EXPERIMENTAL AND THEORETICAL STUDIES ON ASPECTS OF THE LIFE HISTORIES OF SOME MOSQUITOES

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EXPERIMENTAL AND THEORETICAL STUDIES ON ASPECTS OF THE LIFE HISTORIES OF SOME MOSQUITOES

Abstract

Three laboratory investigations concerning the life history of *Aedes togoi* (Theobald), and the results of a theoretical study of mating and blood feeding behaviour in anopheline mosquitoes are reported. Components of the life history of *Ae. togoi* that were examined included adult female survival, the effects of increasingly dense larval populations on some larval and adult attributes, and the effects of other *Ae. togoi* eggs on selection of oviposition site.

Large- or medium-sized adult females were provided with 15% sucrose solution at 2 different feeding rates, *ad libitum* or every other day, and a third group was starved to investigate the effects of size on survival at each feeding rate. Both feeding rate and size had a significant effect on survival, however, there was no interaction between these 2 factors.

Larvae were reared at 50, 200, and 400 per 200 ml of rearing medium to study the effects of larval density on some larval and adult attributes. There was significant larval mortality at the highest density, pre-adult development was always prolonged as density increased, and adult size decreased non-uniformly. The possible effects of these differences on the bionomics of this species are discussed.

Single females provided with 3 water-filled cups containing conspecific eggs and 3 cups with no eggs in a choice experiment, oviposited in twice as many cups containing no eggs as in cups with eggs. There was no statistically significant difference between the number of eggs laid in cups with or without eggs already present. However, most females laid eggs in 2 or more cups rather than depositing all their eggs in one cup. "Risk-spreading", by laying eggs in 2 or more sites, could improve the chances of offspring survival.

The effect of various host and mate densities, and the sequence in which female mosquitoes mate and blood feed on female mosquito survival was modelled in 2 groups of mosquitoes. One group, assumed to mate before blood feeding, was named deterministic, and the other, which could either blood feed or mate first, opportunistic. The opportunistic mosquitoes had higher relative survival under each of the conditions tested. The results are discussed in light of current knowledge of these behaviours.

Dedication

to Naomi, Joseph, Nda and Sunday.

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Chapter 1

Introduction

An animal that specializes to exploit one particular resource may have a competitive advantage over its generalist competitors because the specialist generally is better adapted to utilize the resource (Pianka 1994). On the basis of blood-host and oviposition site preference, Bentley and Day (1989) classified mosquitoes into 2 groups as follows: (i) opportunistic, and (ii) specialists. Opportunistic species, for example, *Culex nigripalpus* (Theobald), oviposit in almost any type of water, while specialists, on the other hand, only breed in certain restricted sites (Bentley and Day 1989).

The mosquito, *Aedes togoi* (Theobald), appears to be an oviposition specialist. Pre-adult development is restricted to shallow, brackish or saline rock pools on the Pacific Coast of North America (Trimble and Wellington 1979; Belton 1980; Belton and Belton 1990). Females often bite humans and breed just above the high tide mark, along the rocky coast, but neither bite nor breed further inland (Belton 1980; Belton and Belton 1990). Curiously, however, the larvae of this species can survive a wide range of salinities, the maximum being equivalent to sea water concentration (Lien 1960). Thus, its restriction to rock pools is likely determined by female selection of oviposition sites rather than a strict larval breeding site requirement.

Unlike opportunistic species that tend to be broadly distributed (Bentley and Day 1989), and like most specialists (Pianka 1994), the distribution of this species is restricted by the availability of its resource, in this case, breeding sites. Moreover, suitable breeding sites along the Pacific Coast are frequently isolated from other rock pools by several kilometers of sand or gravel or open sea (Belton 1980). Accordingly, it seems feasible that, like *Ae. atropalpus* (Coquillett) (Kalpage and Brust 1973), successive generations of females oviposit in the same rock pools repeatedly; in fact, the rock pools at Lighthouse Park, West Vancouver, (49°20'N., 123°15'W.) have continuously held larvae for approximately 20 years (P. Belton personal communication). Consequently, larval populations may build up to high densities, resulting in intraspecific competition, which, in turn, may lead to increased mortality of larvae, prolonged larval development, and small-sized adults that are less successful at blood feeding, and, consequently, lay fewer eggs (Peters and Barbosa 1977; Fish 1985; Nasci 1986, 1987, 1990; Renshaw *et al.* 1994). Moreover, the occurrence of autogeny, the ability to produce a first batch of eggs without a blood meal, in female *Ae. togoi* (Lien 1960), greatly depends on larval nourishment, which, in part, depends on larval population density (Sota and Mogi 1994). These problems fit under the rubric of life history theory; this specialist mosquito benefits from its ability to tolerate salt water, and probably owes its widespread distribution by ship to this ability (Belton and Belton 1990). However, its special breeding site requirements may restrict its dispersal and, consequently, affect quality of breeding habitats.

A major goal in the study of life histories is to detect and analyze differences between individuals, these differences usually concerning survival or fecundity or both and, consequently, fitness (Lebreton *et al.* 1992). This thesis approaches this goal by examining 3 aspects of the biology and behaviour of *Ae. togoi*, namely, adult female survival, the effects of larval habitat quality (based on larval density) on some attributes of this mosquito, and oviposition site selection in response to the presence of conspecifics. In addition, Chapter 5 examines the feeding and mating strategies of mosquitoes of the genus *Anopheles*. Ultimately, the issues addressed herein concern differential fitness among members of a population of these mosquitoes.

Chapter 2 is a survival study. Adult survival is perhaps the most important factor affecting the stability of mosquito populations (Miller *et al.* 1973). Accordingly, longevity of females from larval habitats of different qualities (as determined by female size) has received much attention recently. However, reports in the literature regarding survival of females of different sizes are conflicting; some authors find that large, well nourished females from good quality habitats survive longer than do small, malnourished females from poor ones (Hawley 1985; Nasci 1987; Renshaw *et al.* 1994), while others find no relationship between size and survival (Walker *et al.* 1987; Landry *et al.* 1988). The present study investigated whether survival differed in caged female *Ae. togoi* of different sizes, maintained at different feeding rates as might occur in nature.

The observations in Chapter 2 on the effects of larval density on some attributes of *Ae. togoi*, led to the design of the experiments in Chapter 3. By rearing larvae at increasing densities, larval habitat quality was varied to investigate their response to such stress.

The effects of increasingly dense larval populations observed in Chapter 3 raised questions about how gravid females respond to the presence of conspecifics. Do they select oviposition sites that already contain eggs over sites that do not? If this is the case, then the presence of eggs may somehow indicate to gravid females that such sites can support offspring (Blaustein and Kotler 1993). On the other hand, the presence of eggs might indicate that offspring will face intraspecific competition (Roitberg and Prokopy 1982). These hypotheses are described and tested in Chapter 4.

Chapter 5 reports the results of a theoretical model of mating and blood feeding behaviour in anopheline mosquitoes. After emergence, a female anopheline must obtain carbohydrate (usually in the form of plant nectar) for energy, then mate, seek and obtain a blood meal for developing her eggs, and find an oviposition site at which to lay these eggs. Clearly, resources (such as, energy and time) allocated to one activity are not available to be spent on another (Roitberg and Friend 1992). Thus, mosquitoes face trade-offs. The objective of this chapter, therefore, was to investigate theoretically, whether the sequence in which mating and blood feeding activities are performed may affect a hypothetical attribute, such as survival.

The Biology of *Aedes togoi*

Ae. togoi is widely distributed in Asia, for example, Japan, Hong Kong, China, Korea, Siberia, Malaysia, Thailand, and several other parts of Asia (Lien 1960; Trimble and Wellington 1979; Belton 1980; Sota and Mogi 1994). This mosquito also occurs on the Pacific Coast of North America, where it was probably introduced from Japan (Belton and Belton 1990; Sota and Mogi 1994). The larvae of *Ae. togoi* can tolerate a wide range of salinities, the maximum being equivalent to sea water concentration (Lien 1960). Belton and Belton (1990) suggested that such tolerance of sea water may have facilitated the widespread distribution of this species by ship.

Pre-adult stages develop in saline rock pools above the high tide mark on the Pacific Coast (Trimble and Wellington 1979; Belton and Belton 1990). Autogeny can occur in females (Lien 1960), but to produce subsequent batches of eggs, they obtain blood meals from humans visiting beaches near larval habitats (Belton and Belton 1990), or from other vertebrates (Sota and Mogi 1994). *Ae. togoi* is multivoltine and may overwinter in the egg or larval stage (Sota and Mogi 1994). However, much of the biology of this mosquito in North America is not known (Belton and Belton 1990).

Chapter 2

The effects of size and feeding rate on survival of female *Aedes togoi*

Introduction

An understanding of the population dynamics of any organism requires a knowledge of its survival rates (Lebreton *et al.* 1992). Miller *et al.* (1973) suggested that adult survival is the most important factor affecting stability of mosquito populations and total egg production. In addition, where disease transmission to other animals is involved, the infected female mosquito must survive the developmental period of the parasite to the infective stage and obtain a subsequent blood meal to transmit the parasite (Clements and Paterson 1981).

Several abiotic and biotic factors affect the longevity of animals. They include: temperature, humidity, parasites, predators, diet, and genotype (Lebreton *et al.* 1992). In mosquitoes, the effects of female size on several fitness and bionomic factors have received much attention recently. Though adult body size is partly genetically determined, the quality of the breeding habitat of immature stages greatly influences adult size as well (Nasci 1990). The extent of variation in the body size of individuals in a population depends on the quality of the larval habitat, so that species breeding in rapidly changing habitats, for example, tree holes, flood water, and man-made containers, show a greater variation in adult body size than do species breeding in more stable habitats, such as swamps, and lake margins (Fish 1985; Nasci 1987). Size, in turn, affects the fitness of mosquitoes in several ways. Accordingly, large females are more likely to engage in host seeking behaviour than are small individuals (Klowden *et al.* 1988). Consequently, large females of *Aedes aegypti* (L.), for example, are more successful than are small females at host finding and blood feeding (Nasci 1986, 1990), in part, because small females show less biting persistence than do large females when disturbed by defensive behaviour of the host (Nasci 1991). In addition, fecundity (number of eggs per batch) increases with female size (Briegel 1990, Lyimo and Takken 1993, Renshaw *et al.* 1994).

Perhaps the most important influence of adult size is on survival. However, reports on the relationship between female size and survival are conflicting. Some studies have shown that large females live longer than small ones (Haramis 198`; Hawley 1985; Nasci 1987; Packer and Corbet 1989; Renshaw *et al.* 1994), but others show that survival is size-independent (Walker *et al.* 1987; Landry *et al.* 1988; Pumpuni and Walker 1989). Some of the aforementioned studies estimated survival by correlating the age (based on parous rates, that is, number of oviposition cycles completed) of females attracted to a human bait in the field with size. Parous rates can be determined in field-caught mosquitoes because, for each gonotrophic cycle (the closely related feeding and reproductive cycle of female mosquitoes), a bead-like dilatation is formed in the ovarioles (Detinova 1962; Clements and Paterson 1981). Thus, the more dilatations a female has, the older it is. Therefore, a lower parous rate in small mosquitoes is an indication of decreased survival (Davidson 1954). However, parous rates provide little information on other important variables, such as adult nourishment, that affect survival.

Virtually all mosquitoes of both sexes feed on a plant-based carbohydrate (usually in the form of nectar or honeydew) as a source of energy (Foster 1995). The quality (concentration) of sugar may have differential effects on survival. Accordingly, Andersson (1992) found that a sucrose concentration of 10% was significantly inferior to concentrations of 25% and 50%, in prolonging the lifespan of *Ae. communis* (DeGeer). Similarly, a diet of blood alone can result in starvation (Briegel and Kaiser 1973;

Andersson 1992). Though these studies demonstrate an influence of diet on survival, they do not consider the possible additional effects of body size. Clearly, a study of the effects of either adult size or diet on survival should take the other into account.

Briegel (1990) showed that, at emergence, large females from less crowded larval habitats have a higher caloric content than do small mosquitoes reared under crowded conditions. Therefore, one might expect smaller females to nectar-feed more often than larger females in nature (Roitberg *et al.* 1994). If this is true, then survival should be reduced more so in smaller females than in larger ones if differential feeding rates were applied. Thus, this hypothesis suggests that an interaction between female body size and feeding rates is more important in influencing survival rather than either size or feeding rate alone. Accordingly, this laboratory study investigated the effects of starvation and 2 feeding rates (15% sucrose solution provided *ad libitum*, and every other day) on survival in 2 size classes (large and medium) of female *Ae. togoi* using a factorial design.

Methods

Colonization

The mosquitoes used in this study were obtained from the eggs of females collected as larvae or pupae from rock pools in Lighthouse Park, West Vancouver. These rock pools almost exclusively contain *Ae. togoi* (P. Belton, personal communication). However, *Culiseta incidens* (Thomson) pupae were collected on one occasion from one rock pool in November, 1995. Several collections were made between April and September, 1994, and three between September and October, 1995.

The larvae and pupae were taken back to the insectary at Simon Fraser University and adults were allowed to emerge in 30 x 30 x 30 cm cages. Fifteen percent sucrose solution was provided as a source of carbohydrate, while a mechanically restrained guinea pig served as a blood source. Glass dishes (12.5 cm wide and 6.5 cm deep) lined with paper towels and partially filled with distilled water were provided as oviposition sites (Trimble and Wellington 1979). The oviposition dishes were also wrapped with black plastic from garbage bags. Temperature was maintained at $25 \pm 1\,^{\circ}\mathrm{C}$, the light:dark photoperiod at 16:8 h, and humidity ranged between 63-71%.

Eggs laid by adults emerging from field-collected larvae and pupae were treated as follows (as described by Trimble and Wellington 1979): oviposition dishes containing eggs were removed from the cages daily and incubated for 24 h at $25 \pm 1\,^{\circ}\mathrm{C}$. Next, all the water from the oviposition dishes was drained, the dishes covered with Plexiglas™ lids, and the eggs incubated for 24 h more (Trimble and Wellington 1979). The paper towels, with eggs attached, were air-dried and stored at $25 \pm 1\,^{\circ}\mathrm{C}$ and humidity 63-71% for a maximum of 2-3 weeks until required for use. Due to initial difficulties in getting dried eggs to hatch, some were used without drying. However, in all experiments described in the rest of this thesis, only F_1 generations (mosquitoes reared from eggs laid by adults emerging from field-collected larvae and pupae) were used. This procedure was employed to reduce the likelihood of inadvertently selecting for any characters as a result of continuous laboratory colonization (Mackauer 1976).

Larval rearing procedure

Stored eggs were immersed in the rearing medium (RM) (1:1 solution of distilled and filtered sea water) described by Trimble and Wellington (1979). The salinity of RM was approximately 11g salts/litre, in accordance with the 10-12g salts/litre range recommended by Trimble and Wellington (1979). Using a pair of forceps, a pinch of yeast powder was added to the inundated eggs (Gerberg 1970). Hatching took place within 24 h. Since hatching does not take place simultaneously, unhatched eggs were removed and dried again for subsequent inundations (Trimble and Wellington 1979).

In all experiments described herein and subsequent chapters, larvae were reared in glass bowls (as described for oviposition above) in an environmental growth chamber pre-set at 20°C (Trimble and Wellington 1979) and a light:dark photoperiod of 16:8 h. Larvae were supplied finely ground fish food (TetraMin™ Staple Food for Tropical Fish, Tetra Werke, West Germany) daily until all individuals had pupated. RM had to be changed frequently, approximately every third day from the third-larval instar stage, as recommended by Trimble and Wellington (1979), due to fouling by bacteria and cloudiness of the medium.

Pupae were transferred daily, after moulting, into 200 ml of fresh RM in the aforementioned glass bowls. The bowls were placed in 30 x 30 x 30 cm cages in a room at 25±1°C and humidity 63-71% for adults to emerge. The light:dark photoperiod was 16:8 h.

To rear the different-sized adults used in this study, the following numbers of newly emerged larvae were counted into bowls: 50, 200, 400 larvae. This procedure was employed because adult body size decreases with increasing larval density (Peters and Barbosa 1977). The larvae were reared in 200 ml of RM. Regardless of larval density, all batches received 50 mg of Tetramin™ daily until all larvae had pupated. Table 2.1 summarizes the food and larval density conditions used.

Females reared at the various densities were allowed to emerge into separate 30 x 30 x 30 cm cages which contained males for mating. Female mosquitoes from the same rearing density were removed from rearing cages after 24 h and put in groups of 5 in separate 30 x 30 x 30 cm cages. Groups of 5 were used because sufficiently large numbers of females could not be obtained each day. In fact, due to the extreme asynchrony of development in the 400 density level, the experiment used only females from the 50 and 200 density levels.

Given the wide range of adult sizes that resulted from the 200 density level, wing lengths of daily samples of adults were measured, as described by Nasci (1990), to determine the approximate size of females used in the experiment. Newly-emerged adults were kept for 12 h at 63-71 % humidity and temperature $25 \pm 1^{\circ}$C. They were provided with distilled water in a beaker with dental cotton wick. Subsequently, they were killed with chloroform and, using a binocular microscope with an ocular micrometer, the wing lengths were measured from the axial incision to the apical margin excluding the fringe of scales (Nasci 1990). Only approximately the first 50% of emerging females from the 200 larvae batch were used since females emerging later tended to be larger in size. Similarly, the few stragglers in the 50 larvae batches were not used. The wing length of large females ranged between 4.4-4.6 mm, whereas that of medium-sized females was between 3.4-4 mm.

Each size category was divided into 3 groups, then provided with 15% sucrose solution as follows: (i) *ad libitum* (ii) every other day (iii) starved (none). Therefore, the *ad libitum* groups had access to the sucrose solution (in a beaker with a wick) immediately after emergence until all members of a cohort died. The every other day groups had access to the sugar solution for 24 h after which it was removed for the next 24 h. The starved groups were never provided with sugar solution or water. No group was blood fed. Oviposition was prevented by simply not providing any water for egg laying.

Given the above treatments, dead mosquitoes were removed and counted every 24 h to obtain the numbers surviving and dying per day. Next, a two-factor analysis of variance (taking into account the possible effect of cages, that is, replicates) to test for effects of feeding rate and size on mean survival time (MST) was performed by use of the General Linear Models Procedure (SAS statistical package).

Results

In all size and feeding rate combinations, mortality was highest within the first 3 days (Fig. 2.1). Regardless of feeding rate, large females were always the last to die.

Both size and feeding rate had a significant effect on mean survival time (MST) (Table 2.2). However, there was no interaction between these 2 factors on survival (Table 2.3).

Discussion

In agreement with Hawley (1985), Nasci (1987), Packer and Corbet (1989), and Renshaw *et al.* (1994), the present study shows that body size may affect female survival. In the present study, at each feeding rate, large females consistently lived longer than did medium-sized females. In contrast, Walker *et al.* (1987), Landry *et al.* (1988), and Pumpuni and Walker (1989), found no relationship between female size and longevity in *Ae. triseriatus* (Say), and *Ae. hendersoni* Cockerell. It seems unlikely that the effects of size on female survival is species specific; Haramis (1983), in a field study, observed that large female *Ae. triseriatus* survive longer than do small females.

Where an effect of size on survival has been observed, it is usually suggested that large individuals have more energy reserves than do small ones (Briegel 1990; Renshaw *et al.* 1994). Longer survival of large females from good quality habitats may imply that they are likely to leave more offspring than are small females. The distribution of adult body size is a good indicator of the quality of a mosquito population (Nasci 1987), so that one of predominantly small individuals would suggest that the population is declining in size (Bock and Milby 1981).

As expected, feeding rate had a significant effect on survival of both large- and medium-sized females. This observation is in agreement with that of Briegel and Kaiser (1973) who noted that lifespan is extended when adult *Ae. aegypti* and some *Culex* species are provided with sugar compared with water only (starvation). Similar observations on *Ae. communis* were reported by Andersson (1992). However, in the starved cohorts (not provided sugar solution or water), the shorter survival of medium-sized females may be attributed to faster dehydration since such females have a larger surface area:volume ratio than large females.

The lack of interaction between body size and feeding rate in the present study may indicate that smaller females do not suffer disproportionately higher mortality than do larger females when provided with food less frequently. Based on the present results, the hypothesis that an interaction between feeding and female size is more important in influencing survival than either size or feeding rate alone is rejected.

Certain problems arose during the course of this experiment that may have affected the outcome, the most important of these being synchronizing adult emergence. Larval development was greatly prolonged with larval density. In fact, small-sized mosquitoes (reared at 400 larval density level) were not available for use when the experiment commenced because these were still in the immature stages (see Chapter 3). Since comparative studies on survival must be conducted under contemporaneous and constant antecedent environmental conditions (Briegel and Kaiser 1973), the small-sized females had to be excluded from the study. Much as I tried to keep the antecedent conditions (for example, quantity of food provided, changing the RM, temperature, and other larval rearing conditions) constant, the contemporaneous requirement was not completely satisfied because the medium-sized females were not always available owing to the non-uniform development of larvae at the 200 density level. Therefore, alternative methods of rearing adult mosquitoes of specific sizes are needed. The method must somehow overcome the difficulty of synchronizing adult emergence. Larvae reared at high temperatures develop rapidly and the resulting adults are usually smaller than those reared at lower temperatures (Reisen *et al.* 1984). This method may not be applicable to *Ae. togoi* since Trimble and Wellington (1979) observed almost 100% mortality of larvae of this species at 25^{o}C.

Though size-dependent survival of females in the laboratory has been demonstrated in the present study, these results may not necessarily hold true in field situations. In the laboratory, survival of *Ae. sierrensis* adults does not depend on size, however, the reverse is true in the field (Hawley 1985). Therefore, estimates of survival of female *Ae. togoi* in the field using methods, such as mark-recapture, are needed to verify the present observations.

Table 2.1 Rearing conditions of *Ae. togoi* at 3 larval densities

Larvae/200ml	Larvae/ml	Food (mg)/larva /day*
50	0.25	1.00
200	1.00	0.25
400	2.00	0.125

*50 mg of food per dish per day

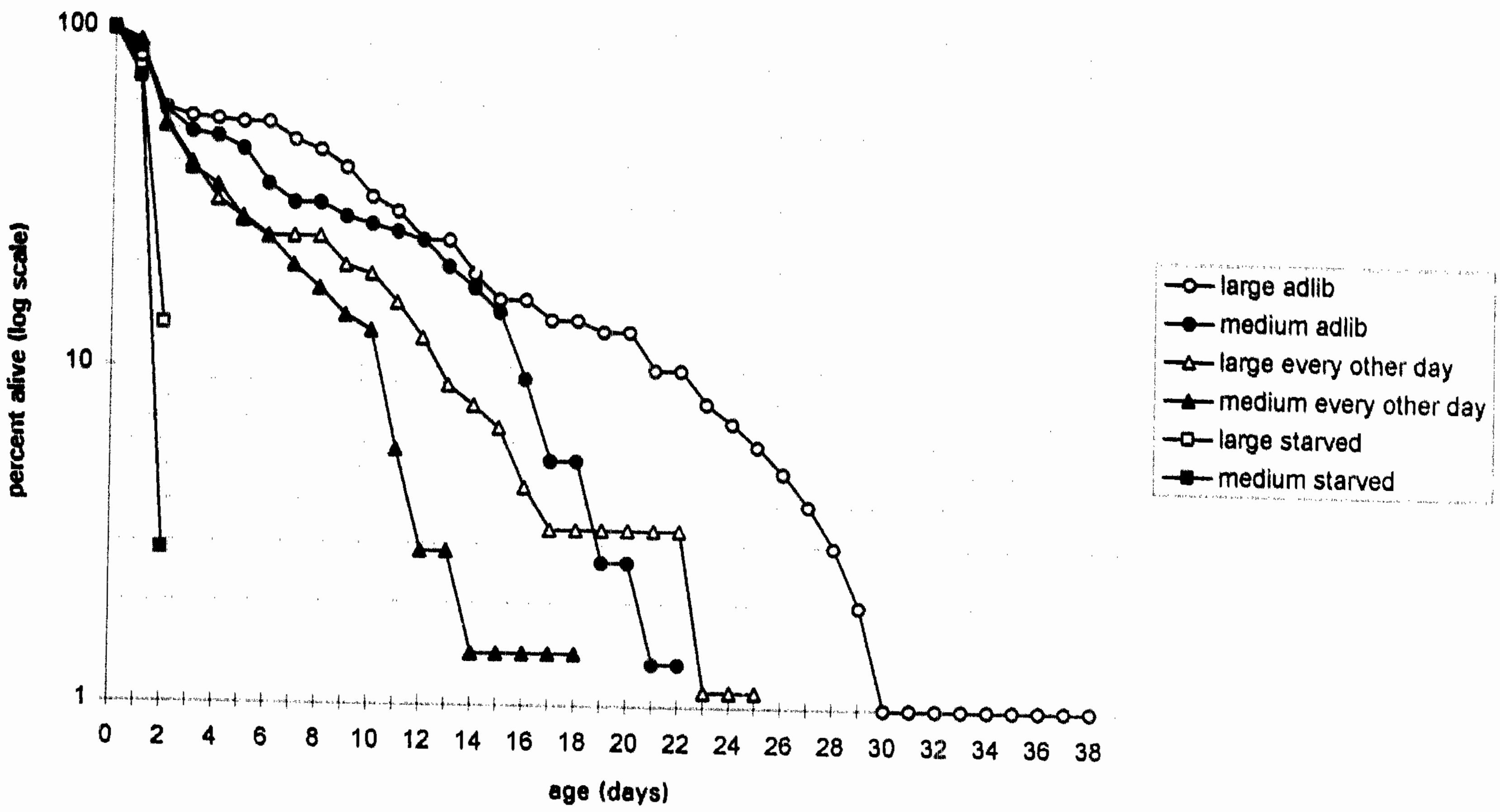

Fig. 2.1. Survival of large- and medium-sized female *Ae. togoi* maintained at 3 feeding rates. Each point on the graph represents the fraction of the original number (100% on day 0) of individuals alive at a given time until all are dead.

Table 2.2. Mean survival time (days) (MST ± S.E) of large- and medium-sized female *Ae. togoi* maintained at 3 feeding rates.

Feeding rate	large	*N**	medium	*N**
ad libitum	8.62±0.844	20	6.67±0.707	15
every other day	5.34±0.578	18	4.46±0.457	14
starved	1.89±0.07	15	1.74±0.06	14

**N* is number of cages; each contained a cohort of 5 females.

Table 2.3. The effects of size and feeding rate on the survival of female *Ae. togoi*.

Source	df	SS	MS	*F*	*P*
Size	1	130.03	130.03	5.96	<0.0150
Feeding rate	2	2762.18	1381.09	63.35	<0.0001
Size * Feeding rate	2	65.31	32.66	1.50	>0.22
Cage (size *feeding rate)	92	5244.55	57.01	2.61	<0.0001

Chapter 3

Effects of the quality of larval habitat on some morphological and physiological attributes of larval and adult *Aedes togoi*

Introduction

When individuals that exploit a finite resource exist at population densities below or above a certain threshold, such individuals may suffer fitness consequences (Peters and Barbosa 1977). Presumably, the various types of mosquito breeding sites (for example, ditches, marshes, flood pools, tree holes, rock pools, man-made containers, such as car tires, and several others) can support only some maximum number of individuals since resources, such as food and space, are limited. In mosquitoes, the consequences of exceeding this number include prolonged pre-adult development and increased larval mortality (Moore and Fisher 1969; Ikeshoji and Mulla 1970; Barbosa *et al.* 1972; Reisen 1975; Frank and Curtis 1977). In addition, adults from densely populated breeding pools are smaller (Barbosa *et al.* 1972; Nasci 1990), imbibe less blood per meal (Reisen 1975), and have a lower fecundity (Briegel 1990; Lyimo and Takken 1993). Thus, it has been suggested that intraspecific competition among immature larvae may regulate the growth rate and size of a mosquito population (Peters and Barbosa 1977; Focks *et al.* 1993).

Three mechanisms by which these density-dependent responses are effected have been suggested as follows: (i) competition for food, (ii) mechanical interference, and (iii) chemical interference. Of these, only intraspecific competition for food is generally accepted. Thus, as density increases, there is a general decrease in quantity and quality of available food per larva (Barbosa *et al.* 1972; Peters and Barbosa 1977; Focks *et al.* 1993; and several others). According to Focks *et al.* (1993), in *Ae. aegypti*, quantity of food per larva rather than larval density *per se*, is more important in affecting the size and growth of a population.

There is little consensus on how the other 2 suggested mechanisms, mechanical and chemical interference, might limit populations. Mechanical interference arises in increasingly dense populations due to competition for space (Dye 1984). Shannon and Putnam (1934) were probably the first to suggest that the mechanical agitation that occurs with increasing larval density (through increased bodily contact), leads to a decrease in feeding rates. Subsequently, Dye (1984) showed that the increased physical contact in dense populations of larval *Ae. aegypti* retards rate of larval development (assessed by larval moulting rates). However, several authors have rejected this hypothesis on the grounds that mechanical interference is of limited consequence in nature (Barbosa *et al.* 1972; Peters and Barbosa 1977; Focks *et al.* 1993).

Moore and Fisher (1969) reported that in dense *Ae. aegypti* populations, larvae produce toxic chemical factors called growth retardant factors (GRF), which slow larval growth, and consequently prolong pre-adult development. However, GRF is produced in larval *Ae. aegypti* under conditions of food shortage regardless of larval density (Moore and Whitacre 1972). Ikeshoji and Mulla (1970) demonstrated that in *Culex pipiens quinquefasciatus* Say, these chemical factors are toxic to first instar larvae of this species, causing a high mortality in them. Similarly, Reisen (1975) showed that *Anopheles stephensi* Liston larvae reared in water that previously held a high density of larvae, develop slower than do larvae at similar density but reared in water in which only a few larvae had previously been held. By contrast, some authors have concluded that chemical interference is of limited importance in nature (Dye 1982, 1984; Focks *et al.* 1993). In laboratory experiments, Dye (1982), working with the 'Liverpool strain' of *Ae. aegypti*, concluded that there was no evidence of chemical interference in mixed populations of first- and fourth-instar larvae of this mosquito reared under competitive conditions; however, he found significant chemical interference using Puerto Rican strains of the same species, though he still concluded that chemical interference in this species is not a major factor in intraspecific competition.

The response of an insect population to stress varies from species to species (Peters and Barbosa 1977). In fact, it may vary between strains of a species, as illustrated by the studies of Dye (1982, 1984) discussed above. Mogi (1984) noted that in parts of Japan, density-dependent miniaturization of larvae and pupae of the leaf axil-breeding mosquito, *Malaya genurostris* Leicester, is not as distinct as it is in another leaf axil-breeder, *Ae.* (*Stegomyia*) *flavopictus miyarai* Tanaka, Mizusawa and Saugstad. Therefore, extrapolating observations from one mosquito species to another must be done with caution.

Frank and Curtis (1977) classified mosquitoes into 2 broad ecological groups based on their response to food shortage. One group, those found in ponds, ditches and marshes, for example, *Cx. nigripalpus* and *Ae. taeniorhynchus* (Wiedemann), grow rapidly and respond to food shortage with high mortality (Frank and Curtis 1977). Therefore, members of this group do not reduce the rate of larval development when food is in short supply (Frank and Curtis 1977). The second group consists of species, such as *Wyeomyia vanduzeei* Dyar and Knab, *Wy. medioalbipes* Lutz, and *Toxorhynchites r. rutilus* (Coquillett), which are container breeders (for example, water-filled leaf axils of plants, tree holes and man-made containers), grow slowly, and can tolerate long periods of food shortage pending the availability of more food (Frank and Curtis 1977). The present study attempts to understand how larval *Ae. togoi* may respond to food shortage.

Since *Ae. togoi* is restricted to breeding in coastal rock pools in North America (Belton 1980; Belton and Belton 1990), successive generations of females, like those of *Ae. atropalpus* (Kalpage and Brust 1973), probably utilize the same breeding pools repeatedly. In fact, suitable rock pools on the Pacific Coast may be several kilometers apart (Belton 1980). Thus, females conceivably lay eggs in the same rock pools over and over, resulting in overlapping generations. Accordingly, it seems feasible that highly dense populations may be attained occasionally, thus resulting in intense intraspecific competition for, in particular, food. The objective of this study, therefore, was to investigate some physiological (rate of larval development) and morphological (adult size) responses to food shortage caused by increasing larval density. The discussion also addresses how the changes of larval habitat quality that accompany increases in larval density may affect the population dynamics of this species.

Methods

Dried eggs were inundated with RM as described in Chapter 2, and within 12 h of emergence, 3 sets, each of 50, 200, and 400 larvae were counted into 9 glass bowls (12.5 cm diameter and 6.5 cm deep). The larvae were reared in 200 ml of the RM described in Chapter 2. Regardless of larval density, all batches received 50 mg/day of Tetramin™ until all larvae had pupated. The rearing conditions were as outlined in Chapter 2.

The relationship between larval density and rate of development was assessed by recording the developmental time from hatch to adult emergence of each sex at each of the 3 densities. Similarly, percent survival to adult emergence at each density level was determined by comparing the total number of live adults with the initial number of larvae per bowl. Adults drowning at emergence were not counted as survivors.

Adult wing length and dry weight were used to determine male and female size at emergence as described by Nasci (1990). After wing lengths were measured as described in Chapter 2, each specimen was dried at 80°C for 3 days and weighed (Nasci 1990) on a Mettler H-31 balance.

Statistical comparisons of developmental rates at the densities used were conducted using the Kolmogorov-Smirnov test (SPSS for Windows, version 6.1). The percent survival to adult emergence in each replicate at the density levels used was arcsine transformed (Zar 1984). Such transformation ensures that the data approximate a normal distribution since the deviation from normality is otherwise great for small or large percentages, such as 0-30% and 70-100% (Zar 1984). A one-way analysis of variance was performed on the transformed data, and on the wing length and dry weight data by use of the General Linear Models Procedure (SAS Institute statistical package). Next, multiple comparisons (by treatment) of the data for each of survival, wing length, and dry weight were made using Student-Newman-Keuls test (SNK).

Results

The percentage of individuals surviving to adult emergence, developmental rate, and adult size were used to assess the response of *Ae. togoi* to increasing larval density. The proportion of individuals surviving to adulthood appeared to decrease with density but this effect was significant only at the 400 larval denstiy level (see Table 3.1 for mean and *P* values).

As shown in Fig 3.1, there was a remarkable reduction in the rate of development to adult emergence as density increased. Regardless of density, males were always the first to emerge; emergence commenced in females 1-2 days later. At the 50 and 200 larval density levels, adult emergence commenced on day 15, whereas the first individual emerged on day 19 in the 400 larval density treatment. In addition, 50% of all individuals surviving to adulthood emerged between day 17-18, day 25-27, and about day 36 in the 50, 200, and 400 larval density levels respectively. Comparison of the rates of development (within sex) in the 3 densities using the Kolmogorov-Smirnov test, showed a significant difference in the rates of development. Furthermore, the range of time to emergence was approximately 15-22 days, 15-41 days, and 19-59 days in the 50, 200, and 400 larval density levels respectively. Thus, development was relatively uniform at the lowest density but was not at the higher densities.

Adult size at emergence was assessed using wing length and dry weight data. In both sexes, average size appeared to decrease with density (Table 3.1). However, the relationship was not linear since, within a treatment, individuals emerging later in the developmental period were larger than those emerging earlier. This is presumably because more food per individual was available as larval population decreased through mortality or recruitment into the pupal population or both. Female wing length (mm) ranged between 4.45-4.69, 3.30-4.50, and 2.92-4.45 at the 50, 200, and 400 larval densities respectively. Male wing lengths followed the same trend. Thus, compared to the 50 larval density level, adult sizes were quite variable at the higher densities used in this study (note increasing coefficient of variation (CV) in Table 3.1). At all 3 density levels, body size differed significantly within sex (Table 3.1).

Discussion

Increasing density of larval *Ae. togoi* led to prolonged pre-adult development, increased larval mortality and, on average, smaller adults. These observations are similar to those on other mosquito species, such as *Cx. p. quinquefasciatus* (Ikeshoji and Mulla

1970), *Ae. aegypti* (Barbosa *et al.* 1972; Dye 1982), and *An. stephensi* (Reisen 1975). The highest density used in the present study (2 larvae/ml) is small compared with the maximum densities used in some of the above studies, 5-7 larvae/ml by Ikeshoji and Mulla (1970), and 16 larvae/ml in Barbosa *et al.* (1972). Thus, the magnitude of the responses observed in the present study may increase at higher densities.

Perhaps the most significant effect of increasingly dense larval populations was the general prolongation of pre-adult development. The synchronous development of individuals at the 50 larval density level gave way to asynchronous development at higher densities. In fact, at higher densities, whereas some individuals had emerged as adults, others were still at the early larval instar stages. Given this observation, *Ae. togoi* appears to employ the strategy of slow growth in response to food shortage, as described by Frank and Curtis (1977). According to these authors, larvae of some species of mosquitoes can tolerate food shortage by prolonging immature development, so that when more food becomes available, they complete their development. Therefore, any unsuccessful attempts at larval control that only partially reduce larval populations, may release such populations from competitive stress, the result being larger females that lay more eggs and, generally, an increase in population size (Renshaw *et al.* 1994).

However, it is possible that rearing the larvae at 20^{O}C is at least partly responsible for the observed prolonged development because low temperatures generally lower rates of development. Protracted pre-adult development would be a useful strategy for surviving food shortage given that rock pools are permanent and hold larvae year round (P. Belton personal communication). Conversely, the consequences of prolonged development should include mortality since, generally, the longer the immature development, the higher the risk of death due to predation, drying out of smaller rock pools, or other causes.

It seems that larval density may affect the population dynamics of *Ae. togoi* directly and indirectly. The direct effect may result from mortality of larvae due to competition for food and from prolonged pre-adult development, as discussed above. Protracted development may delay reproduction, thereby increasing the mean generation time of the population (Pianka 1994). These effects could regulate the size and growth rate of a population of this species.

The indirect effects concern the reduction of adult size. Compared to large females, small adults are less likely to seek hosts (Klowden *et al.* 1988; Nasci 1986, 1990), imbibe less blood (Reisen 1975), have a lower fecundity (Briegel 1990), and are probably shorter lived (Hawley 1985; Nasci 1987; Renshaw *et al.* 1994). Accordingly, Bock and Milby (1981) concluded that a population composed predominantly of small individuals is an indication that it is in decline. *Ae. togoi* can be autogenous (Lien 1960), but autogeny is greatly influenced by the quality of larval habitat, especially temperature and food content, so that poorly nourished adults from poor quality habitats are less likely to be autogenous (Sota and Mogi 1994). Therefore, non-autogenous females of this species must expend energy and time to obtain a blood meal, and must take the risk of death associated with blood feeding. Thus, these indirect effects, through their influence on adult reproductive success, could influence population dynamics.

Clearly, much of the responses demonstrated herein are due to the decrease of quantity of food per larva with increasing density. However, it is feasible that competition for space and chemical interference also mediated these responses. Therefore, I am unable to resolve the extent to which each of these factors may have influenced the observations in this study.

In North America, *Ae. togoi,* as far as is known, breeds only in rock pools along the coast (Belton and Belton 1990). Consequently, it probably breeds in the same rock pools continuously; the rock pools at Lighthouse Park have had larvae continuously for about 20 years (P. Belton, personal communication). In addition, since this species is multivoltine (Sota and Mogi 1994), continuous use of rock pools may lead to overlapping generations breeding concurrently in these rock pools. This could cause intraspecific competition if resources, such as food and space, are limited, resulting in some of the responses I have demonstrated in the laboratory. Such responses could result in a general reduction of population size and growth rates, the population rebounding when more resources become available (Frank and Curtis 1977). However, these observations can hardly be extended to field conditions since density-independent factors, such as temperature (Bock and Milby 1981), also play a critical role in population dynamics. For example, *Ae. togoi* adults emerging in spring (May or thereabouts) are larger than those collected late in summer (P. Belton, personal communication). Field studies on this species in North America (which are largely lacking) should include monitoring larval populations and assessing adult attributes, such as size, in order to correlate these with the population dynamics of this mosquito.

Table 3.1: A comparison of some attributes of *Ae. togoi* reared at 3 larval densities.

	Larval density/200ml (Means + S.E)[1] and [coefficient of variation]					
	50	200	400	df	*F*	*P*
Larval survival (%)*	90 ± 0.17 (a)	84.33 ± 0.21 (a)	46.42 ± 0.28 (b)	8	20.74	<0.002
Female wing length	4.53 ± 0.01 (a) [1.89]	3.55 ± 0.03 (b) [6.71]	3.29 ± 0.08 (c) [15.76]	114	133.53	<0.0001
Female dry weight	0.73 ± 0.02 (a) [19.86]	0.48 ± 0.01 (b) [26.54]	0.23 ± 0.01 (c) [49.05]	114	144.38	<0.0001
male wing length	3.37 ± 0.01 (a) [2.38]	3.16 ± 0.01 (b) [3.28]	2.95 ± 0.01 (c) [4.60]	139	171.37	<0.0001
male dry weight	0.39 ± 0.01 (a) [18.83]	0.27 ± 0.01 (b) [33.03]	0.22 ± 0.01 (c) [42.23]	139	44.27	<0.0001

[1] Means ± S.E followed by the same letter were not significantly different ($P > 0.05$). (GLM Procedure followed by SNK test).

*Percent larval survival was transformed by the arcsine method (Zar 1984) before performing a one-way analysis of variance.

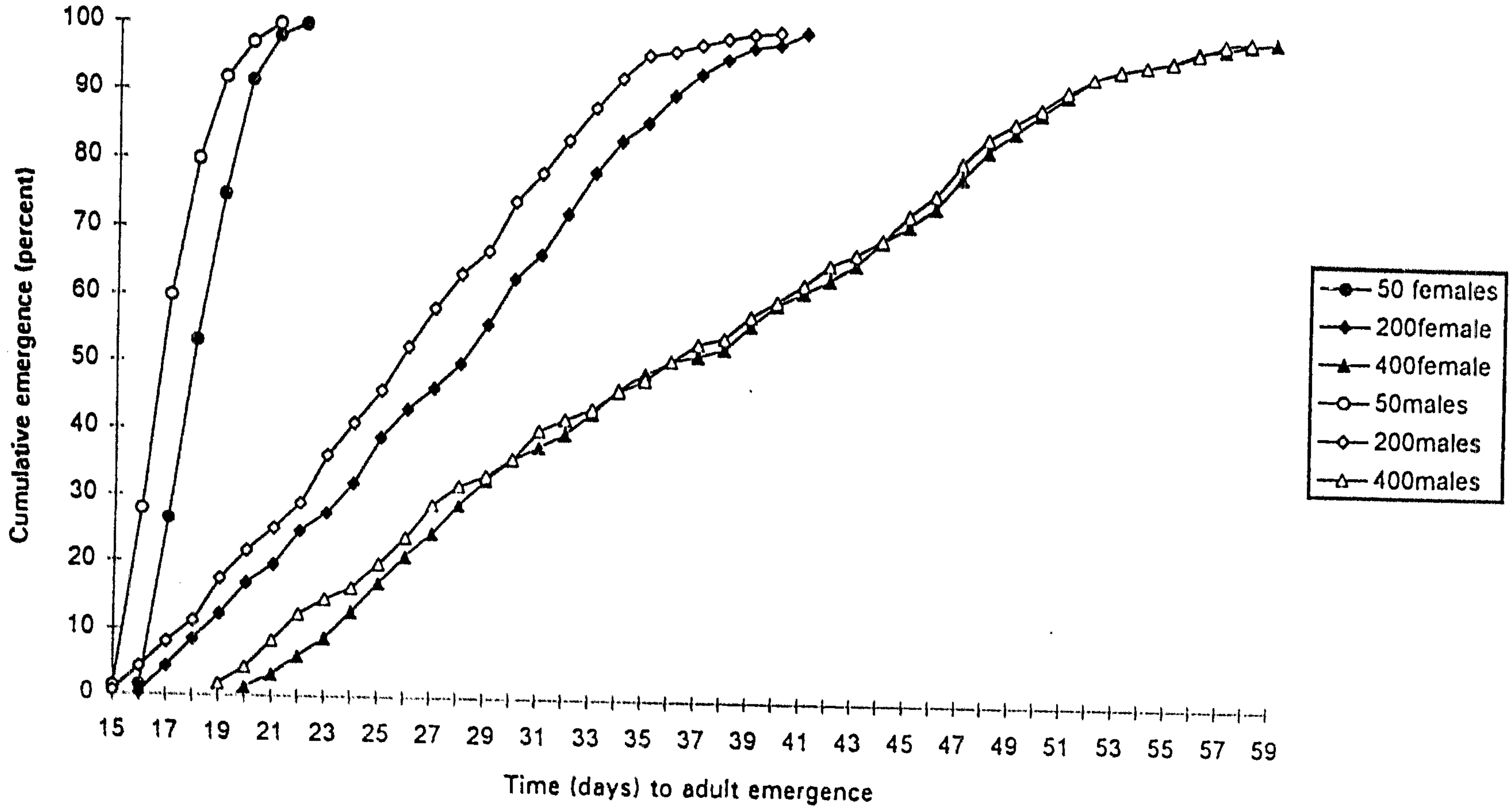

Fig 3.1 Cumulative adult emergence rates of *Ae. togoi* reared at 3 larval densities. Rate of development decreased with increasing larval density. There was a significant effect ($P < 0.05$) of larval density on rate of development within sex (Kolmogorov-Smirnov test, SPSS version 6.1 1994).

Chapter 4

The effect of conspecifics on the selection of oviposition site by *Aedes togoi*

Introduction

It is generally accepted that animals select habitats that maximize their fitness (Rosenzweig 1991). In particular, the selection of oviposition site by egg-laying animals may be influenced by availability of food, abundance of predators, and presence of competitors (Blaustein and Kotler 1993). Therefore, ovipositing animals may be expected to lay eggs in habitats with the best environment for survival and reproduction of their offspring. For example, Ritchie and Laidlaw-Bell (1994) demonstrated that the mosquito, *Ae. taeniorhynchus*, tends to not oviposit in sites with high concentrations of fish that prey on juveniles. However, the ability to detect and select the most favourable habitat for offspring development is not universal. For example, Roitberg and Prokopy (1982) showed that female codling moths, *Cydia pomonella* (L.), readily oviposit in apples regardless of the presence or absence of conspecifics (potential intraspecific competitors).

The selection of where and how many offspring to leave in limited oviposition sites (for example, rock and flood pools, leaf axils, and man-made containers, such as car tires) is critical for the pre-adults of animals like mosquitoes that are unable to move to more suitable habitats if the local oviposition site is unfavourable. Several studies have demonstrated that the selection of oviposition site by mosquitoes is influenced by many factors. Benzon and Apperson (1988) demonstrated that female *Ae. aegypti* in the laboratory oviposit more frequently on water if it contains bacteria that may serve as larval food. Lounibos and Machado-Allison (1983) reported that the shape of cacao fruit husk affects oviposition site selection in *Trichoprosopon digitatum* (Rondani). Ovipositing females of this mosquito are less likely to deposit eggs in husks cut lengthwise than in fruits whose tops are excised. In addition, aquatic vegetation (Angerilli 1980) and the chemical nature of oviposition sites (Lounibos 1978) may repel or attract ovipositing females. Thus, in mosquitoes, the choice of where to lay eggs is affected by a complex of physical and chemical factors rather than a single factor (Bentley and Day 1989).

It appears that ovipositing *Ae. togoi* can detect suitable sites for their offspring. Indeed, in Canada and the U.S.A, eggs are apparently only laid in rock pools just above the high tide mark (Trimble and Wellington 1980; Belton and Belton 1990) since they would be at the mercy of splashing ocean water and wave action at high tide mark and the intertidal zone. In this species, Trimble and Wellington (1980) also observed that females in the laboratory deposit significantly more eggs on water that had previously held fourth-instar larvae when given a choice between it and previously unused water that never held larvae. They concluded that an unidentified oviposition stimulant - probably bacterial metabolites, larval excretory products, or chemical compounds of larval origin - was associated with fourth-instar larvae of this mosquito. In addition, they suggested that such a stimulant would aid gravid females in identifying suitable breeding rock pools.

Given that increasing larval densities of *Ae. togoi* may lead to increased larval mortality, prolonged larval development, reduced adult size (as demonstrated in Chapter 3), and other physiological and bionomic consequences (Ikeshoji and Mulla 1970; Barbosa *et al.* 1972; Reisen 1975; Peters and Barbosa 1977; Lyimo and Takken 1993), how might female oviposition behaviour possibly mitigate these effects? The question is pertinent because the selection of oviposition site largely determines how many and where pre-adults are found in nature.

Ovipositing females that can detect and oviposit less frequently in sites already occupied by conspecifics, potential intraspecific competitors, should presumably be favoured by natural selection. Conversely, the presence of conspecifics may indicate to a female that the habitat is of good quality (Blaustein and Kotler 1993). Another strategy may be to distribute eggs into more than one oviposition site to improve the chances that at least some offspring will survive.

I investigated the response of gravid *Ae. togoi* to the presence of conspecific eggs (Experiment 3) by comparing the ratio of eggs deposited when provided with a choice between oviposition cups containing conspecific eggs and cups with no eggs. First, however, to choose a suitable oviposition medium for the study, it was necessary to test (in Experiment 1) the response of females to different oviposition solutions, namely, larval rearing water (LRW) and rearing medium (RM). RM is the 1:1 sea:distilled water mixture described by Trimble and Wellington (1979). A confounding factor in Experiment 1 led to the design of Experiment 2, in which Experiment 1 was repeated, but this time, with food in the rearing medium (FRM). Furthermore, the egg distribution pattern in each of the 3 experiments was assessed by providing each female with 6 cups (3 of each of the respective treatments used).

Methods

Adults used in the oviposition experiments were reared at the 50 larval density level as described in Chapter 2. Females were offered a blood meal daily from a mechanically restrained guinea pig from 2 days after emergence (Trimble and Wellington 1980) to day 4. Females blood feeding on each day were transferred to separate 30 x 30 x 30 cm cages. Thus, there was one female per cage in all 3 experiments, and each was considered a replicate. Adults were maintained at a temperature of 25 ± 1°C, and humidity of 63-71%.

In Experiment 1, 30 females were each offered 3 plastic cups (height = 8.5 cm, bottom diameter = 4.5 cm, and top diameter = 7 cm), each containing 150 ml of RM (filtered through Number 1 Whatman™ filter paper), and 3 cups of the same quantity of filtered LRW obtained from several batches of larvae reared at the 50 larval density level. Thus, each female was offered 6 oviposition cups in all. All cups were wrapped with plastic from black garbage bags and lined on the inside with paper towel (Trimble and Wellington 1979). Like RM, LRW was derived from a 1:1 mixture of sea:distilled water (Trimble and Wellington 1979) except that 50 fourth-instar larvae had been reared in it for 2 days. Thus, it contained larval food as well as larval associated products, for example, faeces. LRW from all the batches were mixed together to ensure that all 30 females received the same treatment. The RM never previously held larvae or contained food.

Each oviposition cup was marked by assigning it a number and inscribing this on adhesive tape on its underside. The cups were placed, 3 each on parallel sides of the 30 x 30 x 30 cm cages used. However, the exact position of cups was determined by random draw. This was repeated for each replicate. Each female was also provided with 15% sucrose solution.

Eggs were laid no earlier than 4 days after blood feeding. For example, females blood feeding on Monday laid eggs at-the-earliest on Thursday. Those not laying eggs by the fifth day were disqualified. Females bloodfed on the same day did not necessarily oviposit 4-5 days later, as some took up to 10 days to lay eggs. Therefore, many females were disqualified. Each female was used only once. Eggs laid per cup were counted and recorded, and note was taken of rejected cups as well as distribution of eggs among cups.

Experiment 1 was confounded in that LRW contained both larval associated products and food, both of which were absent in RM. Thus, if females accept LRW more freqeuntly than they do RM, it may not be resolved what they are responding to (between food and larval associated products). Accordingly, Experiment 2 repeated Experiment 1 except that 0.25 mg/ml/day of food (this being the equivalent quantity of food in LRW) was put in RM. Like LRW, the food-containing rearing medium (FRM) was filtered before use. Thus, if females respond to food, no statistically significant difference would be expected in the number of eggs laid in the 2 types of treatments. On the other hand, females would lay significantly more eggs on LRW if they were responding to larval associated products.

The average number of eggs laid per female was determined using results from Experiment 1. This estimate was divided by 3 to obtain the mean number of eggs per cup. To investigate female oviposition response to the presence of conspecific eggs, each of 24 females in Experiment 3 was offered 6 oviposition cups each containing 150 ml of filtered LRW. However, 3 of the 6 cups, in addition to LRW, contained 50 freshly laid eggs. The conditions maintained and procedures followed in this experiment were as outlined in Experiment 1.

In all 3 experiments, the following measurements were made: (i) total number of eggs laid per treatment by each female (ii) proportion of cups accepted and rejected per treatment by each female. Statistical comparisons of data from each female were made with the paired-sample *t* test. However, the proportion of cups accepted and rejected was arcsine transformed (Zar 1984) before performing the paired-sample *t* test.

Results

The results of Experiment 1 are summarized in Table 4.1. Females laid over 11 times as many eggs on LRW as they did on RM. This difference was statistically significant ($t = 8.033$, $P < 0.0001$). Only 10 of the 30 females in this experiment laid any eggs on RM. By contrast, all 30 deposited at least some eggs on LRW (Appendix 1).

The ratio of accepted cups, 70 LRW:21 RM, was statistically significant ($t = 6.445$, $P < 0.0001$).

Observations on the distribution of eggs laid showed that 26 females in this experiment deposited eggs in at least 2 of the 6 cups offered, while 4 laid all eggs in one cup (Appendix 1).

In Experiment 2, females were offered a choice between LRW and FRM that previously contained food (Table 4.2). A total of 2625 eggs was deposited on LRW, whereas 1430 was on FRM. This difference was not statistically significant ($t = 1.914$, $P > 0.066$, $t_\beta = 0.20$).

Females accepted 30 but rejected 54 of the FRM cups. Similarly, they oviposited in 43 but rejected 41 of the LRW-containing cups. Again, this difference was not statistically significant ($t = 1.723$, $P > 0.096$, $t_\beta = 0.23$) (Table 4.2). In addition, 9 of the 28 insects in the study deposited no eggs on FRM, while only 4 of 28 did not oviposit in any of the LRW cups offered (Appendix 2).

Of the 28 females in this experiment, only 6 laid all their eggs in 1 cup; 22 distributed their eggs among 2 or more cups (Appendix 2).

Based on the results of Experiment 1, LRW was used in Experiment 3 to test the response of gravid females to the presence of conspecific eggs. Though more eggs were laid in cups that contained no conspecifics (2565) than in cups with conspecifics (1444), the difference was not statistically significant ($t = 1.93$, $P > 0.066$, $t_\beta = 0.391$) (see Table 4.3). Furthermore, 10 of 24 females did not oviposit in any cups containing conspecifics compared with only 1 female that laid no eggs in cups with no conspecific eggs (Appendix 3).

Females accepted twice as many cups containing no conspecific eggs as they did cups with conspecific eggs (48:24) (Table 4.3). In contrast to egg counts, this difference was statistically significant ($t = 3.77$, $P < 0.001$).

Observations on the distribution pattern of eggs in Experiment 3 revealed that 20 of the 24 females used in the study laid eggs in 2 or more of the 6 cups offered, whereas 4 laid all their eggs in one cup (Appendix 3).

Discussion

In Experiment 1, gravid *Ae. togoi* laid more eggs on water in which larvae had been held and fed (LRW) than on water that never held larvae nor contained food. However, in Experiment 2, when offered a choice between LRW and FRM, no tendency to accept one medium more frequently than the other could be demonstrated. These observations indicate that the higher oviposition rate on LRW observed in Experiment 1 was more likely due to the presence of food rather than to an oviposition stimulant of larval origin. Moreover, Blaustein and Kotler (1993), found that gravid *Culiseta longiareolata* Macquart respond with higher oviposition rates to water containing surplus food for larvae. Similarly, it has been demonstrated in other mosquitoes, such as *Ae. atropalpus*, *Ae. triseriatus*, and *An. stephensi* (Kalpage and Brust 1973; McDaniel *et al.* 1979; Reisen 1975), that females frequently deposit more eggs on water that has held conspecifics than on water that has not. However, this behaviour is not common to all mosquitoes since McCrae (1984) showed that ovipositing *An. gambiae* Giles do not deposit more eggs on rearing water that previously held conspecific larvae than on tap water.

Trimble and Wellington (1980) concluded that the higher oviposition rate of gravid *Ae. togoi* on water that had held immature stages could be attributed to bacterial metabolites, larval excretory products, or compounds of larval origin specifically produced to aid gravid females in identifying suitable oviposition sites. The results of Experiment 2 indicate that the higher acceptance of LRW in the present study is more likely due to food or bacteria or both, rather than to a compound of larval origin. Indeed, Benzon and Apperson (1988) demonstrated that the higher oviposition rate on larval holding water (water that previously held larvae) than on distilled water by gravid *Ae. aegpyti* is eliminated when bacterial growth in holding water is prevented.

As discussed in Chapter 3, increasing larval density in *Ae. togoi* populations has adverse consequences; the resulting adults are smaller, pre-adult development is prolonged, and larval mortality increases. Thus, natural selection should presumably favour females that can detect and oviposit less frequently in sites containing conspecifics (potential competitors). In contrast, the presence of conspecifics may be an indication that the habitat can support offspring (Blaustein and Kotler 1993). Although on average, gravid females in Experiment 3 laid more eggs in the absence rather than in the presence of conspecific eggs, the difference was marginally insignificant ($P > 0.066$). The lack of a significant difference may be due to the wide range (59 - 260) and large standard deviation (42.78) of total eggs laid per female. This could have reduced the power of the statistical test. However, females laid eggs in twice as many cups with no conspecific eggs as in cups containing eggs ($P < 0.001$). Moreover, only 1 of 24 females did not oviposit in cups containing no eggs compared with 10 females that did not lay any eggs in cups with eggs

(Appendix 3). Taken together, these results, though equivocal, probably mean that most females can detect conspecific eggs, and oviposit more frequently in sites containing no conspecifics, but whenever they accept sites with conspecifics, they lay just as many eggs as they would in their absence. However, considering that suitable rock pools in the field are limited, it seems gravid females would more often encounter pools already occupied by conspecifics and will oviposit in such pools. Moreover, I have observed that caged females provided with cups containing only RM (an otherwise unsuitable medium judging from the results of Experiment 1) will deposit approximately as many eggs in them as they would in LRW. Similarly, with the aid of oviposition pheromones, gravid females of several entomophagous and phytophagous parasitic insects can detect hosts infected with conspecific eggs and larvae, and respond by rejecting such hosts (Roitberg and Prokopy 1987; Robertson *et al.* 1995). However, the acceptance of parasitized hosts will increase when unparasitized hosts are few or when competition for hosts is severe (Roitberg and Prokopy 1987; Robertson *et al.* 1995).

The results of Experiment 3 are in contrast to the high oviposition rates by gravid *Cx. p. quinquefasciatus* in sites containing conspecific egg rafts (Starratt and Osgood 1973). These authors identified the attractive compounds as a mixture of fatty acids associated with the egg rafts of this mosquito. Subsequently, Bruno and Laurence (1979) identified the apical droplet on each egg as the source of the attractant, while Laurence and Pickett (1982) identified erythro-6-acetoxy-5-hexadecanolide as the major component of the oviposition attractant pheromone of this species. An oviposition pheromone is also associated with the egg rafts of *Cx. tarsalis* Coquillett (Osgood 1971). Osgood and Kempster (1971) observed that females of this species in an olfactometer are preferentially attracted to a chamber with water containing egg-washed materials over one with distilled water only. No egg-associated oviposition pheromone is as yet known in *Ae. togoi.*

Some alternate explanations of the results are possible. The density of eggs per cup may have been too low to elicit a response or so high that typical female response was altered. Egg density per cup in Experiment 3 was based on the mean number of eggs laid per female in Experiment 1. This number was then divided equally among 3 cups. It should be interesting to test female response to a range of egg densities. The cups provided for oviposition may have been so small that they influenced the number of eggs laid per cup since I have observed that rock pools in the field vary widely in size, but some may reach 4 ft long and 6 inches deep. However, I can neither substantiate nor refute these hypotheses.

Judging from the distribution of eggs among cups in all 3 oviposition experiments, gravid females (with a few exceptions) laid eggs in at least 2 of the 6 cups provided for oviposition. This may be some sort of "bet-hedging" behaviour (Seger and Brockmann 1987) in which females do not deposit all their eggs in one site. If this observation holds true under field conditions, then it may tend to distribute offspring among available breeding sites. However, this behaviour would be of little consequence in mitigating intraspecific larval competition since its usefulness would depend on the number of ovipositing females in the adult population and on the number of available breeding sites. More importantly, this behaviour may be interpreted as a "risk-spreading" measure (Seger and Brockmann 1987), so that eggs are laid in as many sites as possible. Clearly, the quality of some of these sites may not be as suitable as others, however, on average, "risk-spreading" would improve the chances that at least some offspring will survive.

A mosquito may be classified, with respect to oviposition site selection, as specialist, rather than opportunistic, if it only breeds in certain restricted sites (Bentley and Day 1989). *Ae. togoi* appears to be a specialist. Indeed, Belton and Belton (1990) noted that in North America, there is as yet no evidence of this species breeding or biting humans further inland other than above the high tide mark on the rocky Pacific Coast. For a species thus restricted, and consequently utilizing the same pools over and over, it may be a more pressing problem to locate a suitable pool than it is to reduce potential intraspecific larval competition. However, depositing eggs in several rock pools will improve the chances that at least some of the offspring of a female will survive.

Finally, many physical and chemical factors that may influence choice of oviposition sites in mosquitoes have been identified (reviewed in Bentley and Day 1989). Accordingly, I expect that the interaction of some (or all) of these factors may be more critical than one factor in influencing the selection of oviposition sites.

Table 4.1. Selection of oviposition site between larval rearing water (LRW) and rearing medium (RM) by female *Ae. togoi* (n = 30).

	LRW	RM	t statistic	P	# laying in at least 2 cups	# laying in 1 cup only
# eggs deposited	4184	362	8.033	<0.0001		
# (%)* cups accepted[1]	70 (77.78%)	21 (23.33%)	6.445	<0.0001	26	4

* Percentages were arcsine transformed (Zar 1984) before performing paired-sample t test.

[1] Each female was offered 6 cups for oviposition, 3 of LRW and 3 of RM.

Table 4.2 Selection of oviposition site between larval rearing water (LRW) and food-containing rearing medium (FRM) by female *Ae. togoi* ($n = 28$).

	LRW	FRM	t statistic	P	# laying in at least 2 cups	# laying in 1 cup only
# eggs deposited	2625	1430	1.914	>0.066		
# (%)* cups accepted[1]	43 (51.19%)	30 (35.71%)	1.723	>0.096	22	6

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*Percentages were arcsine transformed (Zar 1984) before performing the paired-sample t test.

[1] Each female was offered 6 cups for oviposition, 3 of LRW and 3 of FRM.

Table 4.3 Selection of oviposition site between larval rearing water (LRW) with and without conspecific eggs present by female *Ae. togoi* ($n = 24$).

	Conspecifics present	Conspecifics absent	t statistic	P	# laying in at least 2 cups	# laying in 1 cup only
# eggs deposited	1444	2565	1.93	>0.066	20	4
# (%)[*] cups accepted[1]	24 (33.33%)	48 (66.67%)	3.77	<0.001		

[*] Percentages were arcsine transformed (Zar 1984) before performing paired-sample t test.

[1] Each female was offered 6 cups of LRW for oviposition, 3 containing 50 conspecific eggs, and 3 without eggs.

Chapter 5

A theoretical study of the mating and feeding behaviour of virgin anopheline mosquitoes

Introduction

Although the benefits of successfully obtaining a meal or mating are obvious, there are costs associated with these behaviours. To obtain a meal or mate, animals invest time and energy, and avoid predation. Thus, all animals face trade-offs (Roff 1992) and natural selection should favour those that in some way maximize benefits and minimize costs.

After emerging from the aquatic environment, an adult female mosquito must mate, obtain carbohydrate to maintain somatic function, seek out and feed on a vertebrate host for blood to nourish its developing eggs, and locate an oviposition site to lay these eggs. These activities are part of the gonotrophic cycle, so called because of the close relationship between feeding and reproduction (Klowden 1990). Each of these activities has benefits and costs associated with it. For example, there is a trade-off in allocation of search time and energy to blood and nectar feeding because both occur within the same activity window (Roitberg and Friend 1992; Foster 1995).

Although sugar feeding probably occurs frequently, throughout the adult mosquito's life (Foster 1995), it seems that newly-emerged females of many species mate before blood feeding. Thus, the conventional view is that virgin females mate before feeding on blood (Herms 1961; Busvine 1980; Belton 1983). However, it is likely that newly-emerged females are exposed to a variety of stimuli originating, for example, from plants and hosts. Must mating and blood feeding be performed in an obligatory sequence? In other words, will female mosquitoes, if unmated, forgo an opportunity to feed despite the benefits of obtaining a blood meal? It appears to be the case for female *An. balabacensis* Baisas. Iwanaga-Sawabe and Kanda (1990) released 7 and 14 day old virgin females of this species into a cage containing a mouse and male mosquitoes, and observed that mating took place before blood feeding. The authors also noted that mating ability developed before that of blood feeding. On the other hand, mating before blood feeding may not be an obligatory sequence for some species as there are a few examples of blood feeding before mating among other species of anophelines, for example, *An. subpictus* Grassi, *An. gambiae*, *An. culicifacies* Giles, *An. stephensi* (Roy 1940; Gillies 1954; Reisen and Aslamkhan 1976; Reisen *et al.* 1977).

On average, the time and energy costs, and risks associated with host seeking and blood feeding (include death due to defensive behaviour of hosts) are presumably higher than those of locating a mate. Therefore, an unfed, virgin female that opportunistically detects a host and feeds would be saved the costs of host seeking. On the other hand, mating might be required to effect some behavioural and physiological changes in the virgin female, which result in the conversion of flight activity from mate- to host-seeking (Jones and Gubbins 1977), and facilitate the digestion of blood-meals (Downe 1975). However, giving up a feeding opportunity has an apparent cost: any female mosquito that forgoes host feeding in the absence of a mating opportunity should suffer decreased fitness because feeding is further postponed and her future fitness will be discounted by mortality. This is a classic life history problem (Roff 1992).

Selection might therefore favour virgin, female anophelines that can mate either before or after blood feeding. Although there are suggestions of advantages due to the ability to feed opportunistically (see Rowland 1989; Lounibos 1994), these have rarely been demonstrated clearly, perhaps because this is difficult with live insects. Therefore, I have adopted the use of computer modeling to address the problem. The model, based on *An. gambiae,* compares the fitness (survival) of opportunistic females, that can feed before mating, with that of deterministic females that always mate before blood feeding. The main questions addressed are: (1) is it advantageous for virgin females to blood-feed before mating if the opportunity presents itself? (2) if so, under what ecological conditions? (3) why is it less commonly observed than mating before blood feeding? The discussion is largely restricted to anophelines because the different genera of mosquitoes are known to differ widely in their behaviour. Thus, extrapolating information across genera must be done cautiously.

The deterministic behaviour

Most mosquitoes studied appear to mate before blood feeding (Herms 1961, Quraishi 1965, Busvine 1980, Belton 1983). Substances from the male accessory gland contributed along with spermatozoa during copulation effect some physiological changes in the female. For example, in *An. gambiae,* the accessory gland substance may alter the pattern of female circadian flight activity from mate- to host-seeking (Jones & Gubbins 1977). The accessory gland secretion may ensure that females are inseminated only once in their life-time as demonstrated in *An. gambiae* (Goma 1963, Bryan 1968). In addition, the secretion may modify biting behaviour (Judson 1967), stimulate oviposition (Leahy & Craig 1965), probably enhance longevity (Liles 1965), and facilitate the digestion of blood meals (Downe 1975). An inseminated female then can proceed with host seeking and blood feeding.

The cost of this deterministic behaviour should include a discount of fitness by mortality due to postponed feeding. The size of this cost should vary for different ecological situations, thus, the best approach to understanding this phenomenon is probably through theory development and computer modeling. Below is a description of one such approach.

The models

I define a "deterministic" mosquito as a female that always mates before blood feeding, and that would not blood-feed if it encountered a potential host before it had mated. In contrast, I define an "opportunistic" mosquito as a female that can blood-feed if it encountered a host before mating.

To evaluate the evolutionary cost of deterministic relative to opportunistic behaviour, two models were developed, each tracking the fitness of 1000 virgin, female, anopheline-like mosquitoes. The models are essentially the same except that one (deterministic) simulates females that must mate before blood feeding (Fig. 5.1), whereas the other (opportunistic) simulates those that can feed if they encountered a host before they have mated (see Fig. 5.2). Below, I first describe the deterministic model and then detail the changes made to effect an opportunistic simulation.

The simulations begin with 1000, unfed, unmated, anautogenous mosquitoes, each with an initial fitness (F) of 1 (that is, no intraspecific variation).

For 1-3 days after emergence of the adult female mosquito, there is an initial period of inactivity (Laarman 1955; Davis 1984) during which the exoskeleton hardens and lactic acid receptors mature (Davis 1984). I assume that some mortality ($1 - \mu_0$) occurs during this period. Thus, updated fitness (F_1) after hardening is:

$$F_1 = 1 * \mu_0 \tag{1}$$

where: $\mu_0 = 0.97$

I assume that mosquitoes emerge near mating sites and, after the cuticle hardens, engage in subsequent activities at or near emergence sites. Mating is assumed to proceed as follows: males form swarms, females approach the aggregation, pair with one male and leave the swarm (Quraishi 1965). I define p_{mate} as the probability that a searching female would locate a mate within a 10 minute search. p_{mate} was allowed to vary among different runs of the model (see Table 5.1 for variable values). I define p_{host} as the probability that a searching mosquito would locate a host during a similar 10 minute search. Given that hosts and mates are located independent of one another, the following four encounter probabilities were defined:

$$P_{both} = P_{mate} * P_{host} \tag{2}$$

$$P_{mateonly} = P_{mate} * (1 - P_{host}) \tag{3}$$

$$P_{hostonly} = P_{host} * (1 - P_{mate}) \tag{4}$$

$$P_{neither} = (1 - P_{mate}) * (1 - P_{host}) \tag{5}$$

During each ten-minute interval, one of the above four mutually exclusive events was stochastically determined by comparing their values with uniformly distributed (0,1), randomly generated values on a desktop computer (Macintosh IIfx). Whenever females encountered both hosts and mates, I assumed that they would choose to mate; when hosts were encountered, they were ignored. Fitness was further discounted during each ten-minute interval by:

$$F_2 = F_1 * \mu_1 \tag{6}$$

where $\mu_1 = 0.99$

Deterministic mosquitoes were forced to search for mates with appropriate fitness discounting until a mate was encountered. I assumed that encounters with mates always led to insemination, at which point females began a host-search. During each ten-minute search, successful host encounter was again determined stochastically as described above with fitness discounting for each search period as:

$$F_3 = F_2 * \mu_2 \tag{7}$$

where $\mu_2 = 0.99$

Encounters with hosts were assumed to lead to feeding attempts. Successful feeds were determined stochastically by comparing a randomly drawn value (0,1) with the constant probability of landing and feeding, $p_{landfeed}$ (0.60). Each attempt led to further fitness discounting due to the danger associated with feeding, thus:

$$F_4 = F_3 * \mu_3 \tag{8}$$

where $\mu_3 = 0.60$

Anophelines always mate and feed at different locations. Thus, females were allowed to make host seeking trips. The model assumes that the "decision" to leave a habitat to engage in a host seeking trip is based on a function of not finding a host. Thus, the probability that an unsuccessful individual would leave a habitat was defined by:

$$p_{leave} = (1 - e^{(-\theta\nu)}) \tag{9}$$

Where: θ = the innate tendency to abandon a habitat and ν = number of unsuccessful searches in the current habitat.

Three different θ values for different runs representing high, moderate and low tendencies to abandon habitats were considered (Table 5.1). Whether an individual actually abandoned a habitat was again determined stochastically as described above. Each move to a new habitat was accompanied by a corresponding reduction in fitness:

$$F_5 = F_4 * \mu_4 \tag{10}$$

where $\mu_4 = 0.90$

Finally, following blood feeding, females sought out oviposition sites with concomitant survival dependent upon the number of habitat switches made during host search. Thus, fitness was further reduced by:

$$F_{final} = F_6 * e^{-(\rho * \tau)} \qquad (11)$$

Where: ρ = trip cost parameter and τ = number of habitat switches due to host seeking trips. ρ varied among runs (Table 5.1) to reflect differences among environments.

Opportunistic simulations were modelled in the same manner as described above except that if a host was encountered during mate search, feedings were attempted. Whenever females encountered both hosts and mates, however, I assume that they would choose to feed. Blood-fed virgins would continue to search for mates until mated and would then seek out oviposition sites, whereas mated, unfed females would then enter into host search as described for deterministic individuals. Thus, the only difference between "deterministic" and "opportunistic" females was that only the latter could take advantage of encounters with hosts while searching for mates.

I compared the success of the two search strategies by comparing mean F_{final} for different environments where availability of hosts and mates varied (Table 5.1).

Results

Fig. 5.3 shows the relative fitness of opportunistic compared with that of deterministic mosquitoes at various probabilities of finding hosts and mates, with a moderate tendency to abandon a habitat to host-search.

The simulations demonstrate that opportunistic females had a higher fitness than deterministic ones in all cases. This advantage is greatest when both mates and hosts are scarce. As the probability of finding hosts and mates increases, the advantage of opportunistic behaviour decreases. However, opportunistic females always had a higher fitness than deterministic mosquitoes. The same pattern was observed in simulations of mosquitoes with the tendency to abandon a habitat rapidly or slowly. Varying the survival parameters and $p_{landfeed}$ did not change this pattern.

Discussion

Mosquitoes, like all organisms, face trade-offs. The reward for successfully mating and blood feeding are survival and reproduction. The costs associated with locating a host include energy and searching time (considering that they are rather short-lived insects). In addition, blood feeding carries the risk of death due to host defensive behaviours. Though mating swarms are subject to high predation (Yuval and Bouskila 1993), females spend very little time therein (Quraishi 1965). Thus, on average, it would seem that blood feeding is a more costly activity than mating. Accordingly, unfed, virgin females that choose to blood-feed if both hosts and mates are encountered would likely minimize costs while maximizing benefits.

The present model demonstrates a higher payoff for opportunistic mosquitoes that are capable of blood feeding if they encounter a host before mating regardless of abundance of mates or hosts. However, the payoff appeared to be highest where the probability of finding a host was quite low. This agrees with Rowland's (1989) suggestion that it would be advantageous for virgin female *An. stephensi* to blood-feed if they encountered hosts, particularly when the probability of finding or successfully feeding on one is low. Equally, the model demonstrates that opportunistic blood feeding should be very advantageous where males are scarce as suggested by Lounibos (1994). Opportunistic females had higher fitness than deterministic females under all conditions tested in the model. The difference in fitness derives from search costs for blood-hosts.

Though the model did not incorporate multiple blood-feeding, which is common among anophelines, I doubt that this behaviour would significantly alter the results since the difference between opportunistic and deterministic females is largely due to forgoing the opportunity to obtain the first blood meal.

The model did not consider body size variation in simulated females. However, mosquito populations are known to vary in body size, a consequence of the quality of the larval habitat (Fish 1985; Nasci 1987). Moreover, larger females emerge with more metabolic reserves than smaller ones (Briegel 1990), and consequently, may live longer than smaller females (Chapter 2). Therefore, it is probably the case that the cost of deterministic behaviour (that is, reduction of future fitness) would affect smaller-sized females more significantly than larger females.

Although there are reports of blood feeding before mating in the field, for example, *An. subpictus* (Roy 1940), *An. gambiae* (Gillies 1954), *An. stephensi* (Reisen *et al.* 1977), and *An. culicifacies* (Reisen and Aslamkhan 1976), it would seem that it is not commonly observed despite the potential pay-off demonstrated by the present model. Even for those species that can blood-feed before mating, only a fraction of the female population does so. For example, only 16% of female *An. culicifacies* collected at bovid baits by Reisen and Aslamkhan (1976) were virgins. Similarly, Rowland (1989) reported that though female *An. stephensi* can feed opportunistically, they usually mate first. Therefore, one must question why mating before blood feeding is more commonly observed than feeding before mating.

Rowland (1989) demonstrated that flight activity was depressed in mated, blood-fed *An. stephensi* but not in unmated, blood-fed females who remained active. Therefore, the unmated but blood-fed female probably does not lose synchrony with male activity and should be able to mate after blood feeding. Though there are no studies on the effects of a blood meal on mating performance, it is unlikely that blood-fed, virgin females are less attractive to males, since competition for females by swarming males is usually intense with the result that only a few males in a swarm mate. Therefore, a female should not be disadvantaged by blood feeding before mating. However, further studies with live insects are needed to verify this.

Another reason few biting virgins are collected could be that mates may be far more abundant than hosts so that most virgin females simply encounter a mate first. As shown in the present study, where mates are extremely abundant, the advantages of feeding opportunistically decline sharply. Therefore, even if virgin females are capable of feeding before mating, they probably mate first, since they likely encounter mates before hosts.

Another reason the deterministic behaviour is more commonly observed may be due to differential viability of sperm compared with blood. Sperm may remain viable in female mosquitoes for a long time. In fact, most female anophelines mate only once in their life time (Goma 1963, Bryan 1968). On the other hand, blood may not be used for reproduction if a blood-fed female is somehow unable to locate a mate. In addition, the added burden of carrying the weight of blood may offset the advantages of opportunistic behaviour.

The inability of some species to feed opportunistically may be due to physiological constraints. For example, the inability of newly emerged females to host-seek and blood-feed for 1-3 days after emergence has been correlated with non-responsiveness of antennal lactic acid receptors (Davis 1984). As the receptors mature, females appear to become capable of detecting and responding to hosts (Davis 1984). Similarly, unmated females of some species may simply be unable to take a meal because mating might be required to regulate feeding and probably trigger other activities.

Klowden (1990) reported that mosquito behaviour is effected through responses to a complex of endogenous and exogenous factors. I have not incorporated all known variables into this model as this would be a very complex task. However, work is on-going to put the activities of the gonotrophic cycle in a spatial and temporal context, so that it can more closely reflect anopheline behaviour.

Fig. 5.1. Flow chart of deterministic behaviour.

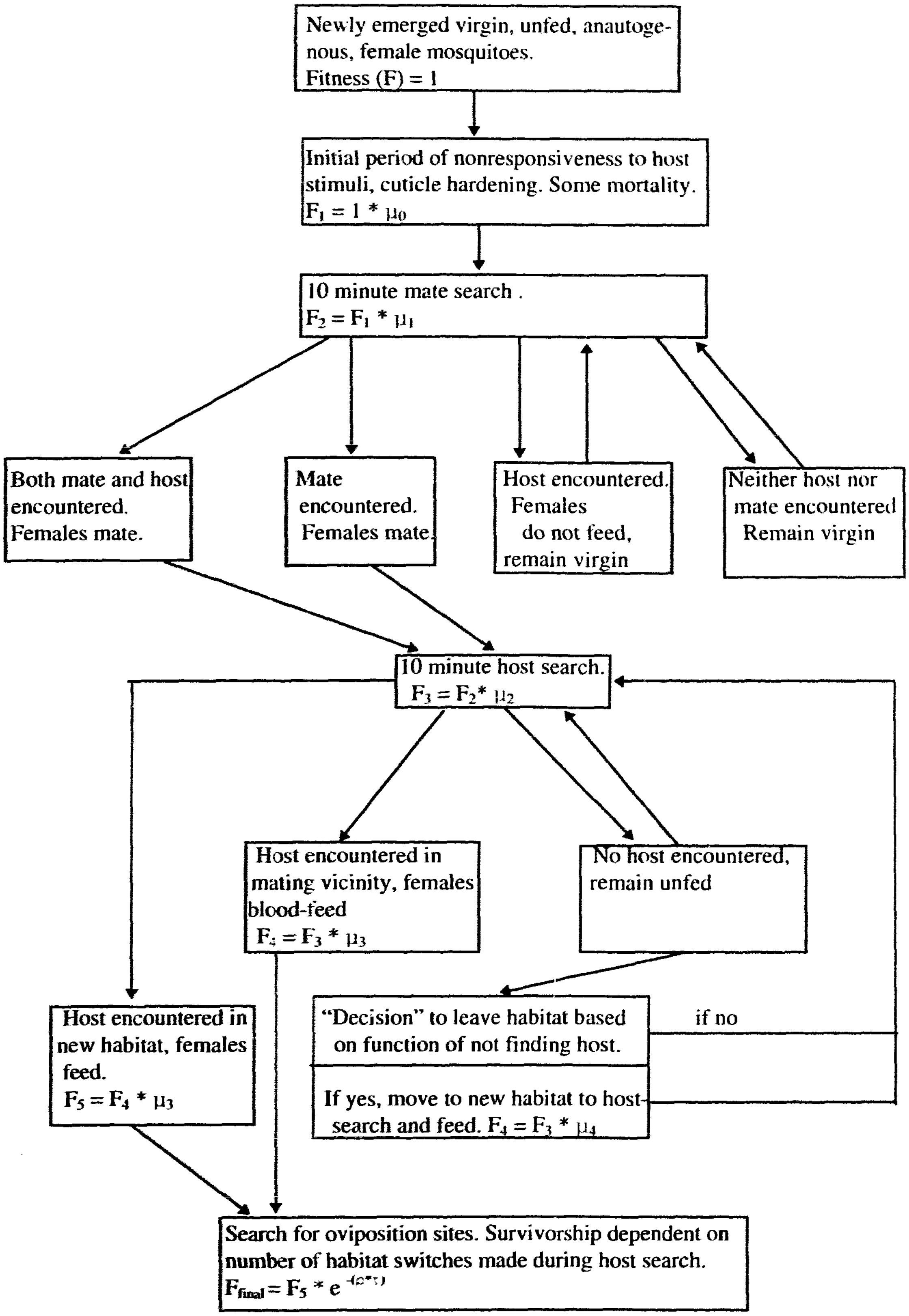

Fig. 5.2 Flow chart of opportunistic behaviour

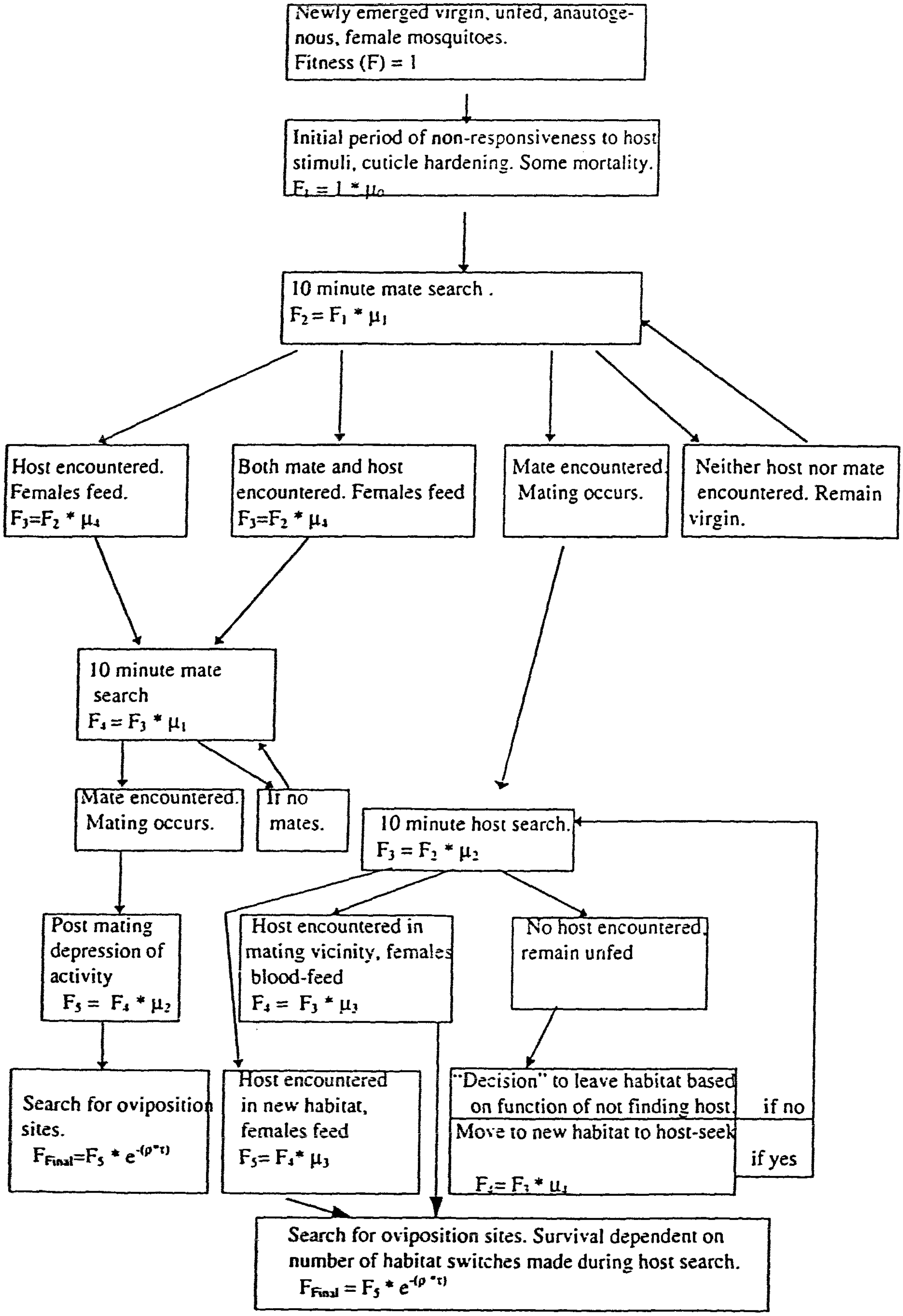

Table 5.1 Parameters and values used in the simulations.

Parameter	Value
p_{mate}	0.01, 0.05, 0.1, 0.2, 0.4
*p_{host}	0.01, 0.05, 0.1, 0.2, 0.4
$p_{landfeed}$	0.60
Tendency to leave habitat to host seek (θ)	rapid (0.2), moderate (0.1), slow (0.05).
Cost of locating oviposition site (ρ) as a function of number of host seeking trips.	high (0.2), moderate (0.1), low (0.05)

*Each p_{host} value was simulated for all p_{mate} values.

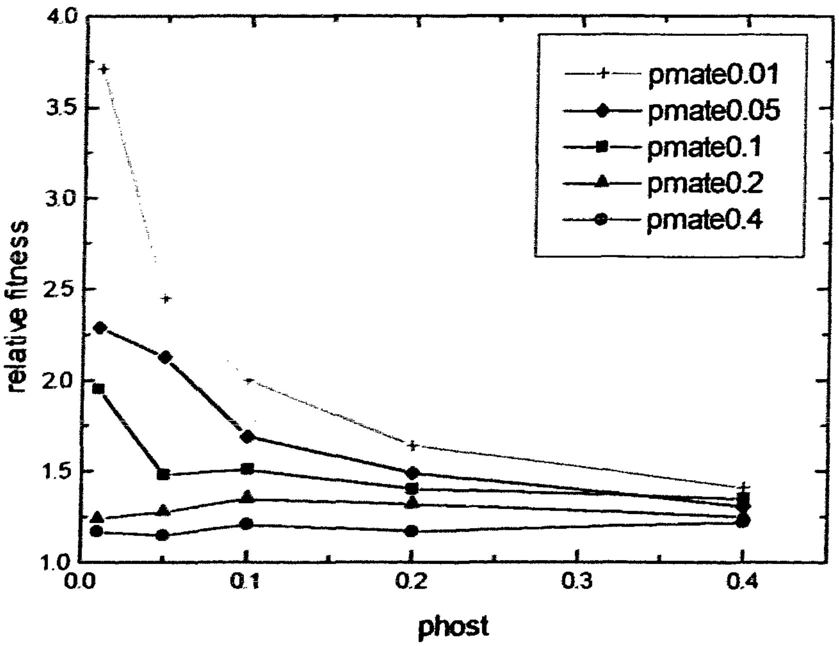

Fig.5.3 Relative fitness (survival) of simulated opportunistic and deterministic female anophelines. Each point on the graph represents the ratio of the mean fitness of 1000 opportunistic and 1000 determinstic females at various mate and host densities.

Chapter 6

General Conclusions

In North America, *Ae. togoi* is restricted to breeding only in rock pools on the Pacific coast. It seems likely that very dense larval populations may occur because it breeds continuously in the same pools. This may lead to food shortage due to larval competition. My laboratory studies show that the probable strategy for dealing with food shortage in this species is to prolong pre-adult development, so that when more food becomes available, larvae complete their development. However, females reared under more densely populated conditions are smaller and probably have reduced survival compared with females from less densely populated conditions. Although caged females responded to the presence of conspecific eggs by ovipositing less frequently in such cups, it seems they are more likely to encounter pools that already contain conspecifics, but will probably oviposit in several rock pools to enhance the survival of at least some of an individual's offspring.

My models of mating and feeding strategies of anopheline mosquitoes show that host and mate availability probably influence the chronology of events in the lives of these mosquitoes. However, several other endogenous and exogenous variables also influence mosquito behaviour. Some of these will be built into the models, hopefully, to better understand the more important factors.

Appendix 1. Selection of oviposition site by female *Ae. togoi*[1] provided with larval rearing water (LRW) and rearing medium (RM).

Female I.D	LRW cup 1	LRW cup 2	LRW cup 3	LRW total	RM cup 1	RM cup 2	RM cup 3	RM total
1	8	53	76	137	2	16	16	34
2	17	17	7	41	0	0	0	0
3	51	79	55	185	0	0	0	0
4	102	41	0	143	0	0	0	0
5	32	41	41	114	0	0	0	0
6	45	0	134	179	0	0	0	0
7	10	132	89	231	0	0	0	0
8	130	58	17	205	0	0	0	0
9	0	3	150	153	0	0	0	0
10	101	61	40	202	2	3	2	7
11	0	167	0	167	0	0	17	17
12	199	0	0	199	0	0	0	0
13	50	13	207	270	0	0	0	0
14	35	67	43	145	0	0	0	0
15	19	32	13	64	0	0	0	0
16	86	0	0	86	0	0	0	0
17	7	51	98	156	0	0	0	0
18	10	0	0	10	4	0	0	4
19	0	36	24	60	0	0	0	0
20	201	0	0	201	0	0	0	0
21	29	22	89	140	20	24	25	69
22	6	0	0	6	63	19	86	168
23	111	65	24	200	0	0	0	0
24	11	3	1	15	0	0	0	0
25	34	0	56	90	0	8	0	8
26	25	0	52	77	17	0	16	33
27	44	45	82	171	10	1	2	13
28	142	0	0	142	0	0	0	0
29	46	67	105	218	0	0	0	0
30	35	48	94	177	0	0	9	9
Total	1586	1101	1497	4184	118	71	173	362

[1] Each individual was provided with 3 cups of LRW and 3 of RM.

Appendix 2. Selection of oviposition site by female *Ae. togoi*[1] provided with larval rearing water (LRW) and food-containing rearing medium (FRM).

female I.D	FRM cup1	FRM cup2	FRM cup3	FRM Total	LRW cup1	LRW cup2	LRW cup3	LRW Total
1	0	89	0	89	0	0	107	107
2	0	0	0	0	0	224	0	224
3	0	0	0	0	0	78	0	78
4	24	0	0	24	28	27	0	55
5	0	0	107	107	0	0	115	115
6	81	25	55	161	0	0	0	0
7	0	0	0	0	0	0	97	97
8	2	0	0	2	113	20	0	133
9	0	0	54	54	0	0	30	30
10	0	0	0	0	29	0	106	135
11	27	0	0	27	84	55	14	153
12	0	7	4	11	3	110	0	113
13	0	84	51	135	13	27	32	72
14	0	0	0	0	42	0	101	143
15	21	9	19	49	41	0	0	41
16	58	0	165	223	0	0	0	0
17	0	0	0	0	0	0	64	64
18	0	0	0	0	146	41	30	217
19	0	0	0	0	0	177	0	177
20	148	69	0	217	13	0	0	13
21	29	16	0	45	0	0	0	0
22	0	0	0	0	187	24	0	211
23	20	0	0	20	48	48	65	161
24	0	0	72	72	0	0	0	0
25	0	0	16	16	1	16	49	66
26	0	10	33	43	0	2	40	42
27	89	0	15	104	0	0	131	131
28	0	0	31	31	18	16	13	47
Total	499	309	622	1430	766	865	994	2625

[1] Each individual was provided with 3 cups of LRW and 3 of FRM.

Appendix 3. Selection of oviposition site by female *Ae. togoi*[1] between cups with and without conspecific eggs.

Female I.D	with eggs cup 1	with eggs cup 2	with eggs cup 3	with eggs total	no eggs cup 1	no eggs cup 2	no eggs cup 3	no eggs total
1	71	0	0	71	0	41	46	87
2	108	6	18	132	31	12	0	43
3	0	0	0	0	0	0	173	173
4	41	0	0	41	90	57	21	168
5	0	0	0	0	16	0	140	156
6	0	0	0	0	1	138	0	139
7	0	0	0	0	157	0	36	193
8	0	9	0	9	92	33	0	125
9	0	0	132	132	14	39	0	53
10	51	13	0	64	43	23	3	69
11	0	0	0	0	32	113	40	185
12	129	22	0	151	0	14	24	38
13	0	0	24	24	15	76	92	183
14	12	13	31	56	16	41	66	123
15	117	0	0	117	0	0	72	72
16	0	0	0	0	53	70	0	123
17	0	191	0	191	0	0	0	0
18	0	0	0	0	38	20	1	59
19	67	63	46	176	15	12	0	27
20	0	0	0	0	139	45	0	184
21	0	45	70	115	51	9	0	60
22	0	31	134	165	0	46	49	95
23	0	0	0	0	0	85	0	85
24	0	0	0	0	125	0	0	125
Total	596	393	455	1444	928	874	763	2565

[1] Each female was isolated and provided with 6 cups of larval rearing water (LRW), 3 containing 50 conspecific eggs, and 3 with no eggs.

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