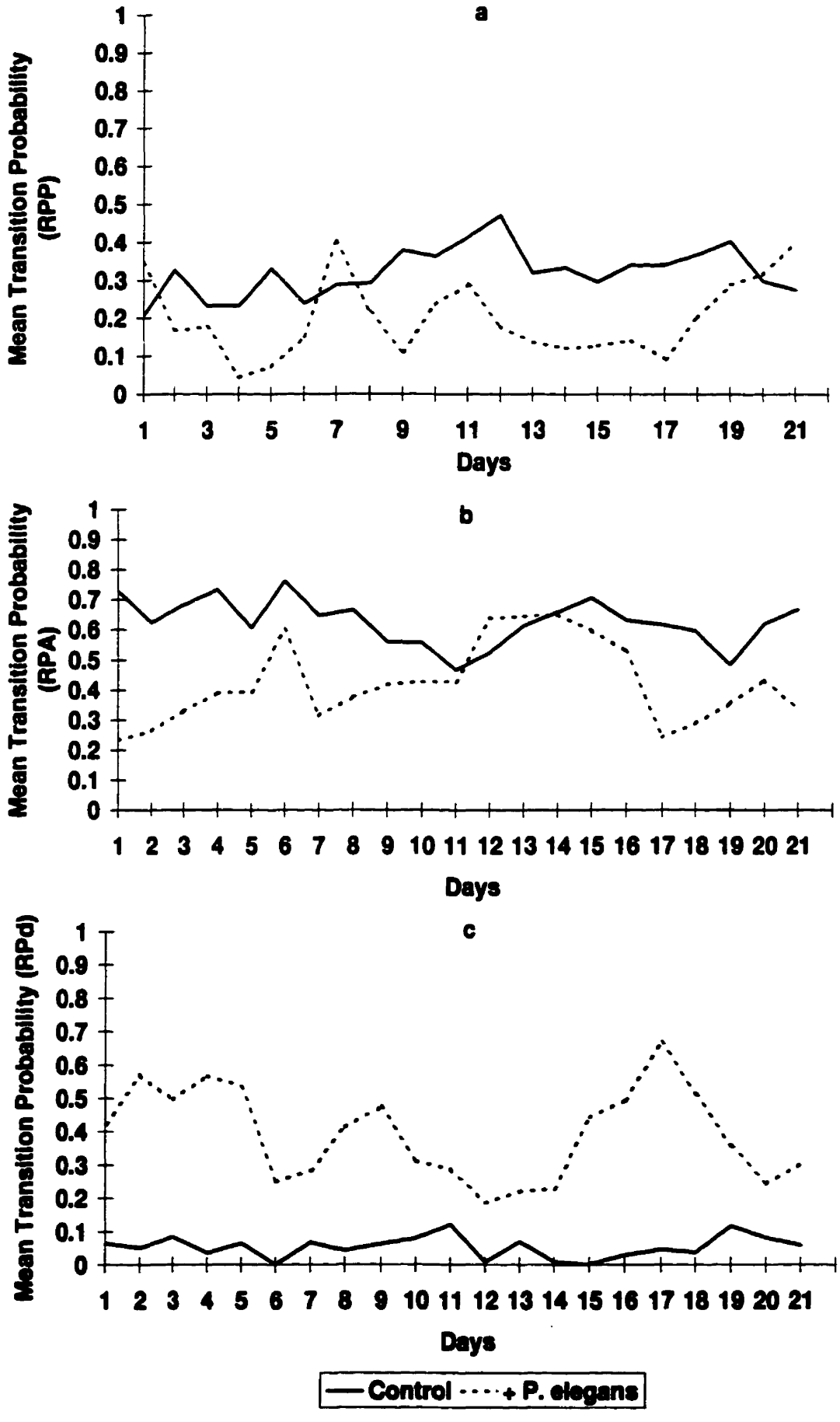
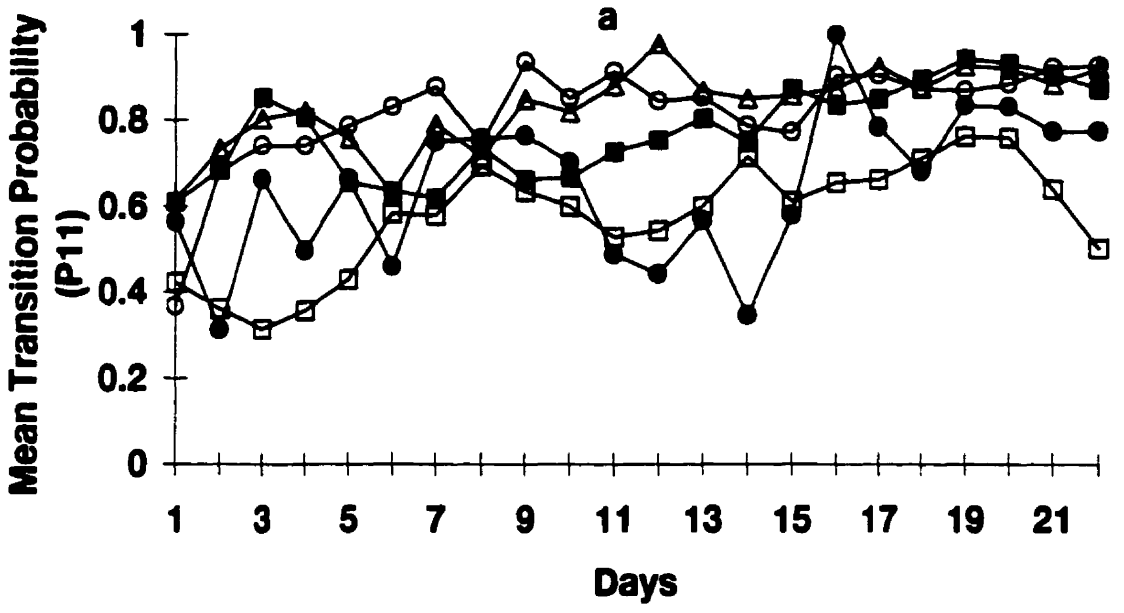

a
Mean Transition Probability (RPP)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (RPA)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
c
Mean Transition Probability (RPd)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
Control
+ P. elegans

Figure 17: **Food and parasite interactive effects transition probabilities of first instar *Aedes aegypti* larvae to remain first instars within 24 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (P11)
1
0.8
0.6
0.4
0.2
0
1 3 5 7 9 11 13 15 17 19 21
Days

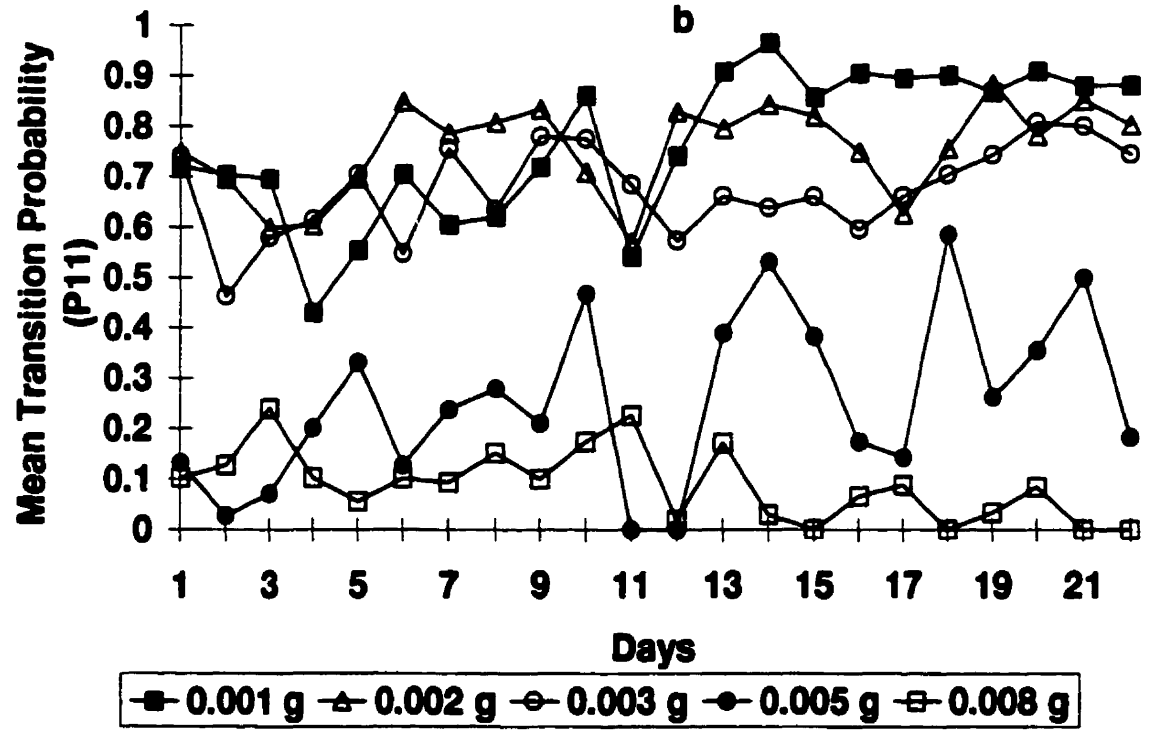

b
Mean Transition Probability (P11)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 18: **Food and parasite interactive effects transition probabilities of first instar *Aedes aegypti* larvae to become second instars within 24 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

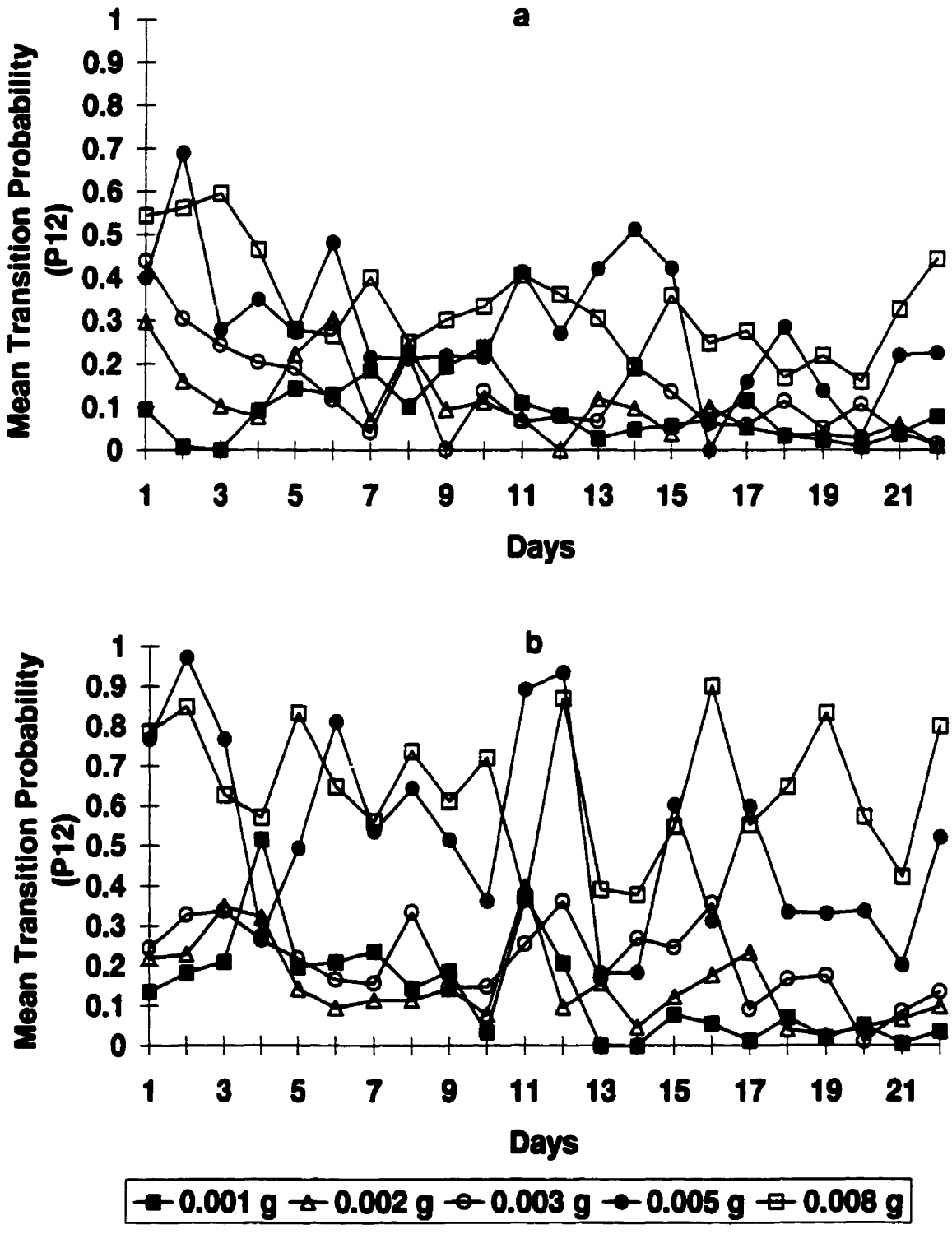

a
Mean Transition Probability (P12)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (P12)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 19: **Food and parasite interactive effects transition probabilities of first instar *Aedes aegypti* larvae to die within 24 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

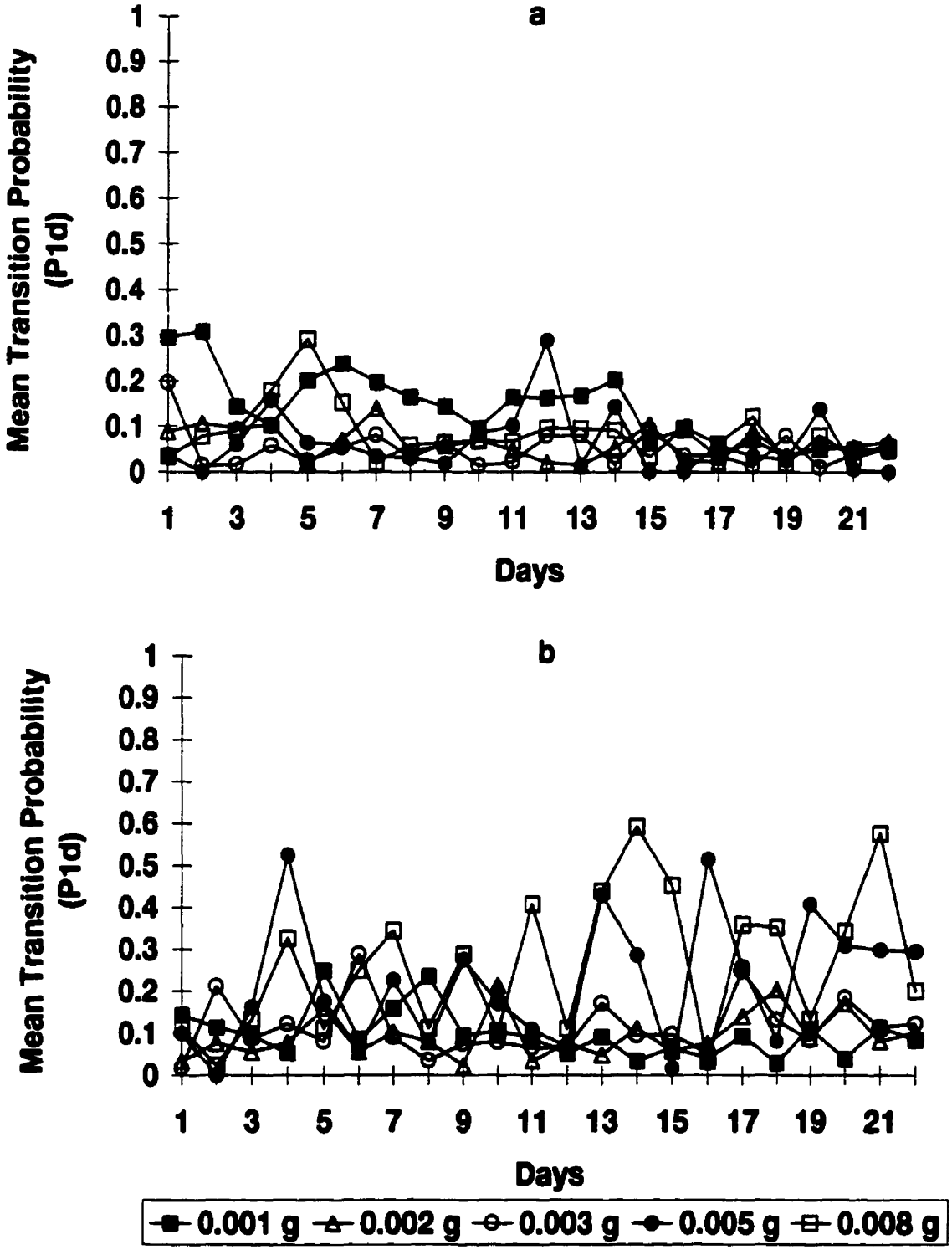

a
Mean Transition Probability (P1d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (P1d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 20: **Food and parasite interactive effects transition probabilities of first instar *Aedes aegypti* larvae to remain first instars within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

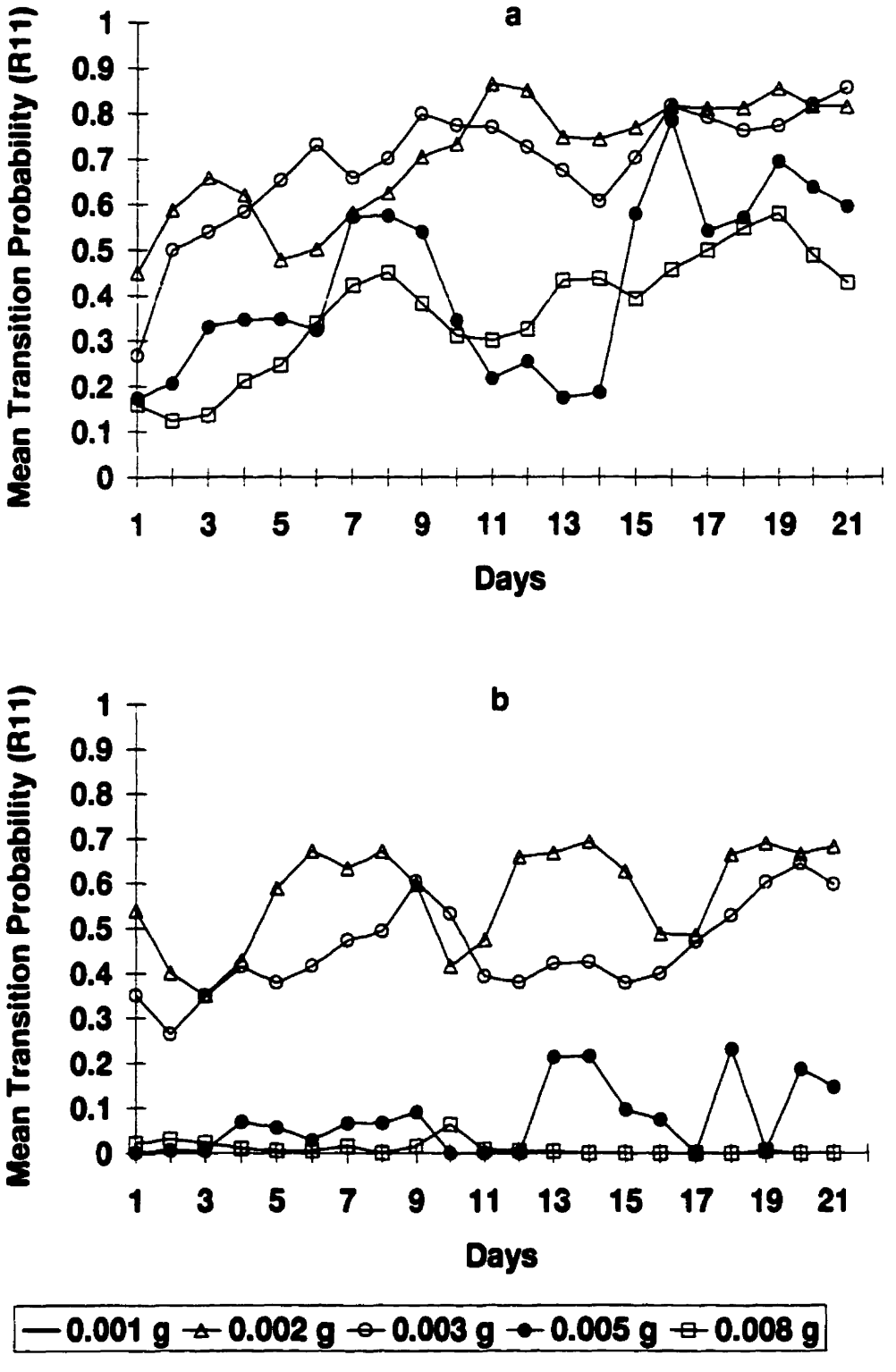
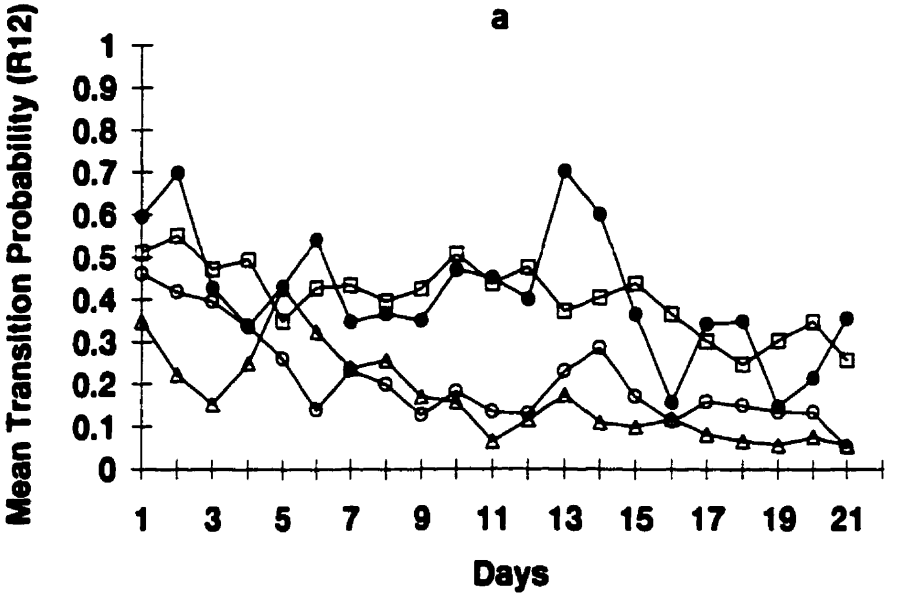
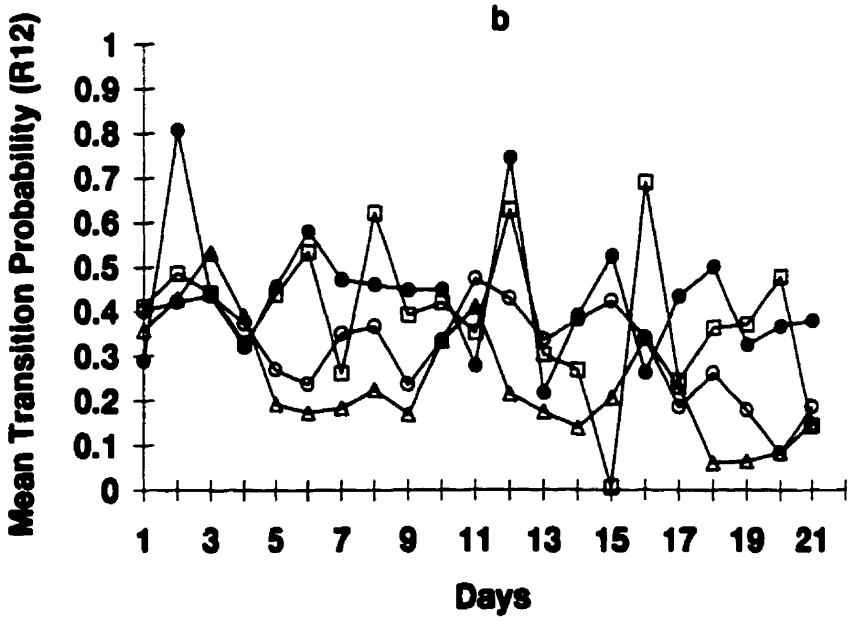

a
Mean Transition Probability (R11)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (R11)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 21: **Food and parasite interactive effects transition probabilities of first instar *Aedes aegypti* larvae to become second instars within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (R12)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days


b
Mean Transition Probability (R12)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

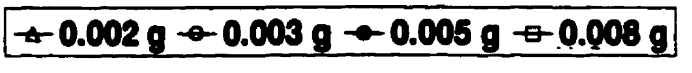

0.002 g
0.003 g
0.005 g
0.008 g

Figure 22: **Food and parasite interactive effects transition probabilities of first instar *Aedes aegypti* larvae to become third instars within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

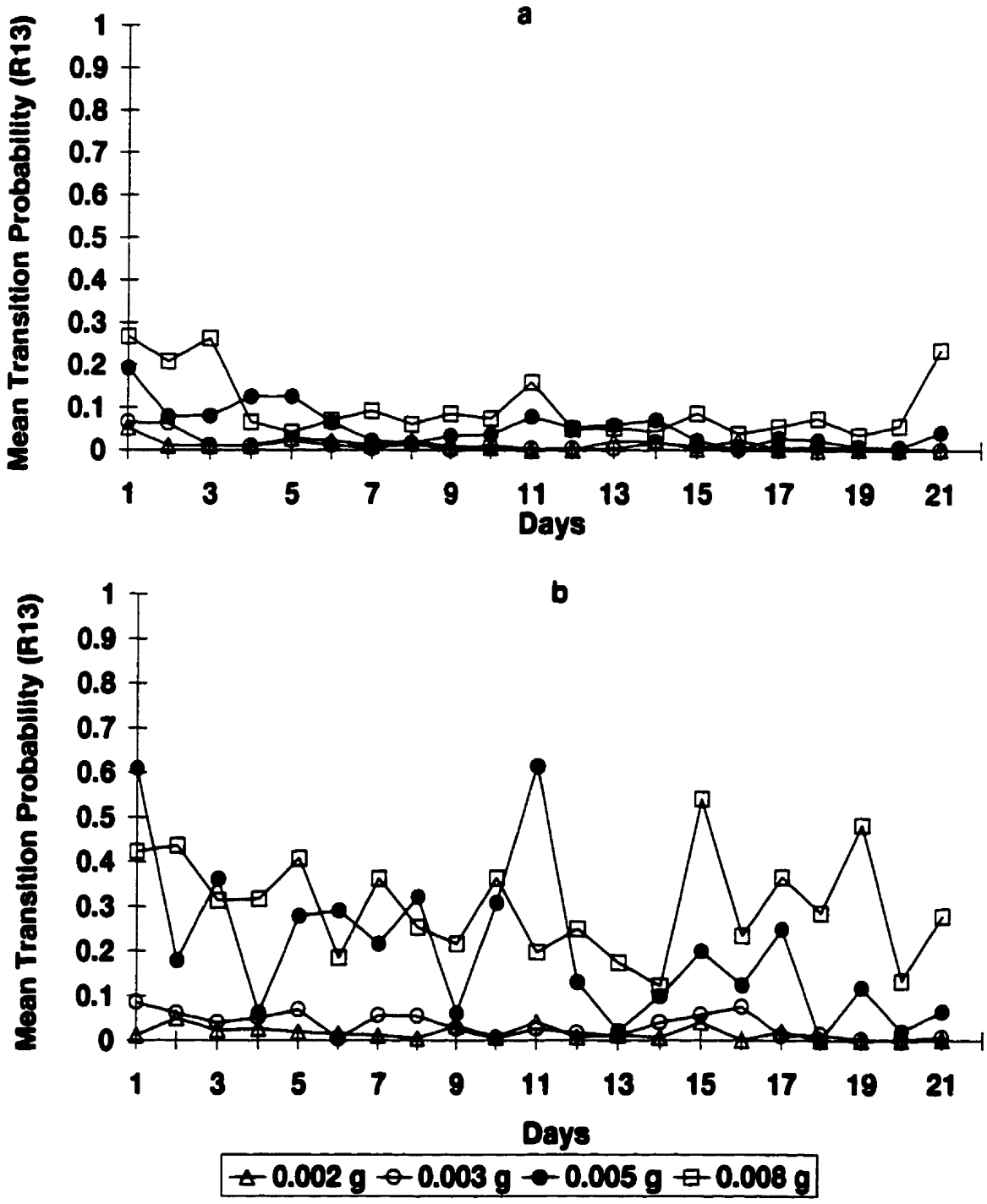
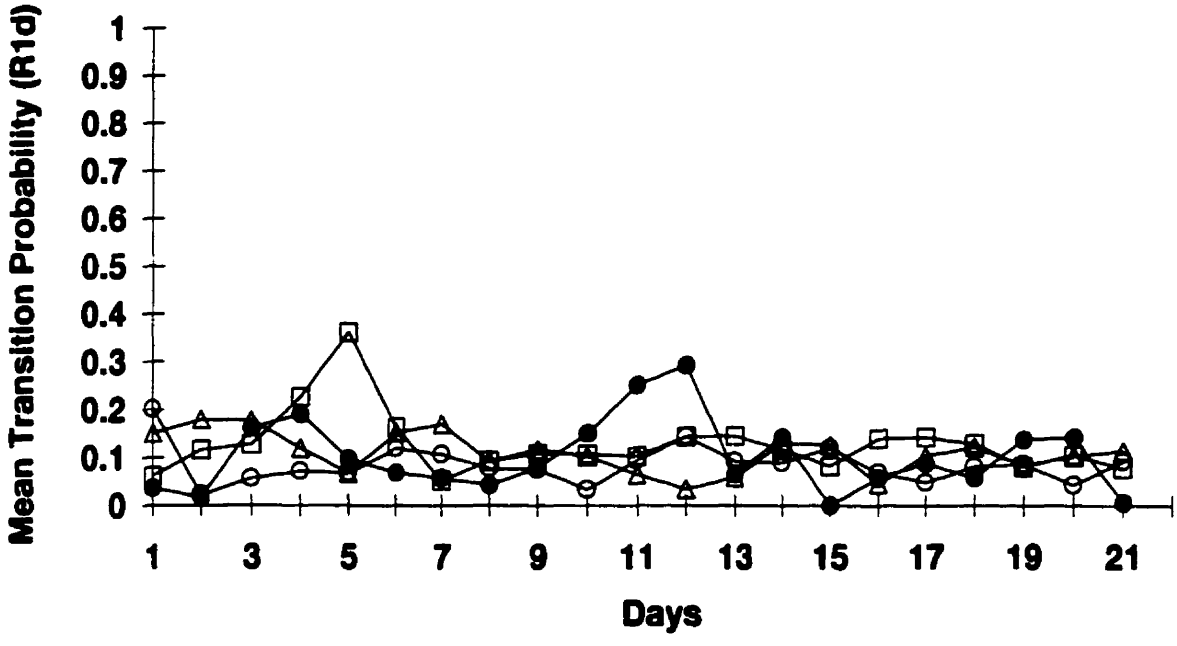

a
Mean Transition Probability (R13)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (R13)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 23: **Food and parasite interactive effects transition probabilities of first instar *Aedes aegypti* larvae to die within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

a


Mean Transition Probability (R1d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

b

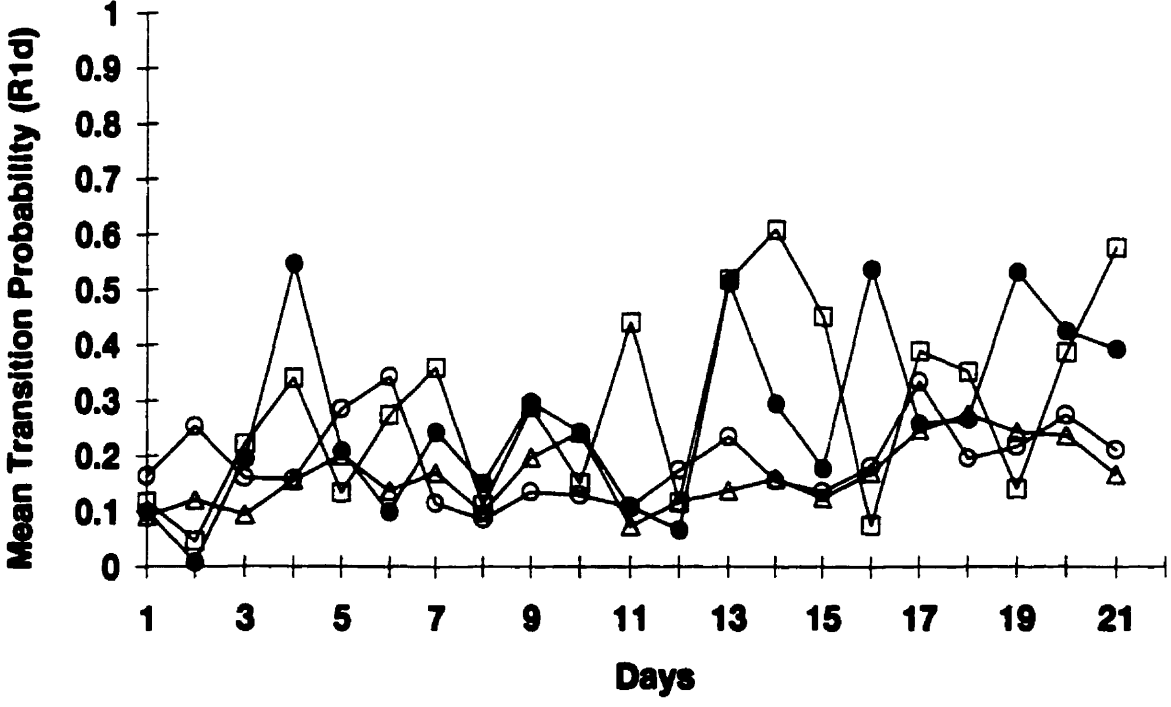
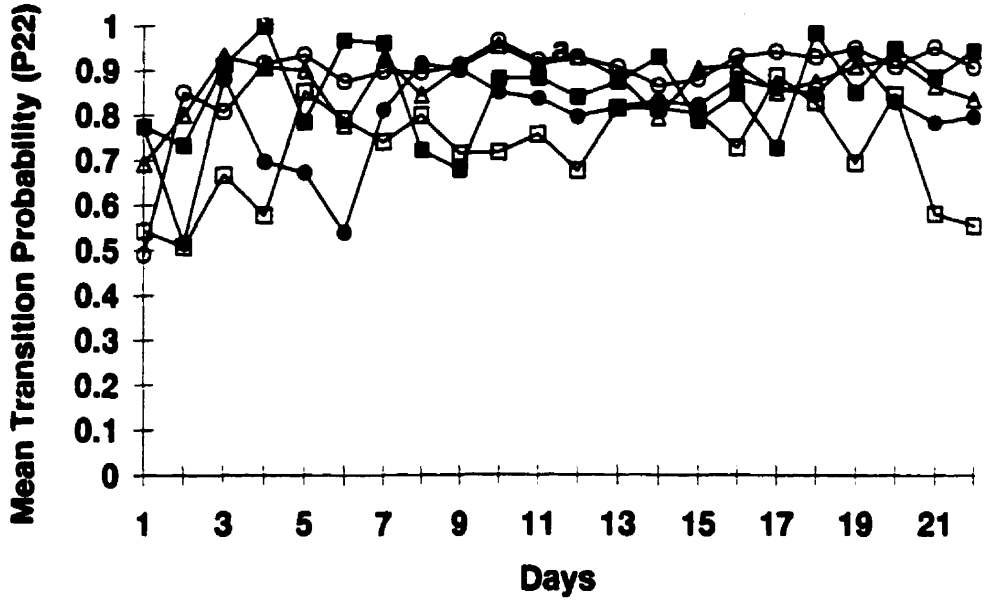

Mean Transition Probability (R1d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 24: **Food and parasite interactive effects transition probabilities of second instar *Aedes aegypti* larvae to remain second instars within 24 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


Mean Transition Probability (P22)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

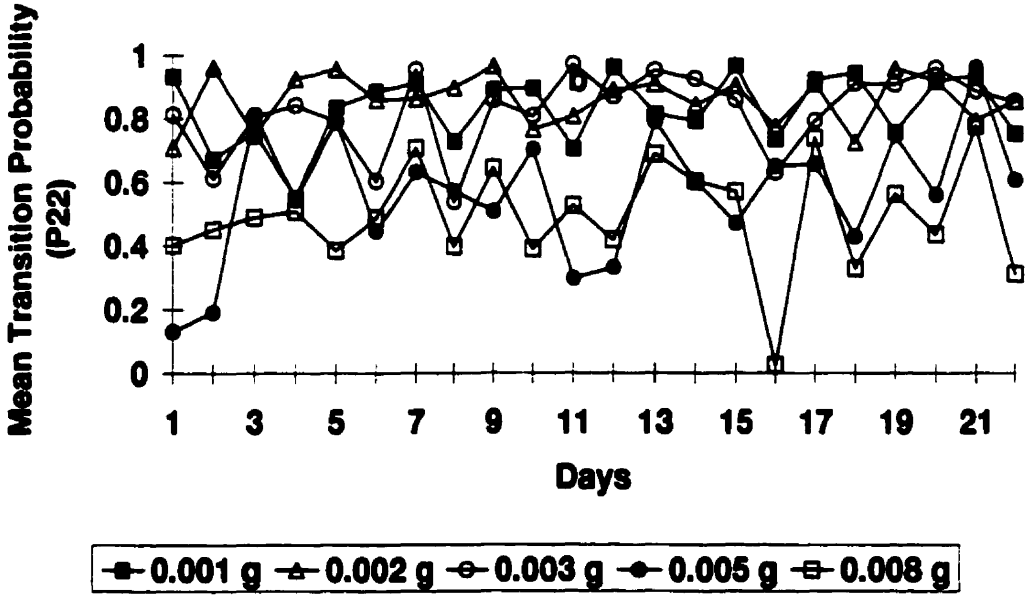
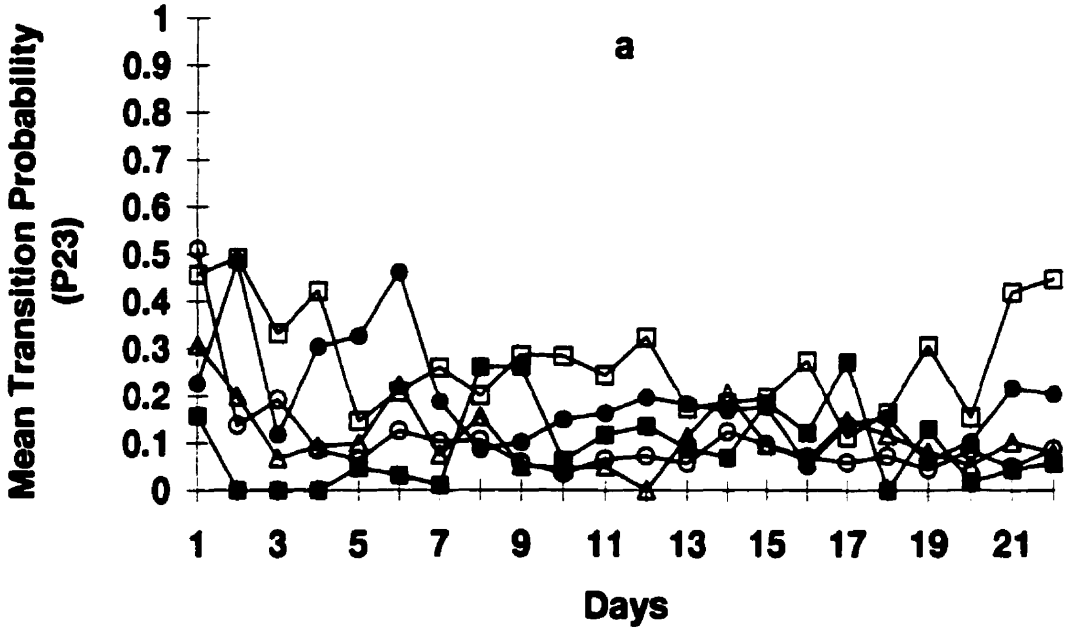

Mean Transition Probability (P22)
1
0.8
0.6
0.4
0.2
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 25: **Food and parasite interactive effects transition probabilities of second instar *Aedes aegypti* larvae to become third instars within 24 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (P23)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

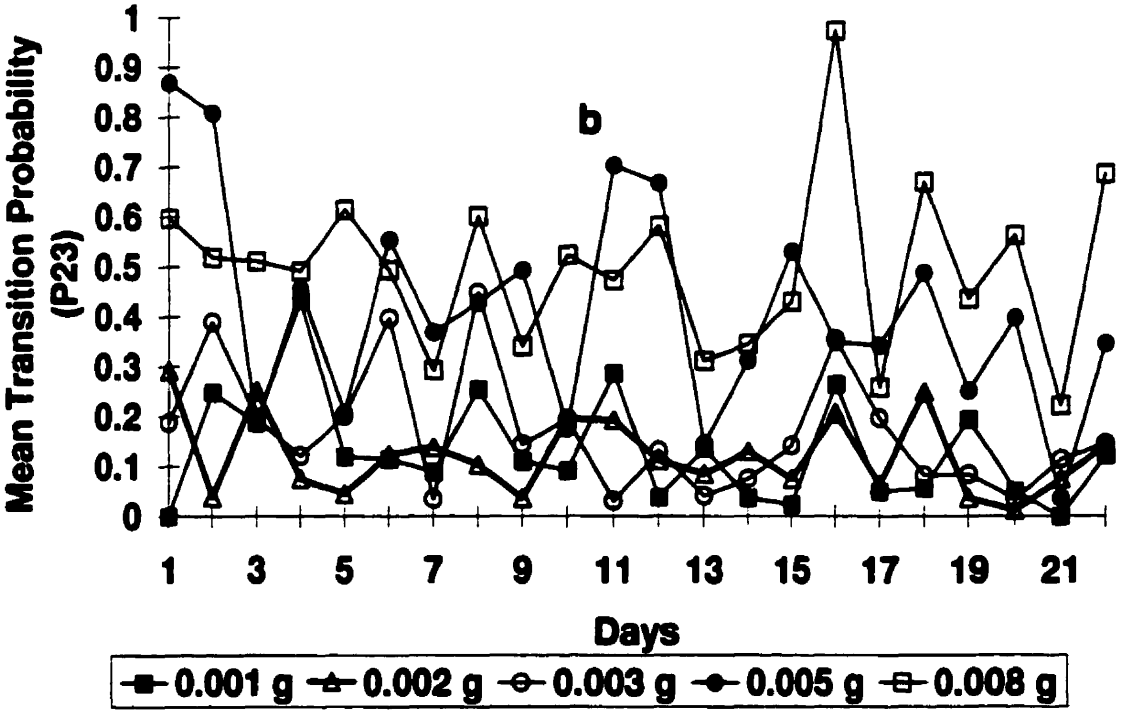

b
Mean Transition Probability (P23)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 26: **Food and parasite interactive effects transition probabilities of second instar *Aedes aegypti* larvae to die within 24 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

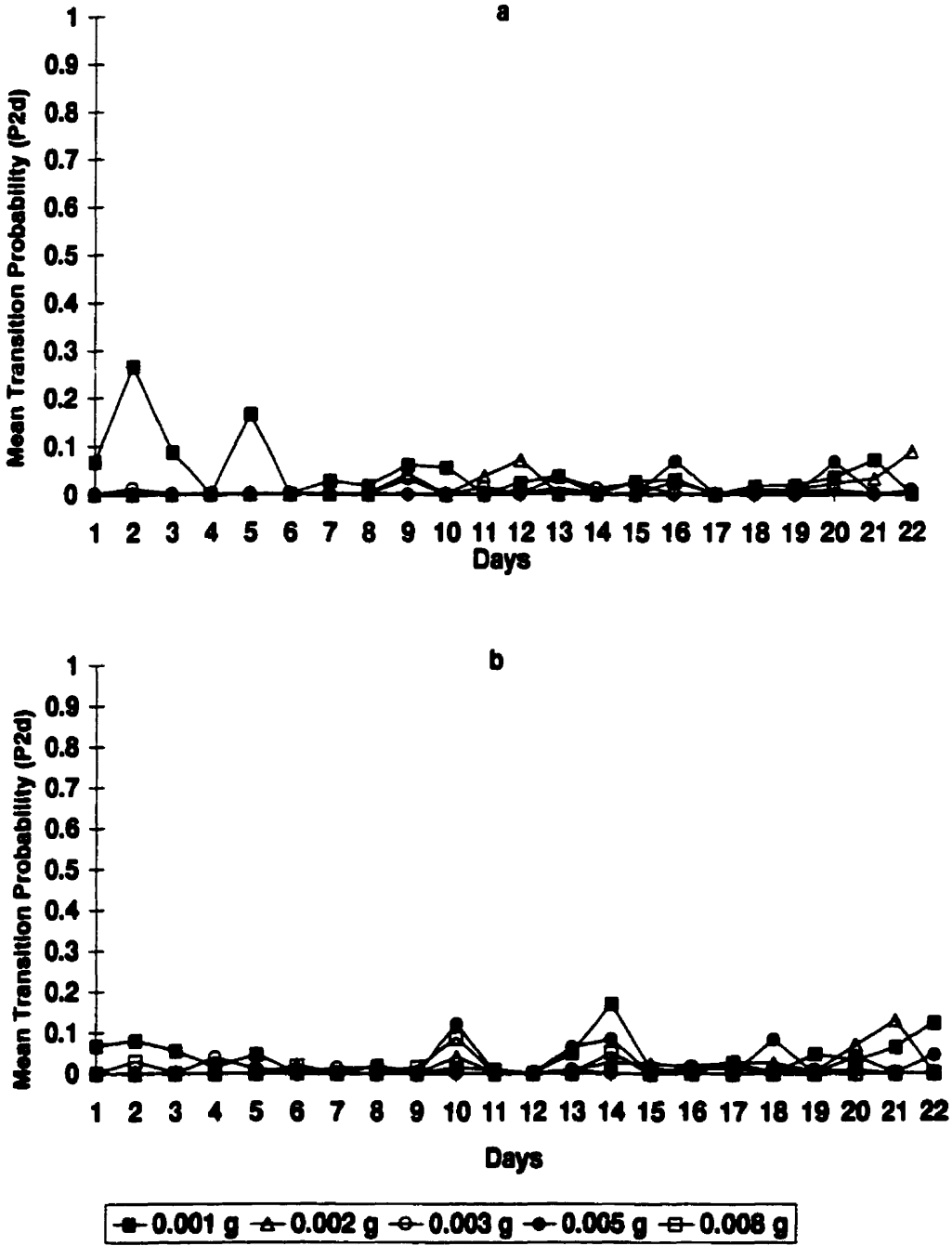

a
Mean Transition Probability (P2d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
b
Mean Transition Probability (P2d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 27: **Food and parasite interactive effects transition probabilities of second instar *Aedes aegypti* larvae to remain second instars within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

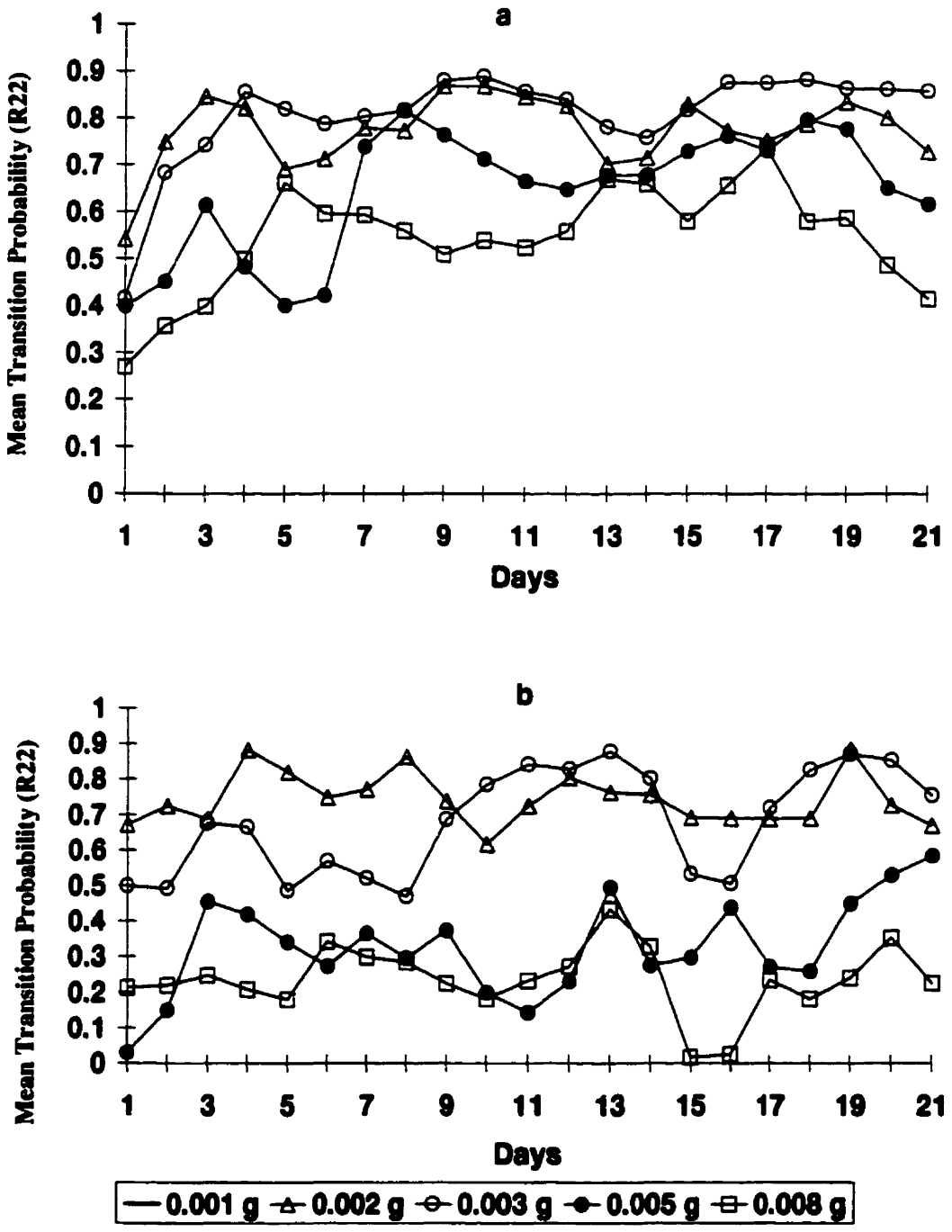
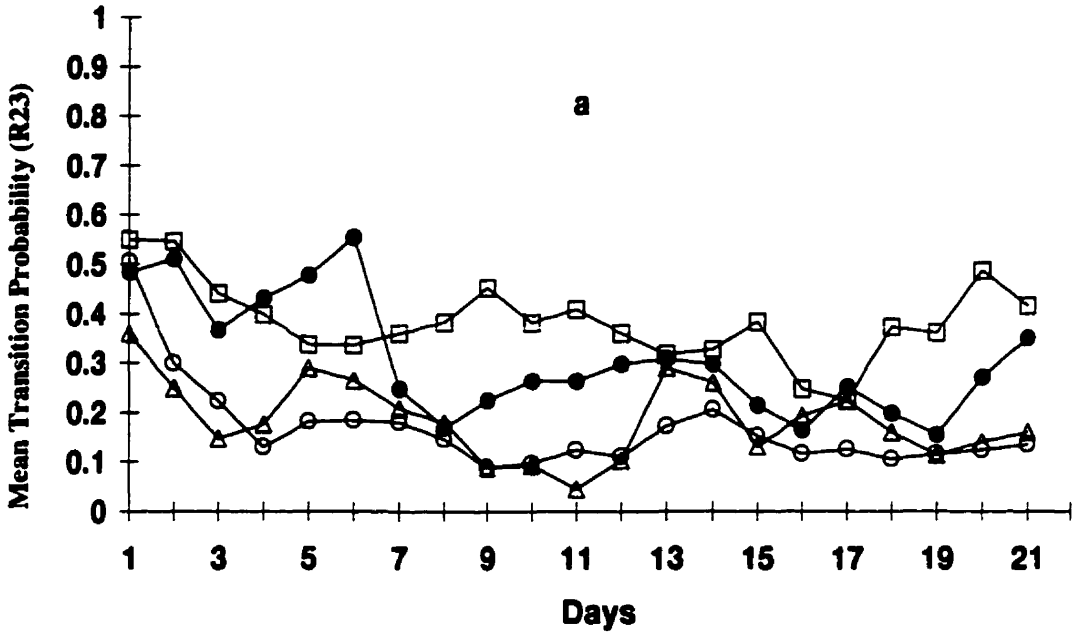

a
Mean Transition Probability (R22)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (R22)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 28: **Food and parasite interactive effects transition probabilities of second instar *Aedes aegypti* larvae to become third instars within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (R23)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

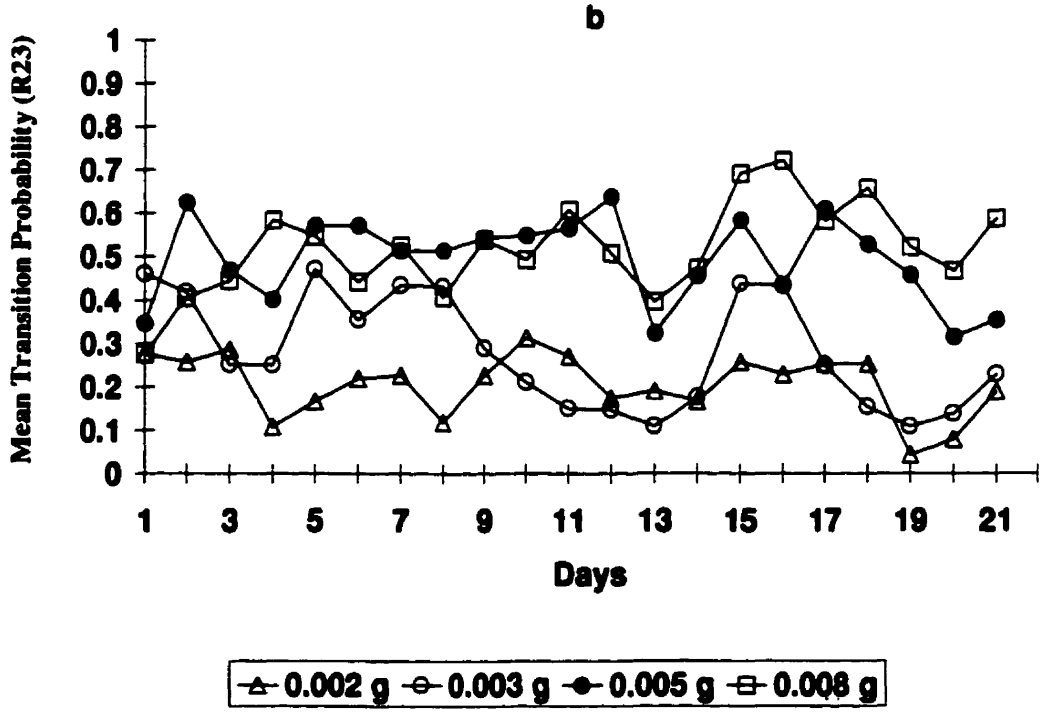
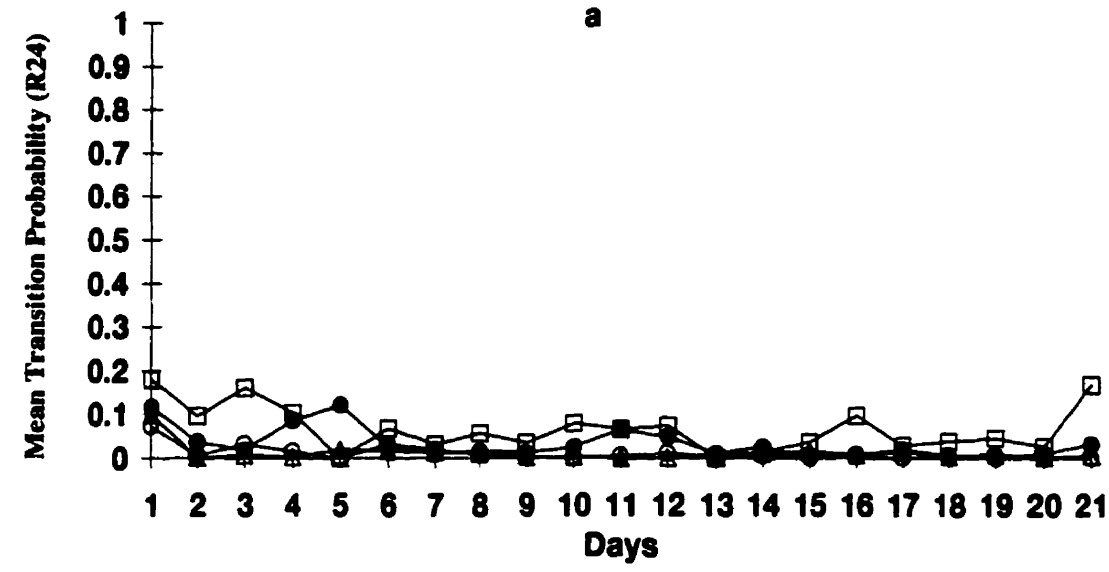

b
Mean Transition Probability (R23)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 29: **Food and parasite interactive effects transition probabilities of second instar *Aedes aegypti* larvae to become fourth instars within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (R24)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days

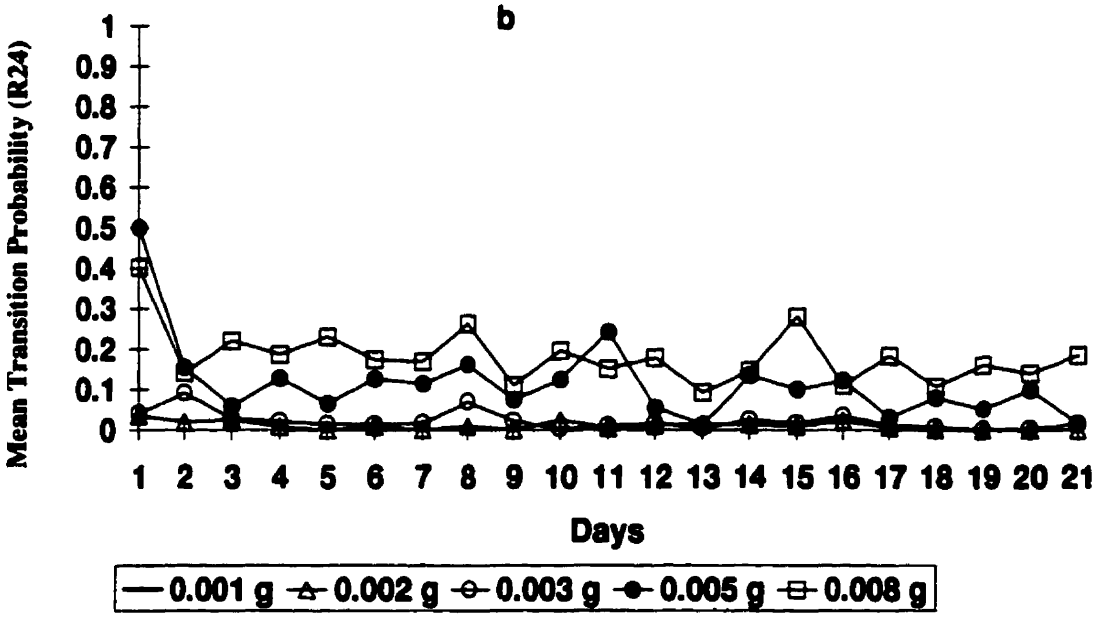

b
Mean Transition Probability (R24)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days
0.001 g
0.002 g
0.003 g
0.005 g
0.008 g

Figure 30: **Food and parasite interactive effects transition probabilities of second instar *Aedes aegypti* larvae to die within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

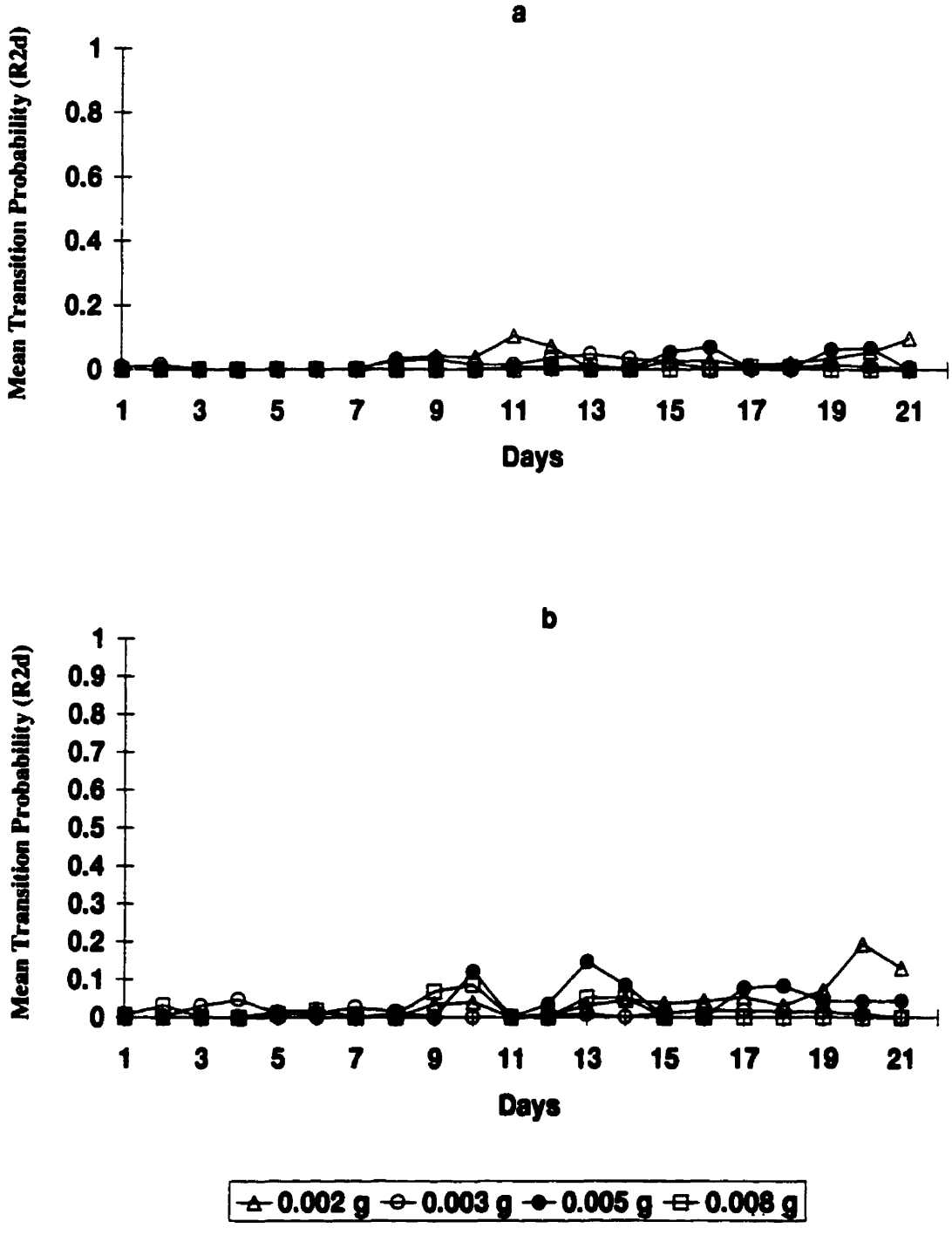
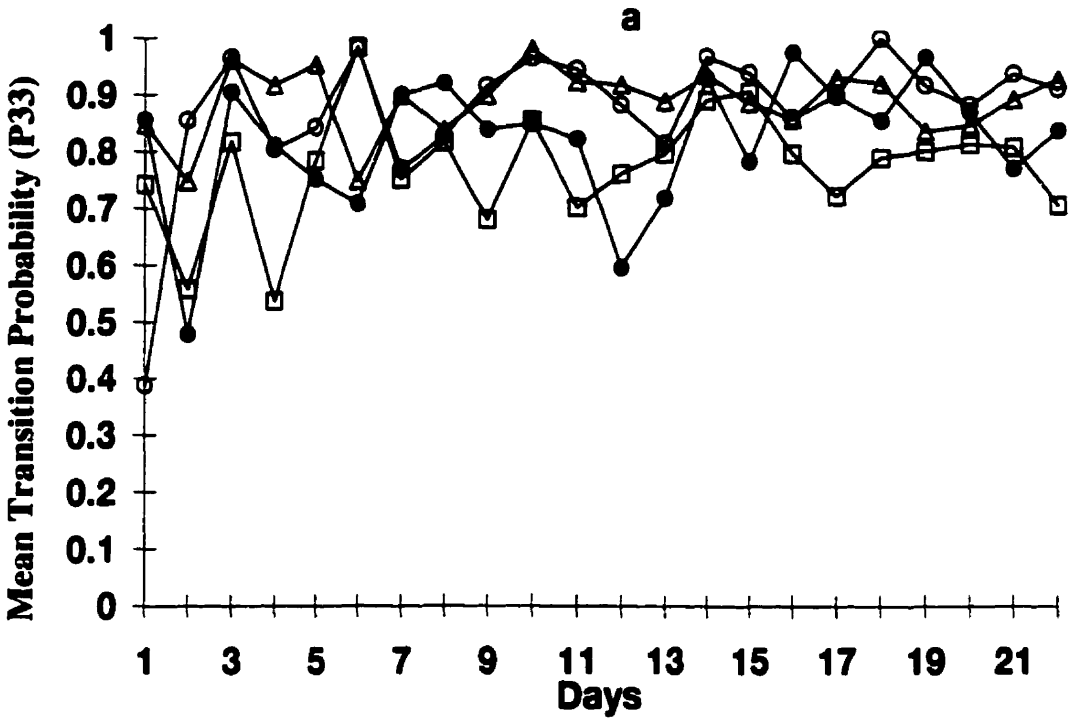

a
Mean Transition Probability (R2d)
1
0.8
0.6
0.4
0.2
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (R2d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 31: **Food and parasite interactive effects transition probabilities of third instar *Aedes aegypti* larvae to remain third instars within 24 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (P33)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

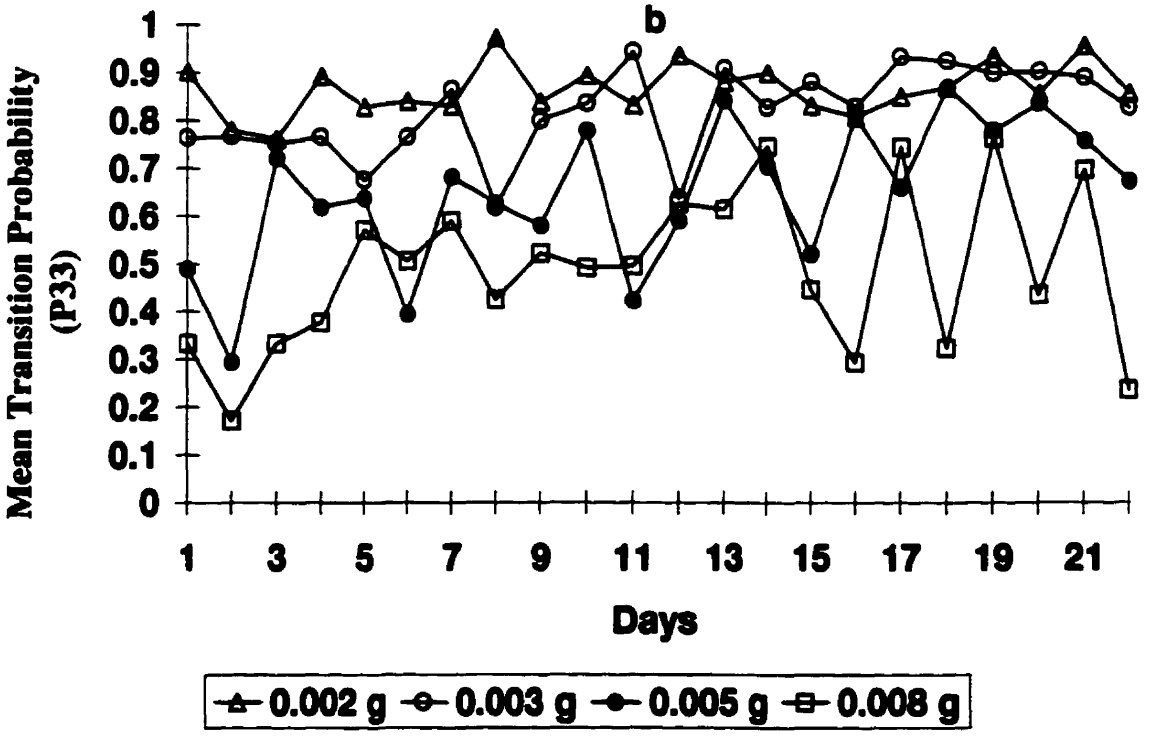

b
Mean Transition Probability (P33)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 32: **Food and parasite interactive effects transition probabilities of third instar *Aedes aegypti* larvae to become fourth instars within 24 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

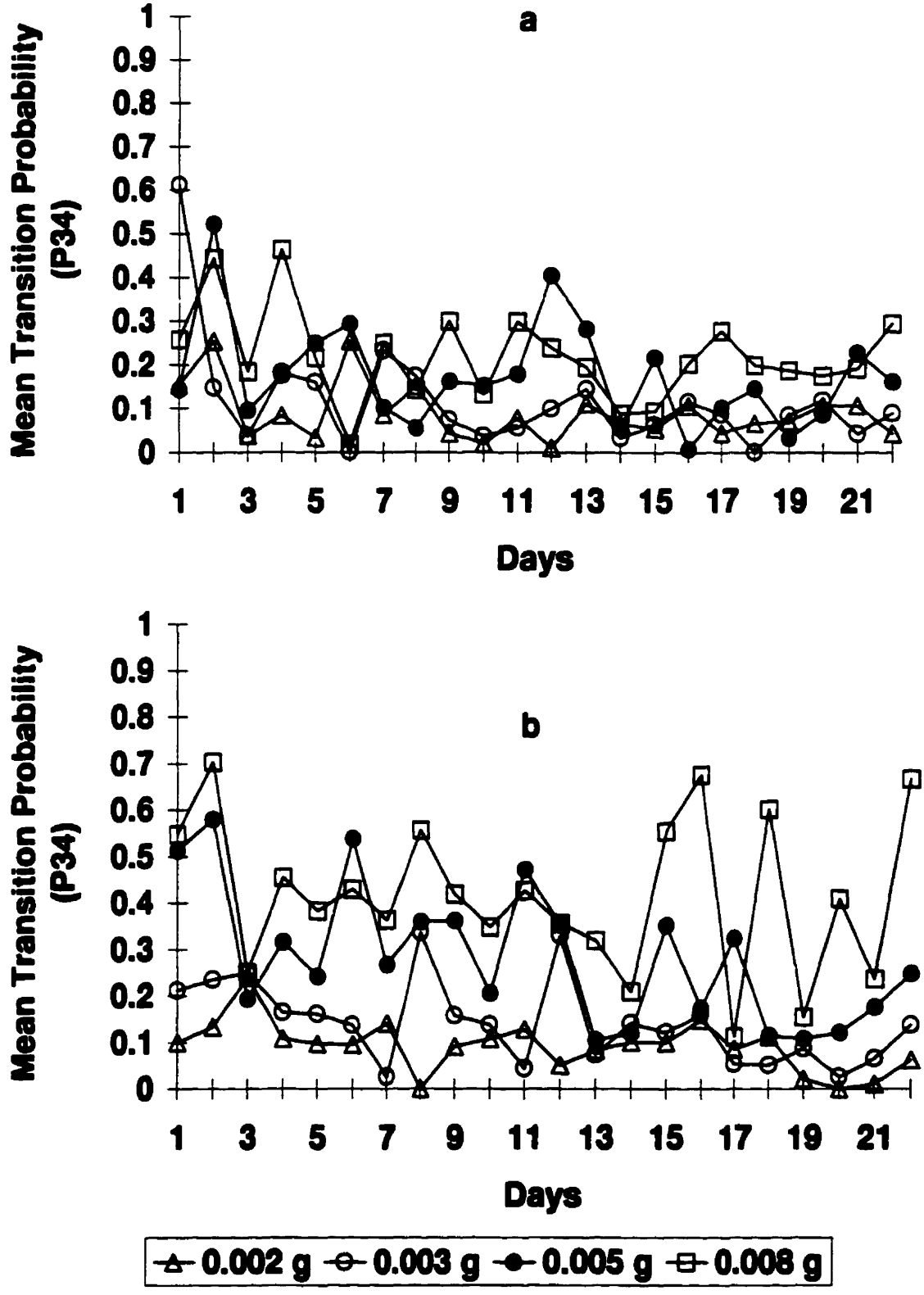

a
Mean Transition Probability (P34)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (P34)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 33: **Food and parasite interactive effects transition probabilities of third instar *Aedes aegypti* larvae to die within 24 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

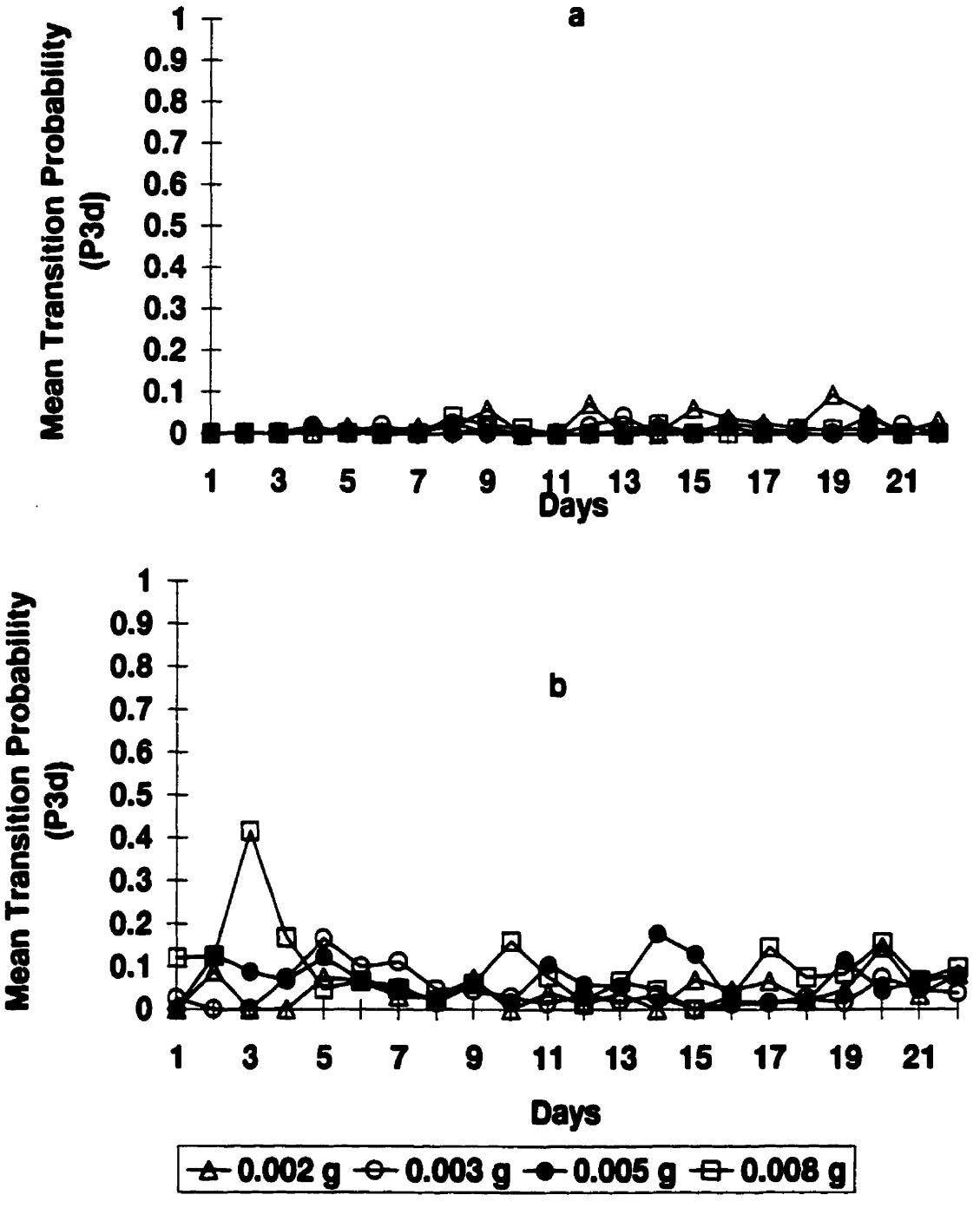
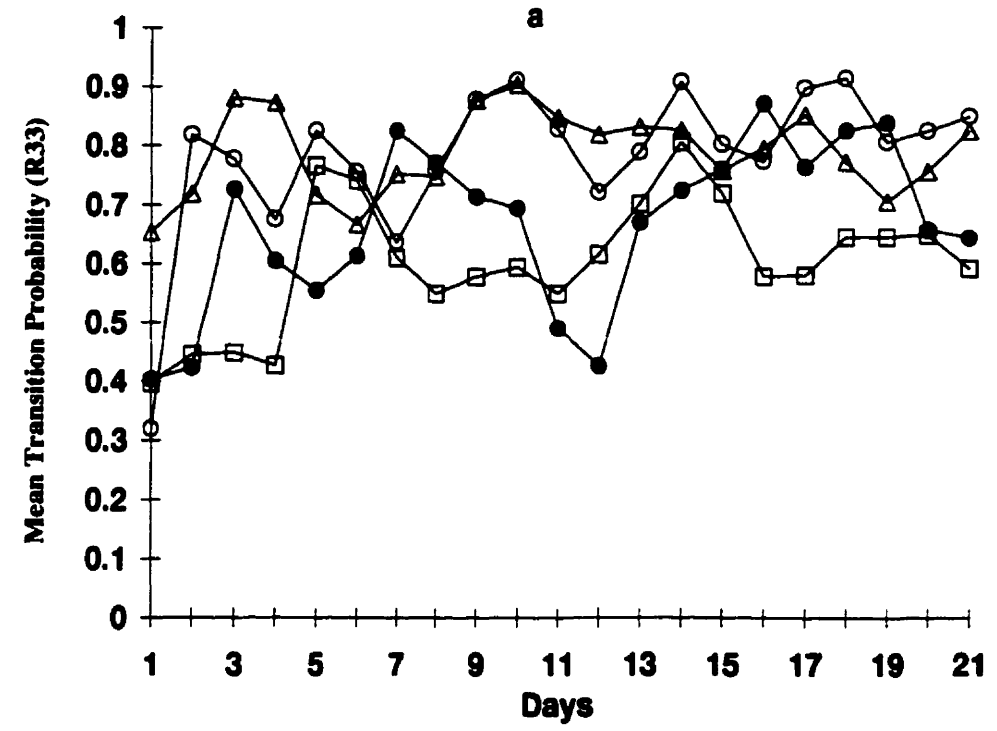

a
Mean Transition Probability (P3d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (P3d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 34: **Food and parasite interactive effects transition probabilities of third instar *Aedes aegypti* larvae to remain third instars within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (R33)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

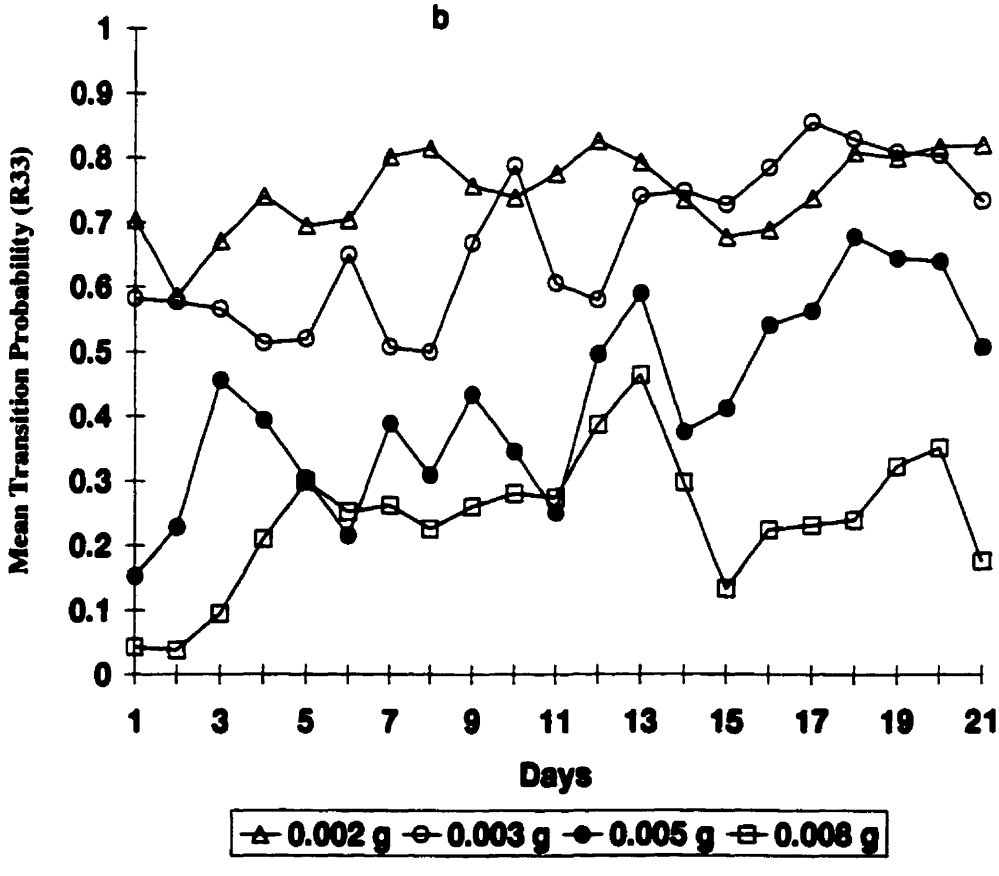
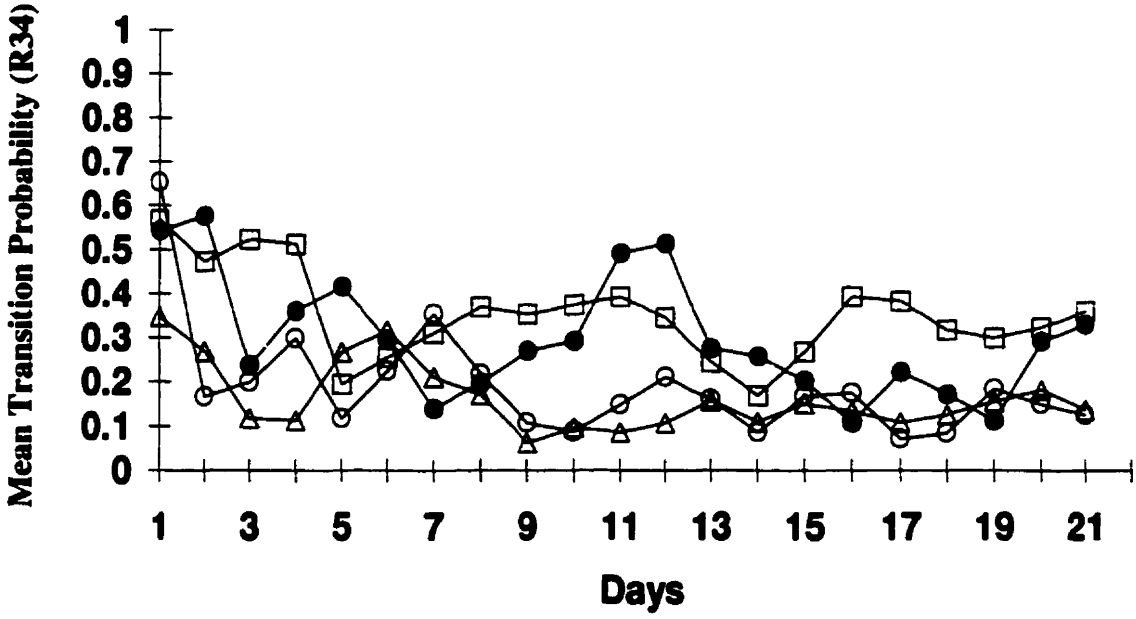
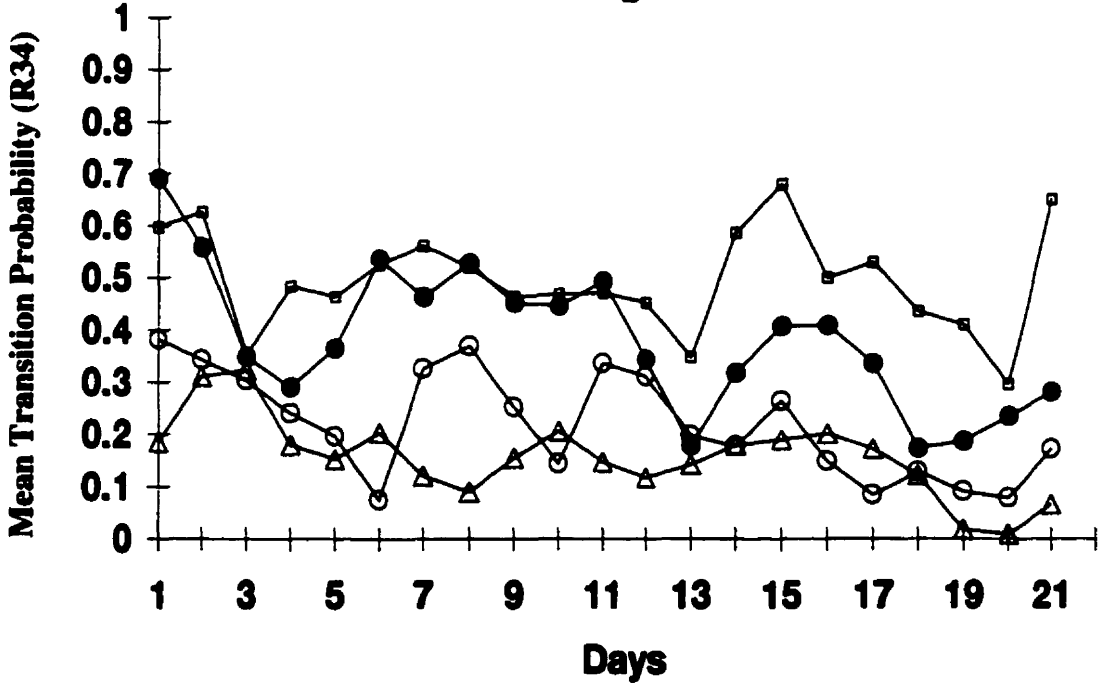

b
Mean Transition Probability (R33)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 35: **Food and parasite interactive effects transition probabilities of third instar *Aedes aegypti* larvae to become fourth instars within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

a


Mean Transition Probability (R34)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

b


Mean Transition Probability (R34)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

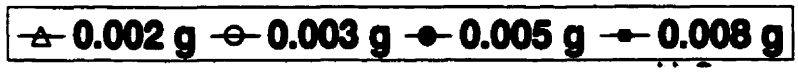
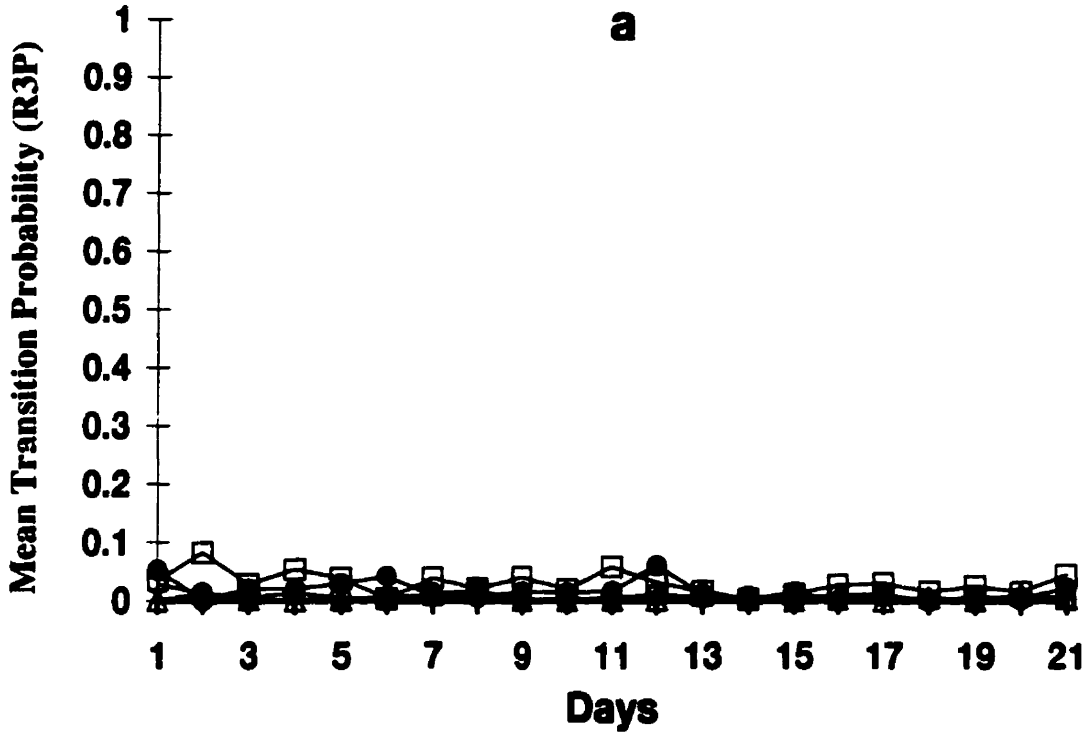

0.002 g
0.003 g
0.005 g
0.008 g

Figure 36: **Food and parasite interactive effects transition probabilities of third instar *Aedes aegypti* larvae to become pupae within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (R3P)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

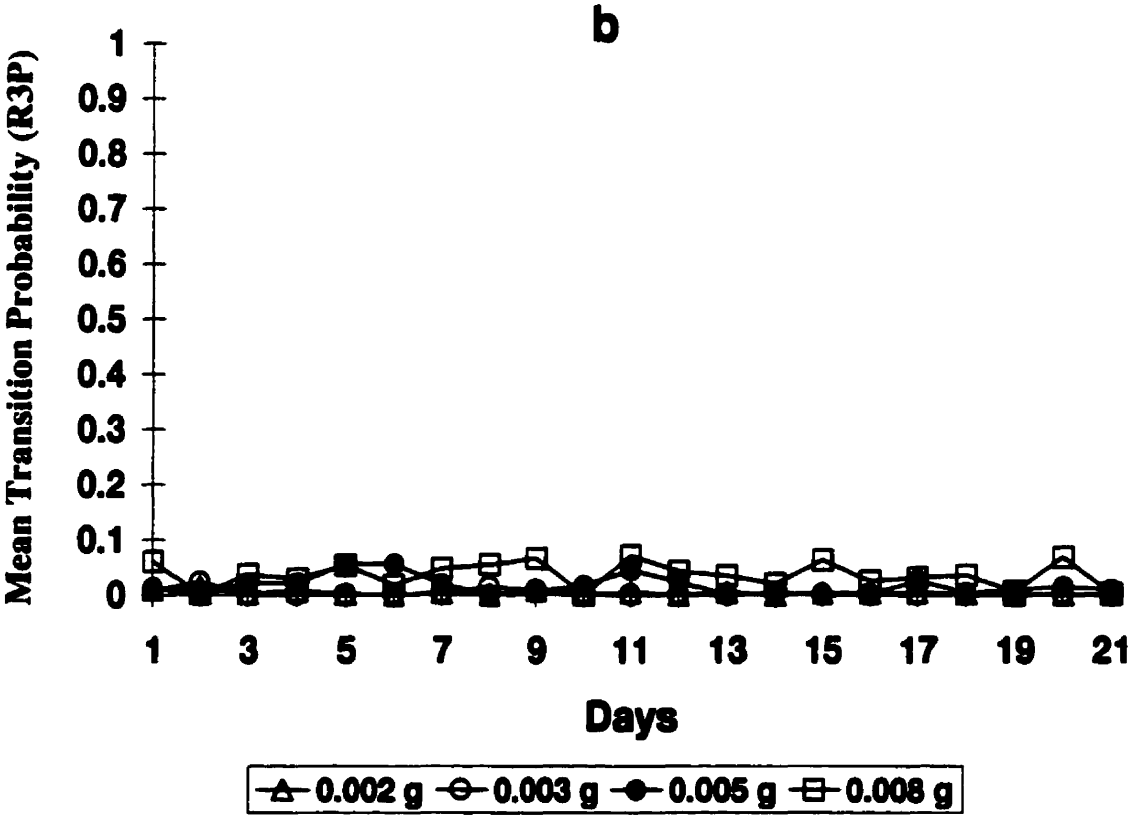
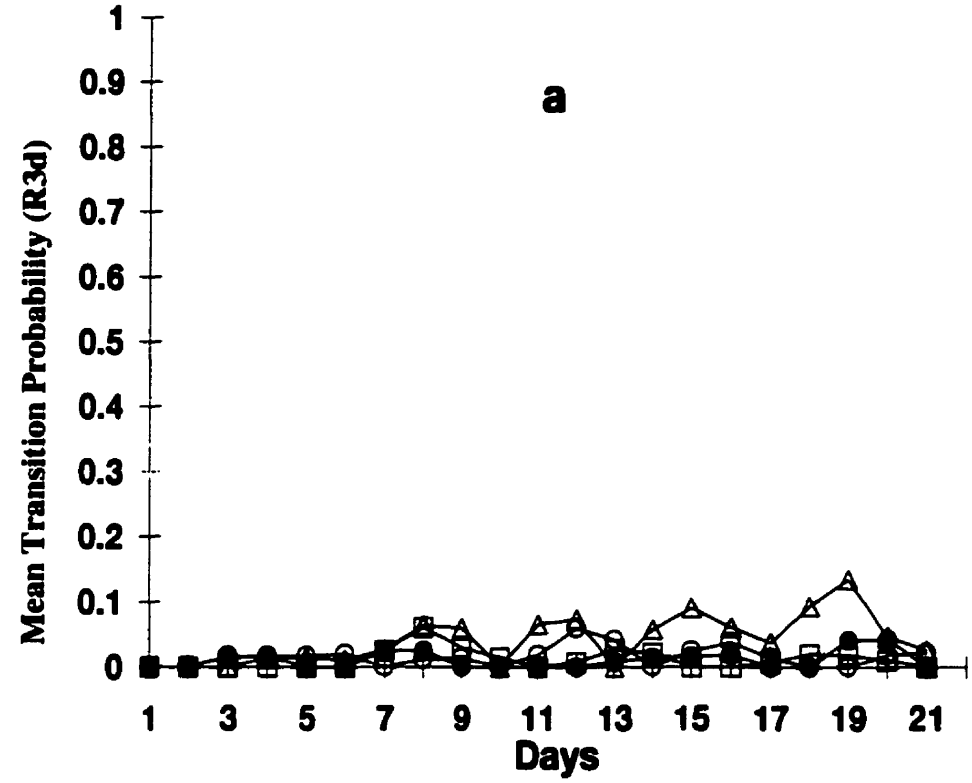

b
Mean Transition Probability (R3P)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 37: **Food and parasite interactive effects transition probabilities of third instar *Aedes aegypti* larvae to die within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (R3d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

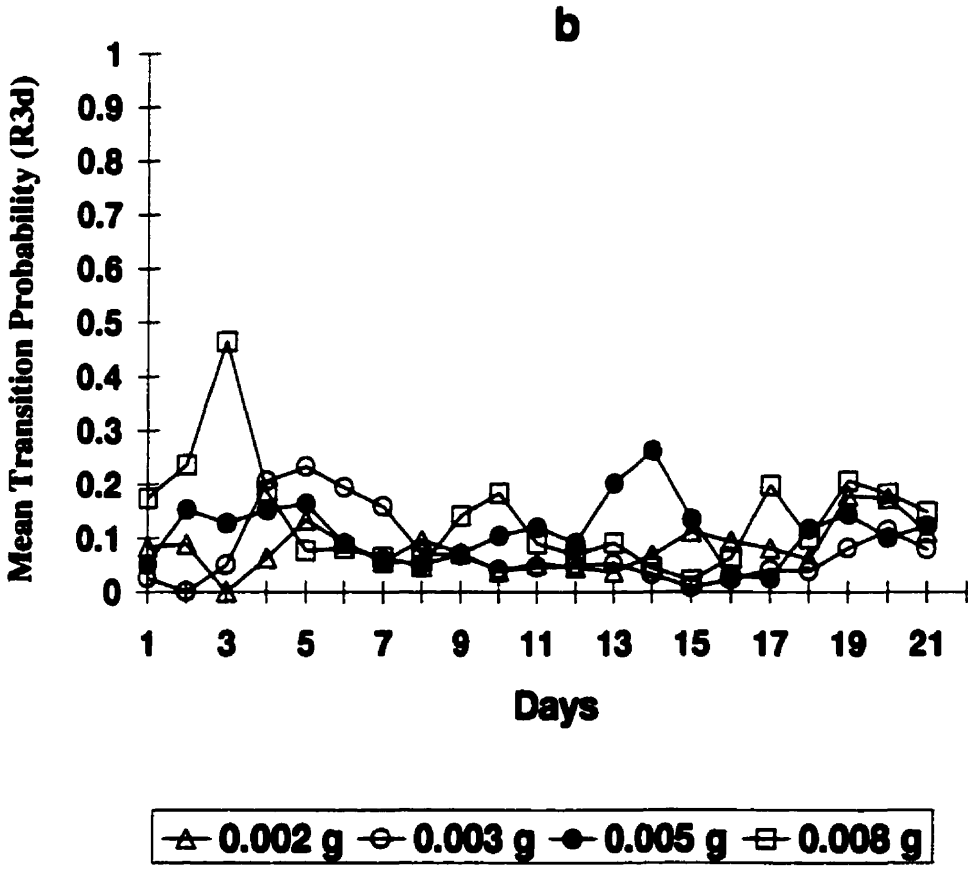

b
Mean Transition Probability (R3d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.003 g
0.005 g
0.008 g

Figure 38: **Food and parasite interactive effects transition probabilities of fourth instar *Aedes aegypti* larvae to remain fourth instars within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

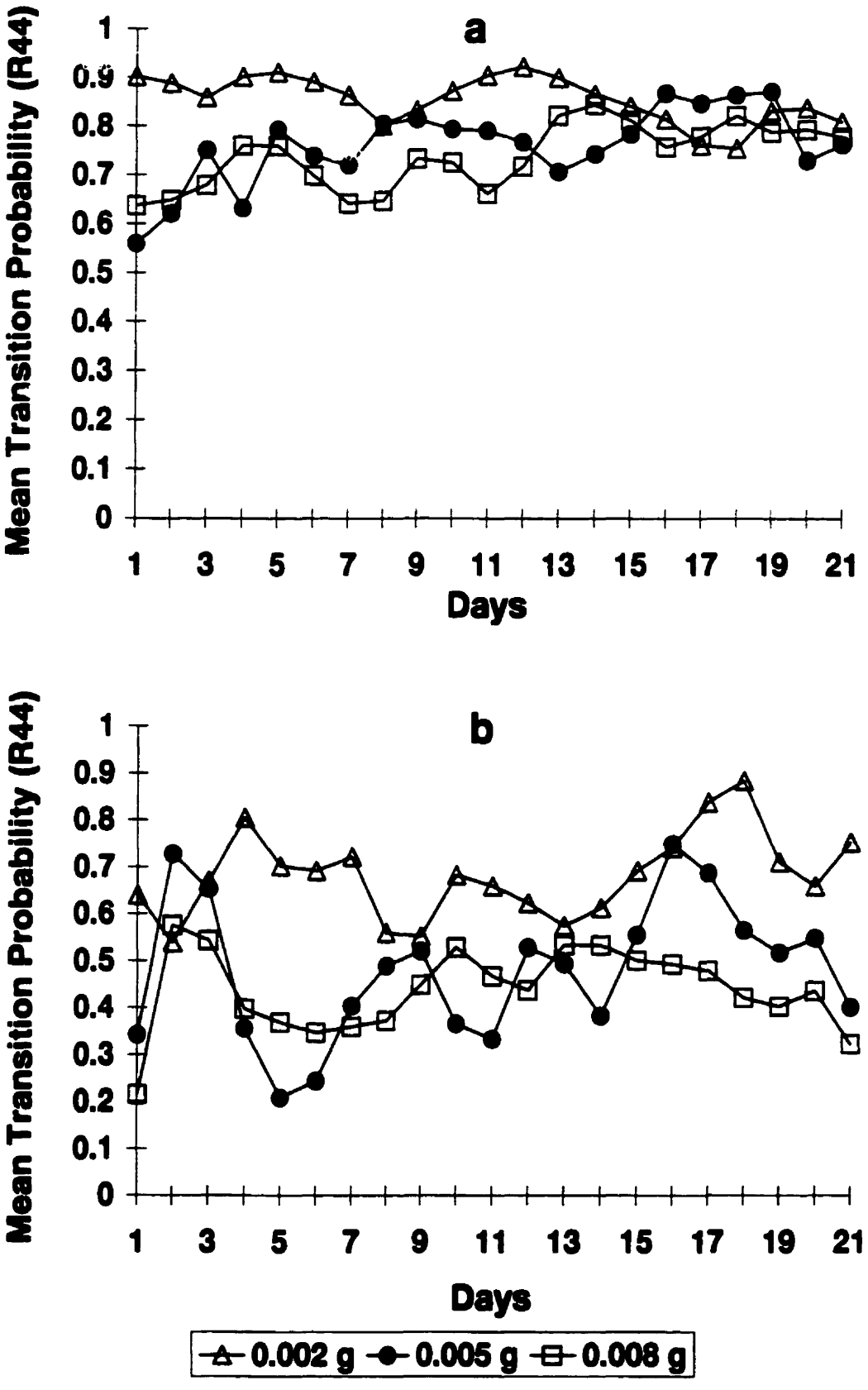
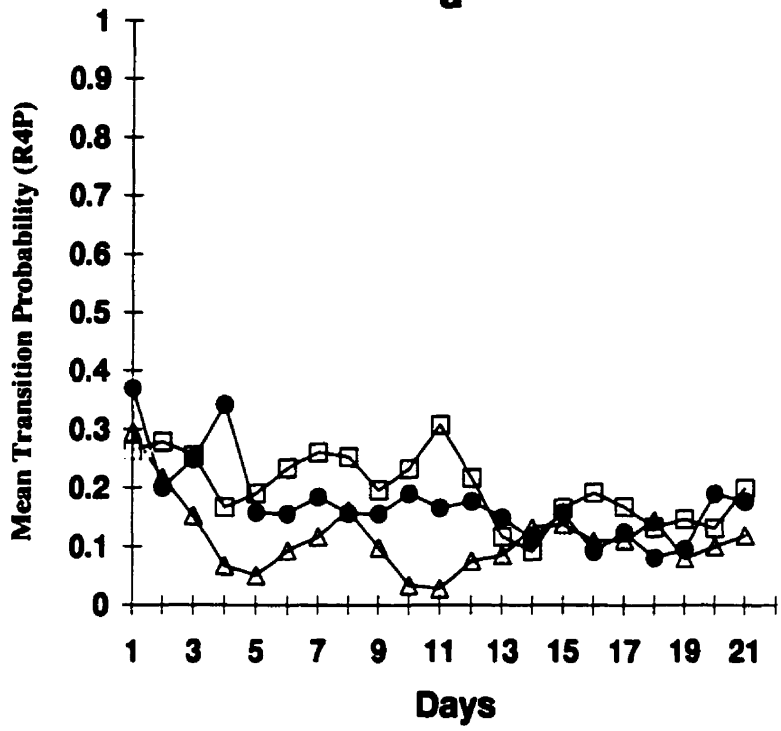

a
Mean Transition Probability (R44)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (R44)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.005 g
0.008 g

Figure 39: **Food and parasite interactive effects transition probabilities of fourth instar *Aedes aegypti* larvae to become pupae within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

a


Mean Transition Probability (R4P)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days

b

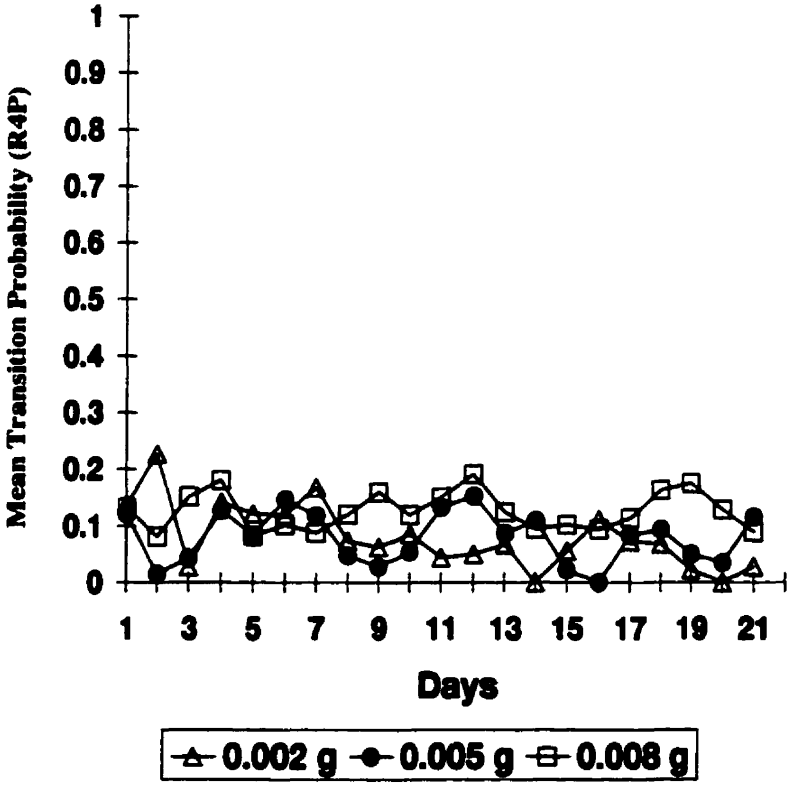

Mean Transition Probability (R4P)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.005 g
0.008 g

Figure 40: **Food and parasite interactive effects transition probabilities of fourth instar *Aedes aegypti* larvae to become adults within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

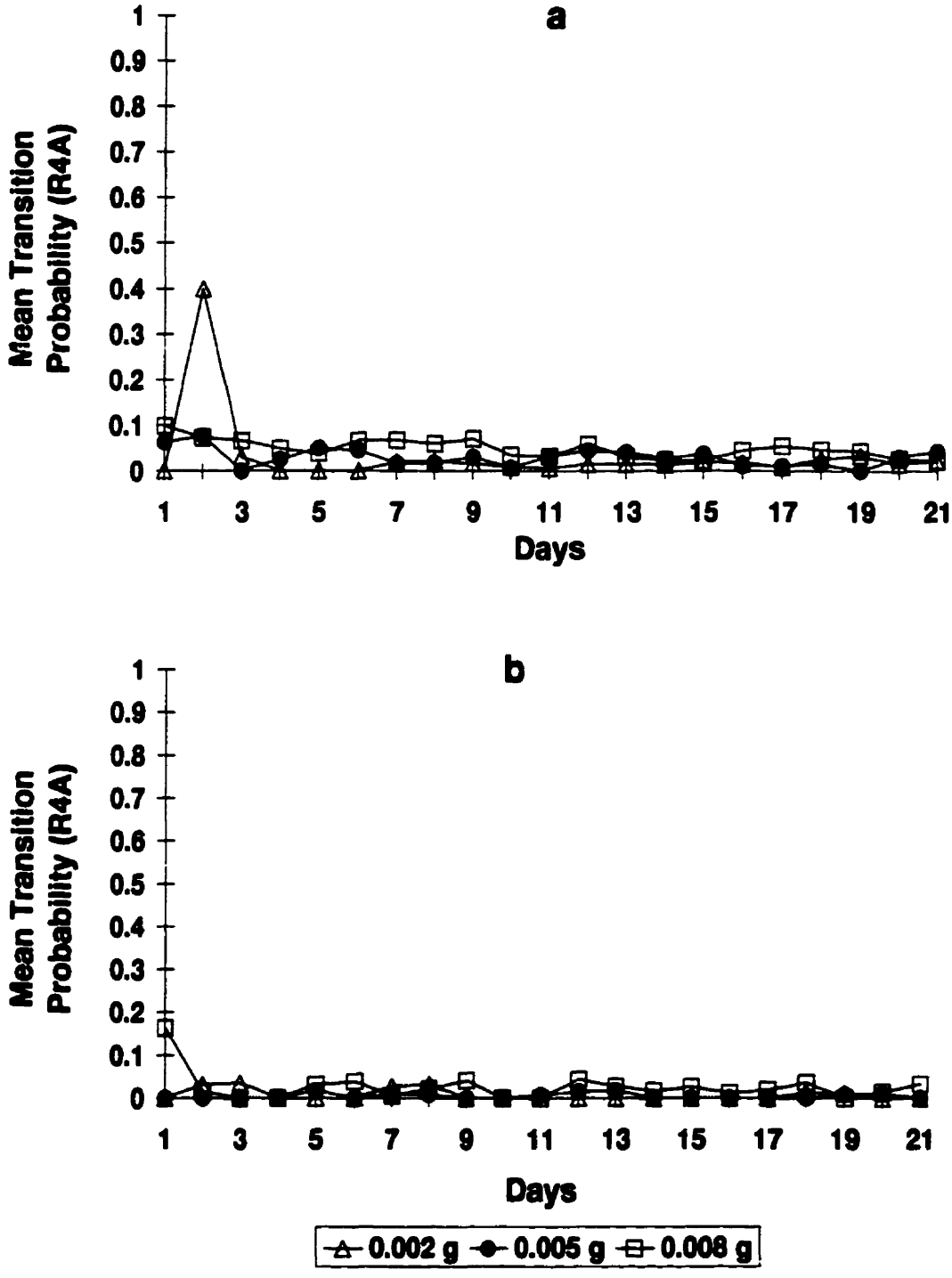

a
Mean Transition Probability (R4A)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (R4A)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.005 g
0.008 g

Figure 41: **Food and parasite interactive effects transition probabilities of fourth instar *Aedes aegypti* larvae to die within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

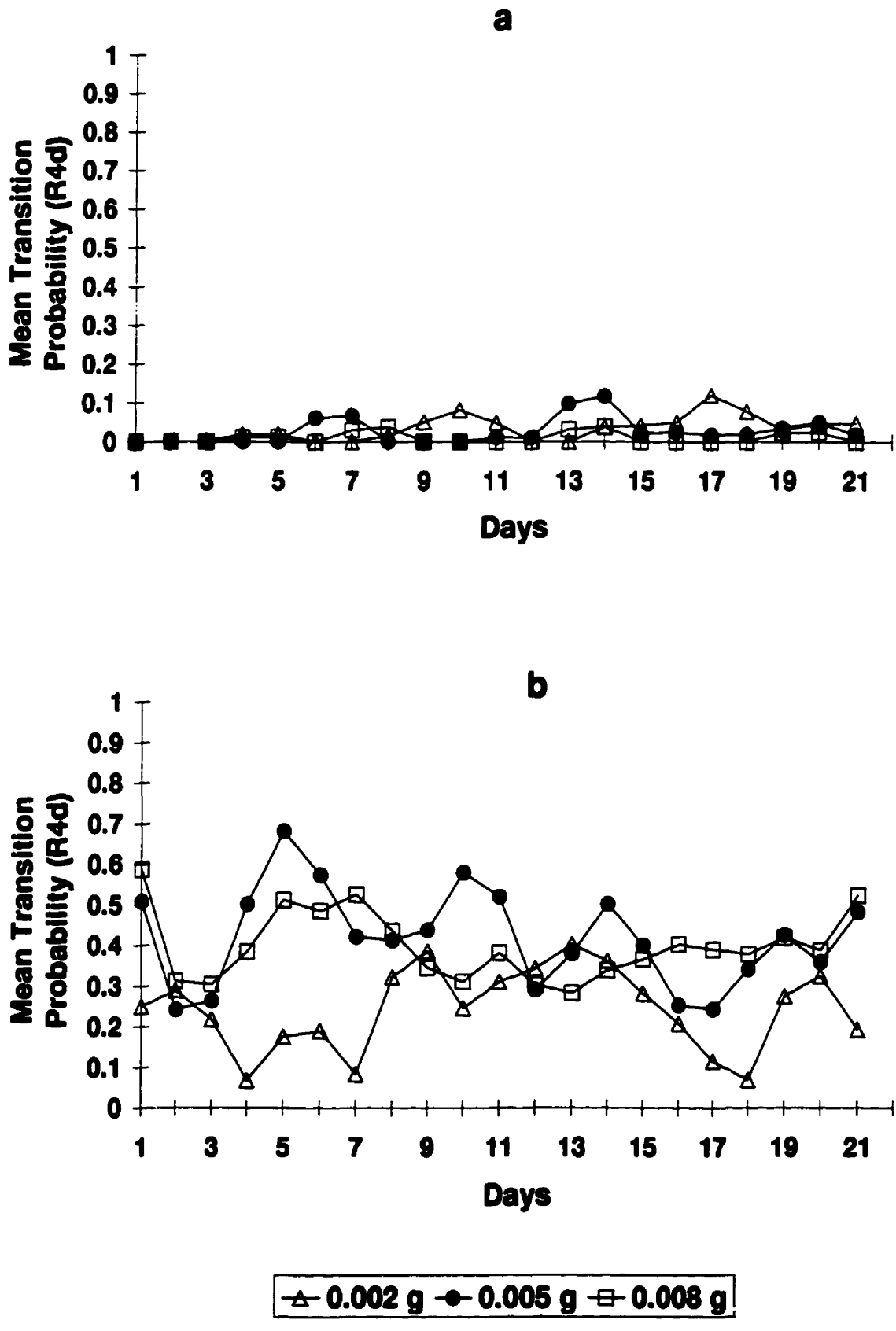

a
Mean Transition Probability (R4d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
b
Mean Transition Probability (R4d)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21
Days
0.002 g
0.005 g
0.008 g

Figure 42: **Food and parasite interactive effects transition probabilities of *Aedes aegypti* pupae to remain pupae within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**

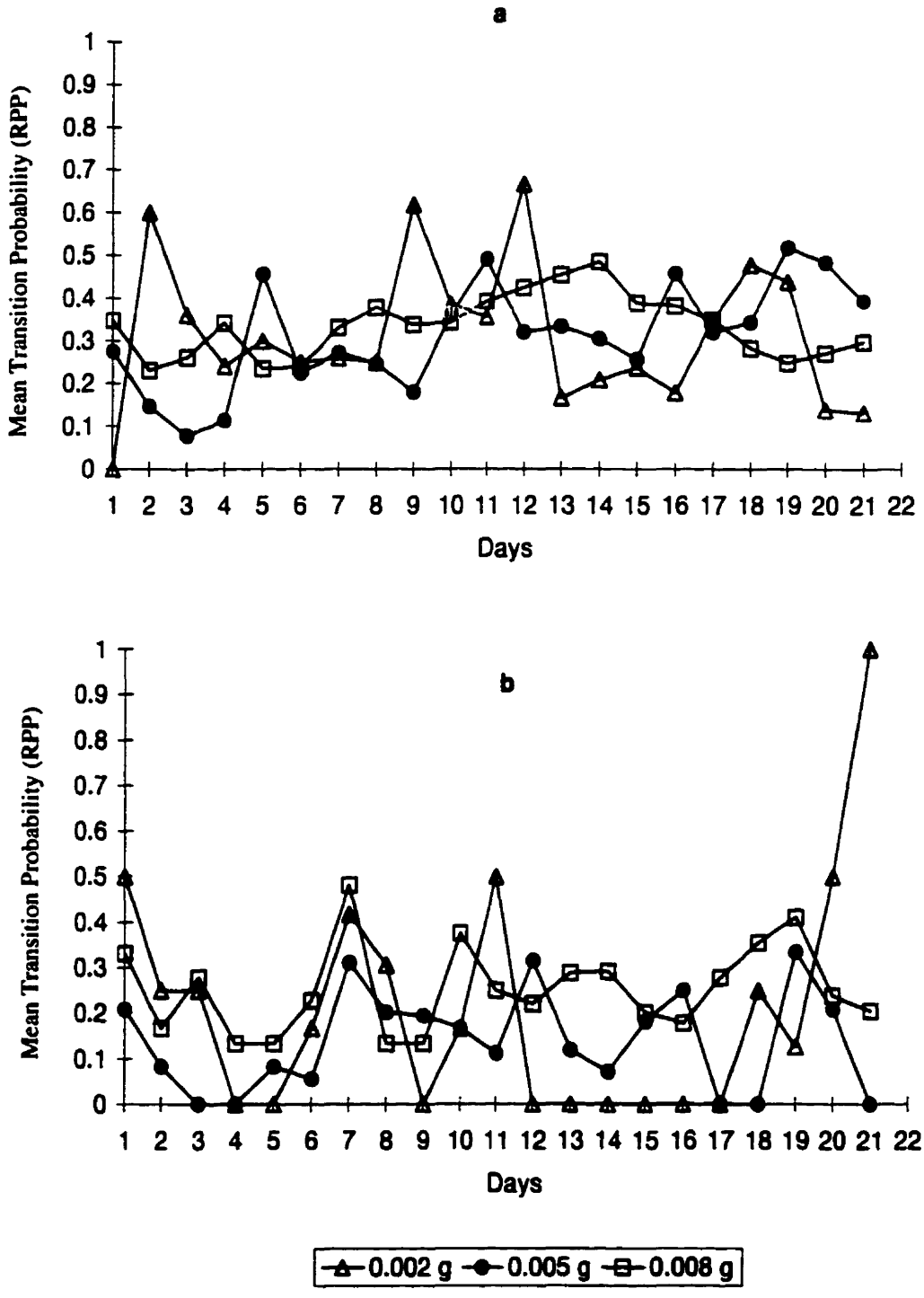
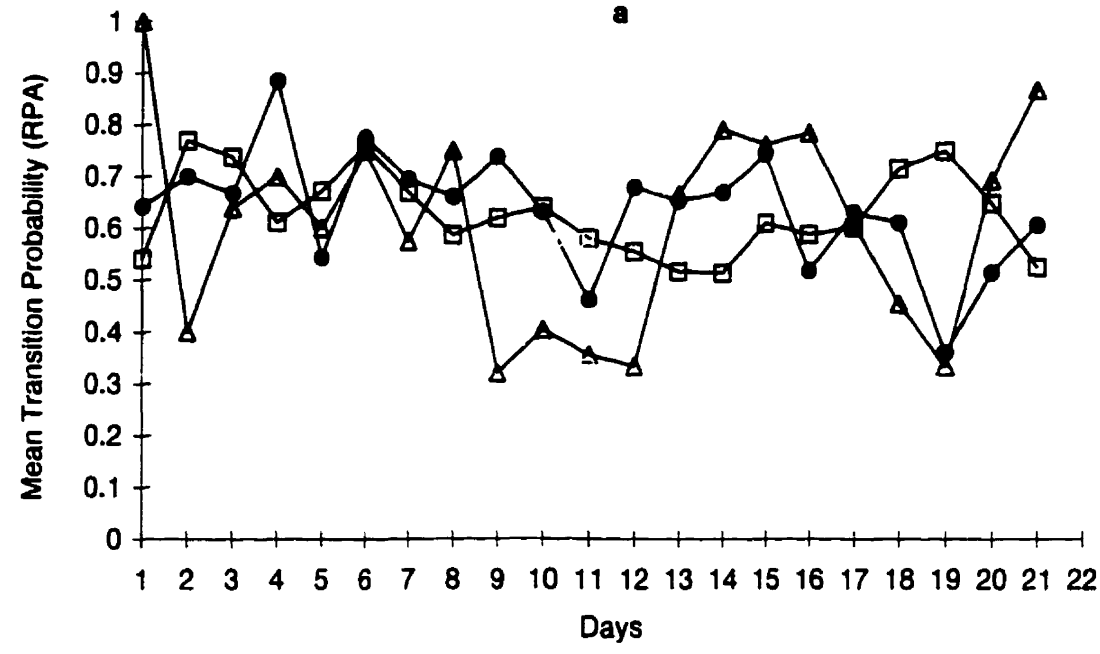

a
Mean Transition Probability (RPP)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
b
Mean Transition Probability (RPP)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
0.002 g
0.005 g
0.008 g

Figure 43: **Food and parasite interactive effects transition probability of *Aedes aegypti* pupae to become adults within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (RPA)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days

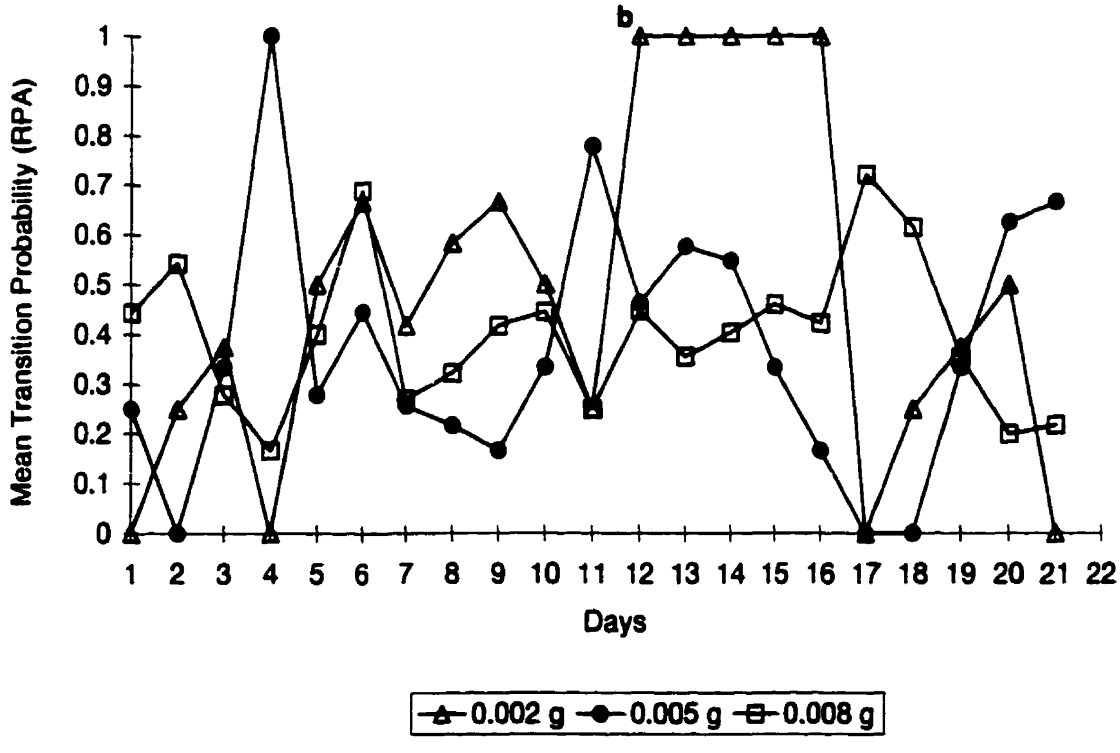
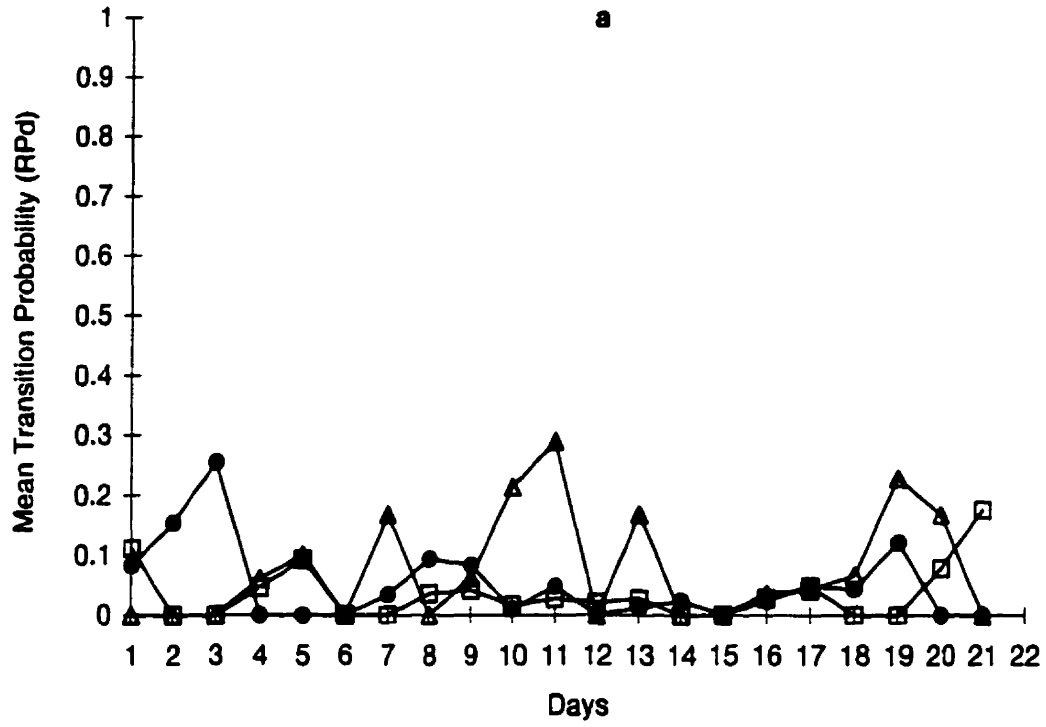

b
Mean Transition Probability (RPA)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
0.002 g
0.005 g
0.008 g

Figure 44: **Food and parasite interactive effects transition probability of *Aedes aegypti* pupae to die within 48 hours: (a) control, (b) with *Plagiorchis elegans* (2 cercariae/ml)**


a
Mean Transition Probability (RPd)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days

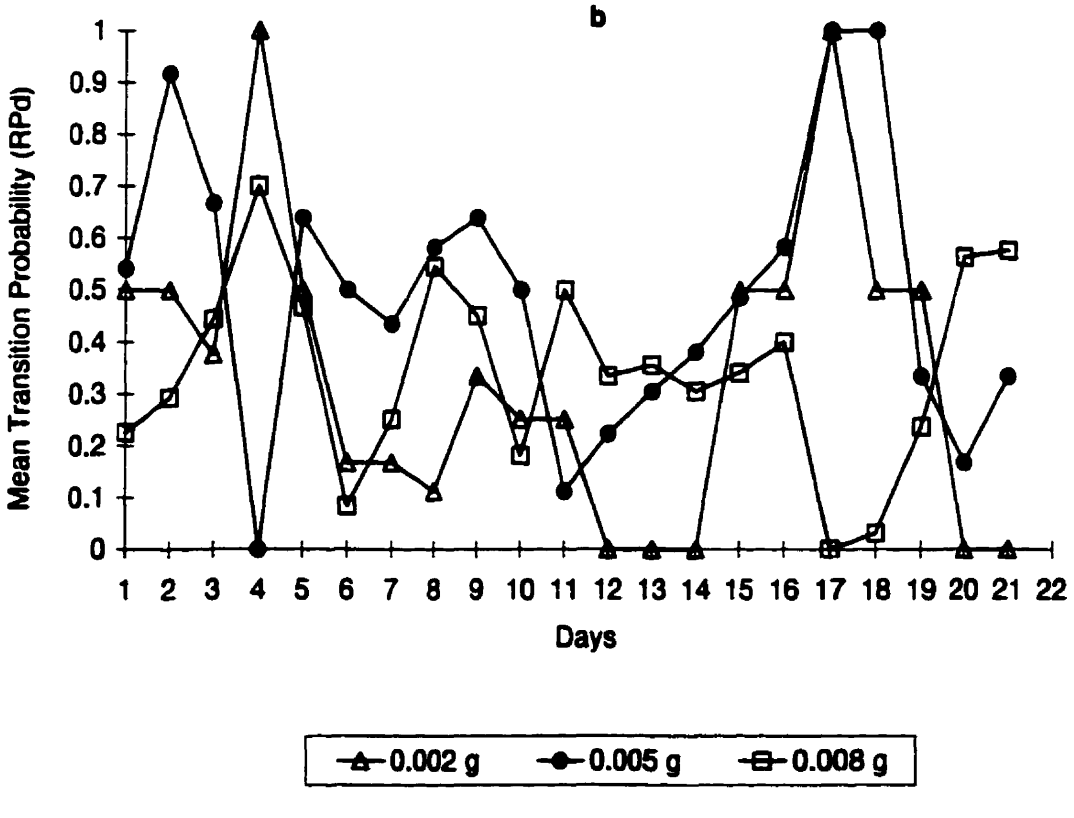

b
Mean Transition Probability (RPd)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Days
0.002 g
0.005 g
0.008 g

Figure 45. Population Structure of *Aedes aegypti* at 0.001 g of Food/450ml: (a) Control, (b) With *Plagiorchis elegans* cercariae

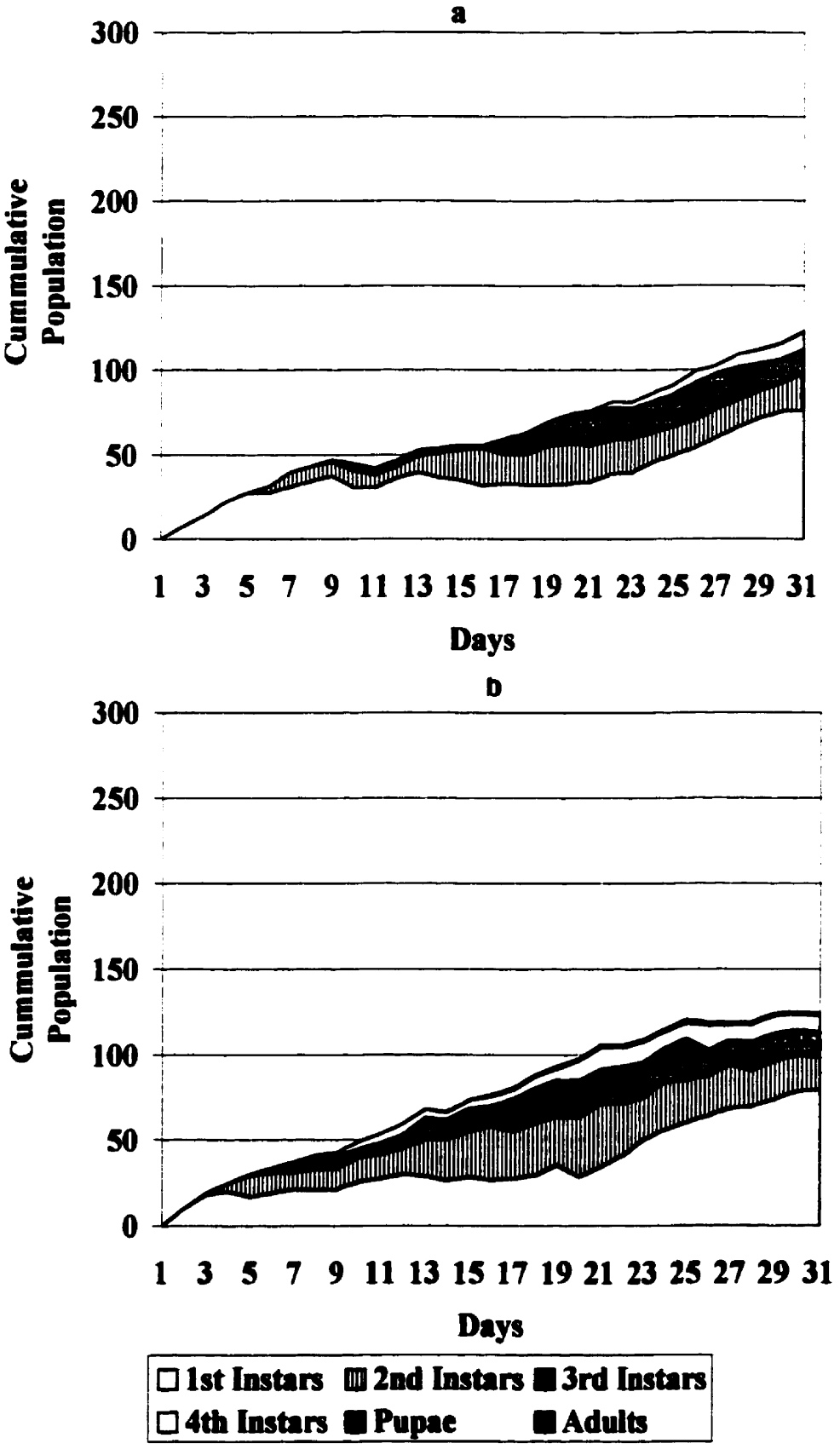

a
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
b
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
1st Instars
2nd Instars
3rd Instars
4th Instars
Pupae
Adults

Figure 46. Population Structure of *Aedes aegypti* at 0.002 g of Food/450ml: (a) Control, (b) With *Plagiorchis elegans* cercariae

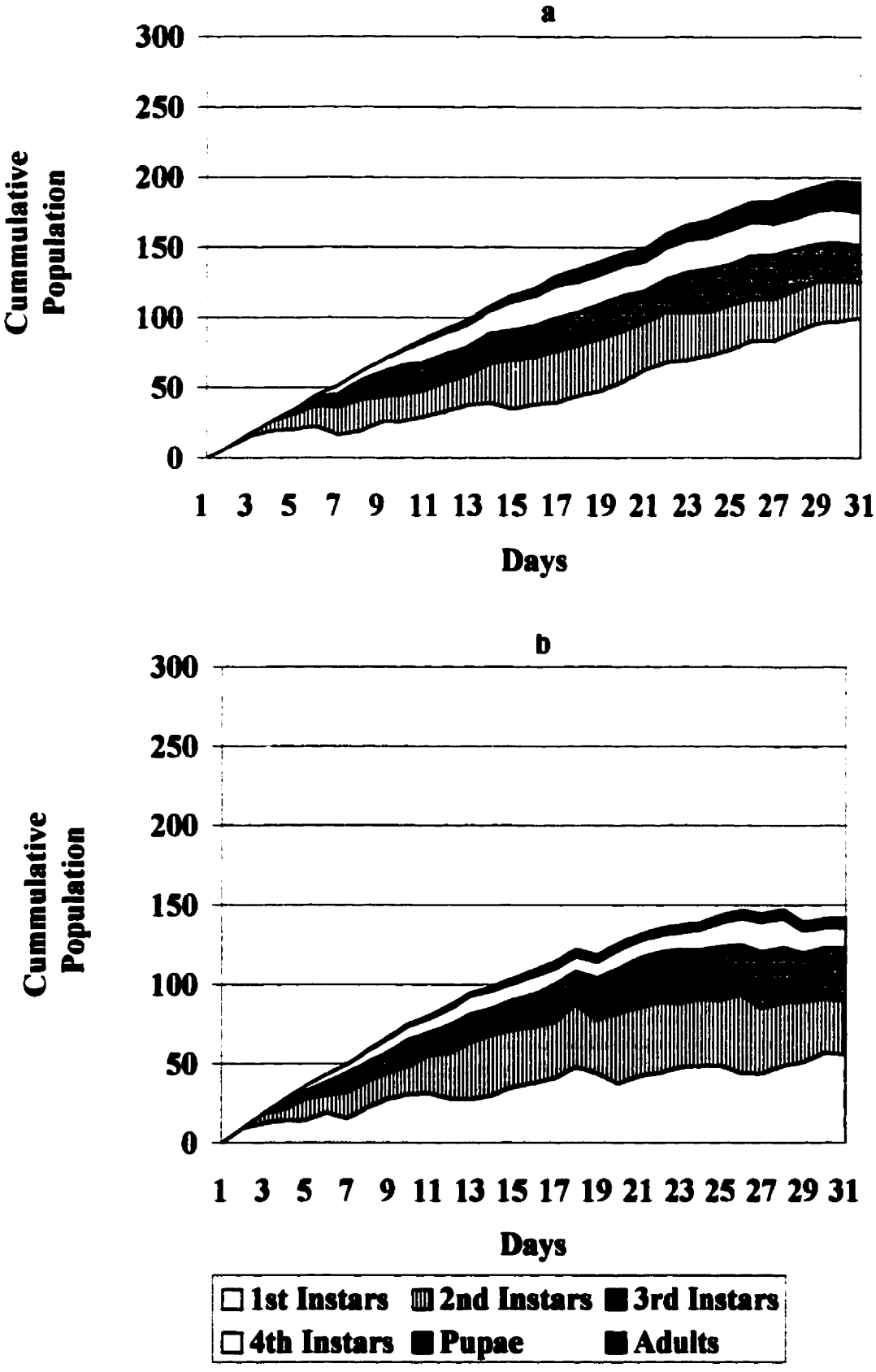

a
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
b
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
1st Instars
2nd Instars
3rd Instars
4th Instars
Pupae
Adults

Figure 47. Population Structure of *Aedes aegypti* at 0.003 g of Food/450ml: (a) Control, (b) With *Plagiorchis elegans* cercariae

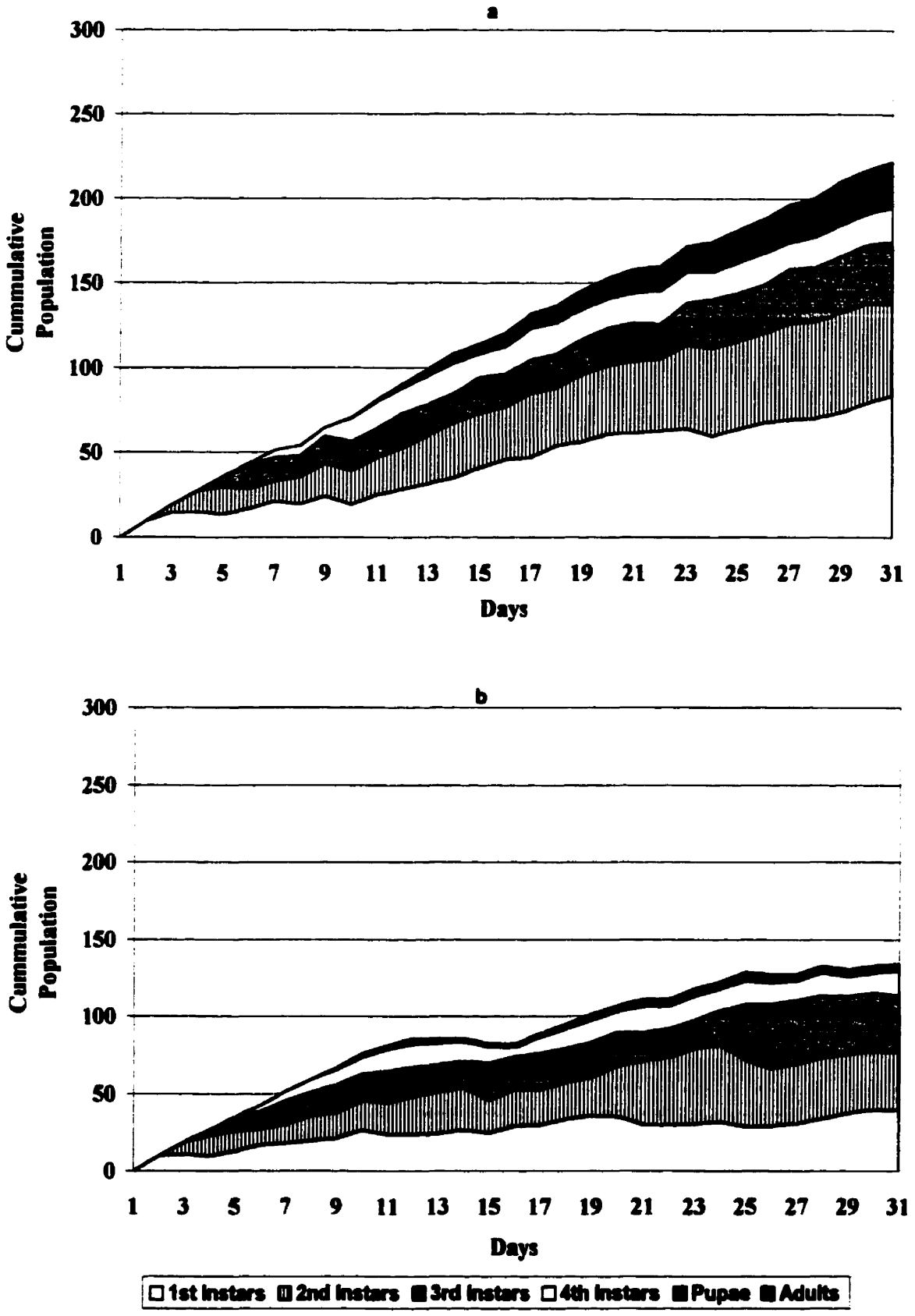

a
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
b
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
1st Instars
2nd Instars
3rd Instars
4th Instars
Pupae
Adults

Figure 48. Population Structure of *Aedes aegypti* at 0.005 g of Food/450ml: (a) Control, (b) With *Plagiorchis elegans* cercariae

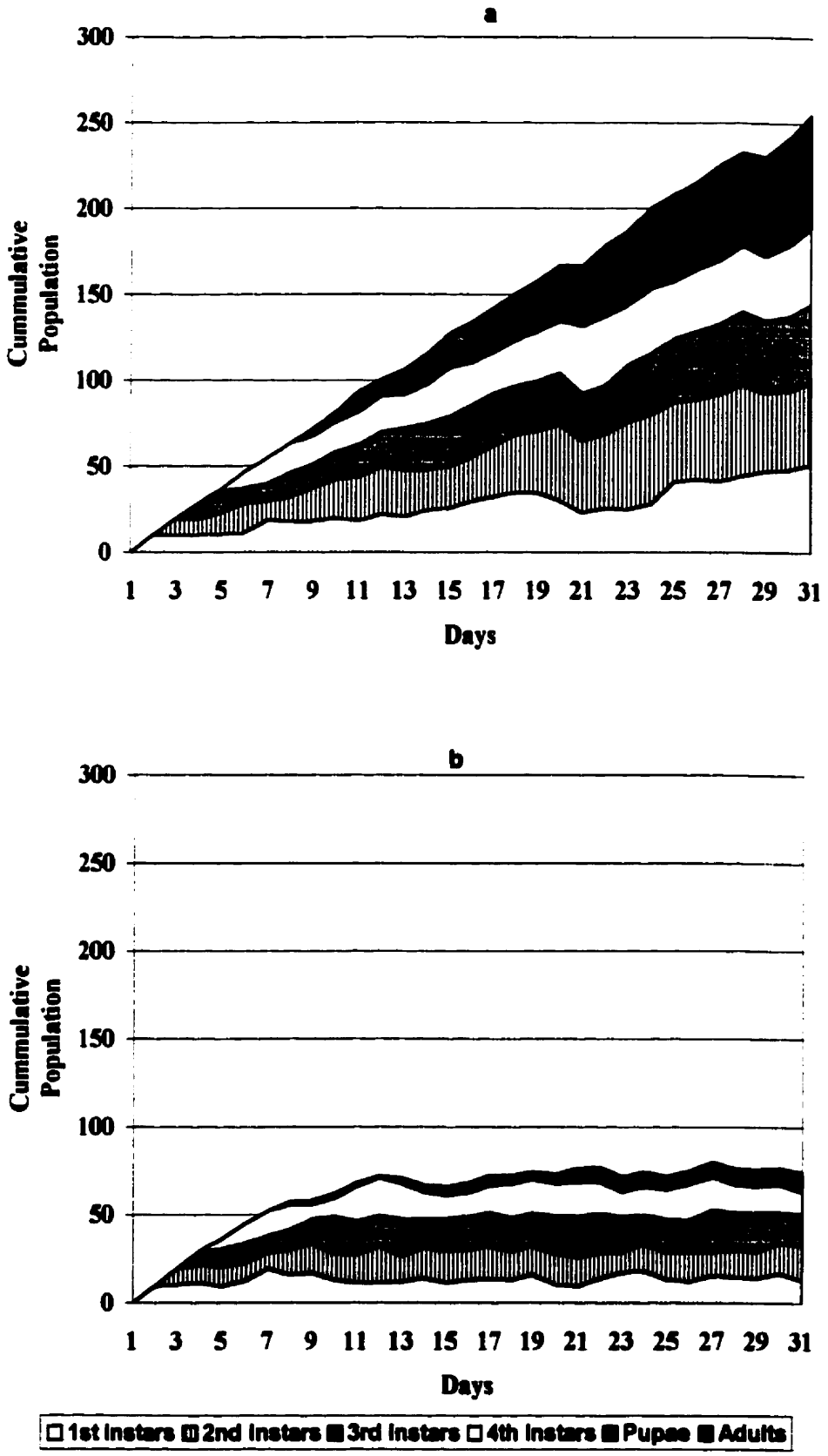

a
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
b
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
1st Instars
2nd Instars
3rd Instars
4th Instars
Pupae
Adults

Figure 49. Population Structure of *Aedes aegypti* at 0.008 g of Food/450ml: (a) Control, (b) With *Plagiorchis elegans* cercariae

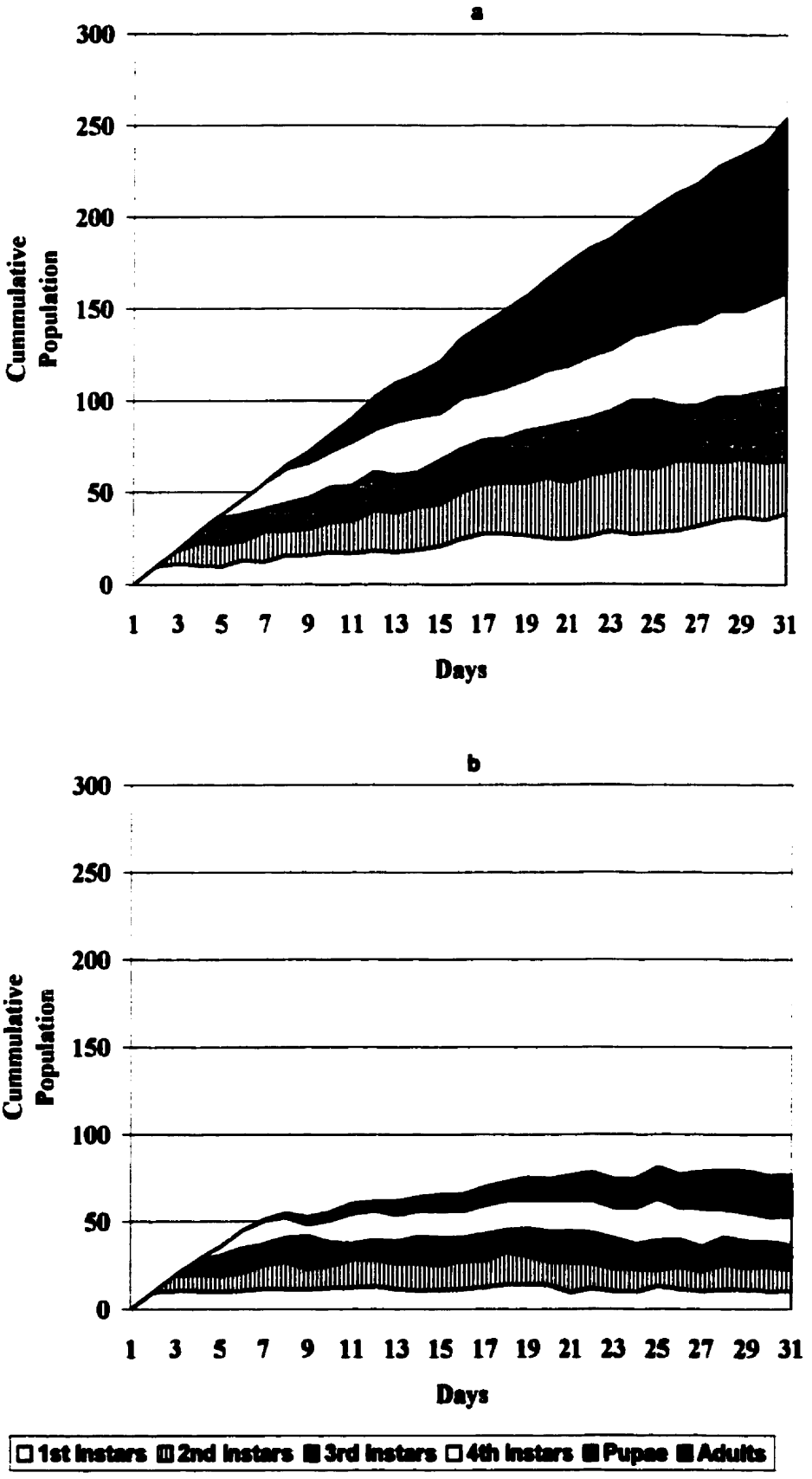

a
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
b
300
250
200
150
100
50
0
Cummulative Population
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
1st Instars
2nd Instars
3rd Instars
4th Instars
Pupae
Adults

Table 1

Tests of Between-Subjects and Within-Subject Effects for Time-Dependent Transition Probabilities Over 24 Hours, Using Repeated Measures Analysis of Variance

Effect	Prob > F		
	first instar	second instar	third instar
	(P11)	(P22)	(P33)
FOOD	0.0001 ***	0.0001 ***	0.0001 ***
PARASITE	0.0001 ***	0.0001 ***	0.0001 ***
FOOD*PARASITE	0.0001 ***	0.0002 ***	0.0001 ***
TIME	0.0001 ***	0.0001 ***	0.0001 ***
TIME*FOOD	0.1882 ns	0.0428 *	0.0156 *
TIME*PARASITE	0.0461 *	0.0784 ns	0.219 ns
TIME*FOOD*PARASITE	0.0587 ns	0.0392 *	0.2608 ns
Greenhouse-Geisser Epsilon	0.3409	0.4172	0.336
	(P12)	(P23)	(P34)
FOOD	0.0001 ***	0.0001 ***	0.0001 ***
PARASITE	0.0001 ***	0.0001 ***	0.0001 ***
FOOD*PARASITE	0.0001 ***	0.0002 ***	0.0005 ***
TIME	0.0001 ***	0.0001 ***	0.0001 ***
TIME*FOOD	0.0494 *	0.0225 *	0.059 ns
TIME*PARASITE	0.0734 ns	0.0435 *	0.294 ns
TIME*FOOD*PARASITE	0.1688 ns	0.0449 *	0.1817 ns
Greenhouse-Geisser Epsilon	0.3791	0.3804	0.3096
	(P1d)	(P2d)	(P3d)
FOOD	0.0001 ***	0.0011***	0.0019 **
PARASITE	0.0001 ***	0.5687 ns	0.0001 ***
FOOD*PARASITE	0.0001 ***	0.6994 ns	0.0001 ***
TIME	0.3631 ns	0.3619 ns	0.5897 ns
TIME*FOOD	0.0504 ns	0.4879 ns	0.3731 ns
TIME*PARASITE	0.0486 *	0.3588 ns	0.4106 ns
TIME*FOOD*PARASITE	0.092 ns	0.712 ns	0.4761 ns
Greenhouse-Geisser Epsilon	0.3457	0.1678	0.1981

ns = non significance
* = significance at p = 0.05
** = significance at p = 0.01
*** = significance at p = 0.001

Table 2

Tests of Between-Subjects and Within-Subject Effects for Time-Dependent Transition Probabilities Over 48 Hours, Using Repeated Measures Analysis of Variance

Effect	Prob > F				
	first instar	second instar	third instar	fourth instar	pupa
	(R11)	(R22)	(R33)	(R44)	(RPP)
FOOD	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.1513 ns
PARASITE	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.0007 ***
FOOD*PARASITE	0.0228 *	0.0002 ***	0.0003 ***	0.0661 ns	0.3506 ns
TIME	0.0001 ***	0.0001 ***	0.0001 ***	0.0536 ns	0.0503 ns
TIME*FOOD	0.2462 ns	0.1732 ns	0.0222 *	0.1421 ns	0.0399 *
TIME*PARASITE	0.0013 ***	0.035 *	0.1807 ns	0.2035 ns	0.0256 *
TIME*FOOD*PARASITE	0.0341 *	0.3668 ns	0.7582 ns	0.1906 ns	0.002**
Greenhouse-Geisser Epsilon	0.2677	0.3294	0.3636	0.2994	0.3302
	(R12)	(R23)	(R34)	(R4P)	(RPA)
FOOD	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.3038 ns
PARASITE	0.041 *	0.0001 ***	0.0007 ***	0.0001 ***	0.0001 ***
FOOD*PARASITE	0.2199 ns	0.0049 **	0.0157 *	0.0201 *	0.0877 ns
TIME	0.0001 ***	0.0099 **	0.0001 ***	0.0018 **	0.0469 *
TIME*FOOD	0.0344 *	0.1841 ns	0.2322 ns	0.0725 ns	0.0005 ***
TIME*PARASITE	0.0321 *	0.0025 **	0.1372 ns	0.0975 ns	0.0178 *
TIME*FOOD*PARASITE	0.0494 *	0.5129 ns	0.2731 ns	0.1047 ns	0.0006 ***
Greenhouse-Geisser Epsilon	0.3708	0.3639	0.3609	0.3012	0.2922
	(R13)	(R24)	(R3P)	(R4A)	(RPd)
FOOD	0.0001 ***	0.0001 ***	0.0001 ***	0.0005 ***	0.0882 ns
PARASITE	0.0001 ***	0.0002 ***	0.9924 ns	0.0001 ***	0.0001 ***
FOOD*PARASITE	0.0003**	0.0031 **	0.5015 ns	0.9297 ns	0.0571 ns
TIME	0.0061 **	0.0038 **	0.1344 ns	0.0078 **	0.062 ns
TIME*FOOD	0.0792 ns	0.3859 ns	0.2369 ns	0.0089 **	0.0033 **
TIME*PARASITE	0.2299 ns	0.4911 ns	0.5603 ns	0.0265 *	0.056 ns
TIME*FOOD*PARASITE	0.1737 ns	0.5439 ns	0.4026 ns	0.0621 ns	0.0155 *
Greenhouse-Geisser Epsilon	0.1806	0.1866	0.2284	0.1268	0.2533

Table 2 (cont...)

Effect	Prob > F				
	first instar	second instar	third instar	fourth instar	pupa
	(R13)	(R24)	(R3P)	(R4A)	(RPd)
FOOD	0.0001 ***	0.0001 ***	0.0001 ***	0.0005 ***	0.0882 ns
PARASITE	0.0001 ***	0.0002 ***	0.9924 ns	0.0001 ***	0.0001 ***
FOOD*PARASITE	0.0003**	0.0031 **	0.5015 ns	0.9297 ns	0.0571 ns
TIME	0.0061 **	0.0038 **	0.1344 ns	0.0078 **	0.062 ns
TIME*FOOD	0.0792 ns	0.3859 ns	0.2369 ns	0.0089 **	0.0033 **
TIME*PARASITE	0.2299 ns	0.4911 ns	0.5603 ns	0.0265 *	0.056 ns
TIME*FOOD*PARASITE	0.1737 ns	0.5439 ns	0.4026 ns	0.0621 ns	0.0155 *
Greenhouse-Geisser Epsilon	0.1806	0.1866	0.2284	0.1268	0.2533
	(R1d)	(R2d)	(R3d)	(R4d)	
FOOD	0.0003 ***	0.002 **	0.0553 ns	0.0112 **	
PARASITE	0.0001 ***	0.0137 *	0.0001 ***	0.0001 ***	
FOOD*PARASITE	0.0054 **	0.2226 ns	0.0012 **	0.004 **	
TIME	0.0667 ns	0.0864 ns	0.4348 ns	0.2962 ns	
TIME*FOOD	0.1394 ns	0.2399 ns	0.1737 ns	0.3339 ns	
TIME*PARASITE	0.0258 *	0.2836 ns	0.2228 ns	0.286 ns	
TIME*FOOD*PARASITE	0.0766 ns	0.3387 ns	0.3184 ns	0.279 ns	
Greenhouse-Geisser Epsilon	0.2902	0.2094	0.2539	0.2115	

For notations, see Table 1

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