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dissertation entitled

AEDES TRISERIATUS AND TREEHOLES; TROPHIC INTERACTIONS AND FACTORS INFLUENCING LARVAL GROWTH

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Entomology

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Date 24 August, 2000

AEDES TRISERIATUS AND TREEHOLES; TROPHIC INTERACTIONS AND FACTORS INFLUENCING LARVAL GROWTH

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Natural Science

2000

ABSTRACT

AEDES TRISERIATUS AND TREEHOLES; TROPHIC INTERACTIONS AND FACTORS INFLUENCING LARVAL GROWTH

By

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The eastern treehole mosquito, *Aedes triseriatus* (Say), and the ecosystem it inhabits provide a valuable model system for studying ecosystem processes and factors affecting growth and development. Studies reported here were directed at two aspects of the biology of this mosquito in treeholes: (1) trophic interactions among larvae and microorganisms in treehole water; and (2) an experimental analyses of interacting factors affecting growth and metamorphosis of larvae. With regard to trophic interactions, the trophic cascade hypothesis, and the "top-down/bottom-up" hypothesis were tested. When a trophic cascade operates in an ecosystem, changes in abundance of organisms in higher trophic levels result in a cascade of changes in abundance or biomass of organisms in lower trophic levels, with alternating directional responses in alternating lower trophic levels. By contrast, top-down/bottom-up forces operate such that abundance or biomass of organisms at any given trophic level is dependent upon the input of nutrients into the lowest trophic level in the ecosystem. Theory predicts that these inputs should have positive but diminishing effects of the same direction with succeeding, higher trophic levels. A series of laboratory and field experiments

showed that larvae of *A. triseriatus* are a keystone predator in treehole ecosystems due to the significant effect of their presence on the abundance of protists and bacteria. There was no clear evidence for a trophic cascade in treeholes. Experiments involving nutrient manipulations showed the primary nutrient affecting higher trophic levels was organic resources (senescent leaf detritus); anionic nitrogen and sulfur were not stimulatory.

Other experiments were directed at growth and metamorphosis of *A. triseriatus* larvae when temperature and basal food resources were manipulated. Theory on the population reaction norm of ectothermic animals predicts that animals should have decreased development rates and larger body sizes at metamorphosis at low temperatures compared to the converse at high temperatures; but that development rates and body sizes should be negatively correlated when food supply is varied. Experiments showed that these contrasting predictions held true when cohorts of larvae were subjected to experimental combinations of food and temperature; but there were no direct statistical interactions between food supply and temperature. However, when larvae were exposed to variable food and temperature individually, these reaction norm responses differed: body size was similar among larvae reared at different temperatures. Larval cohort mortality may affect these responses by dynamically affecting the ration of food supply for larvae as temperature dependent mortality occurred. Overall, the results indicated that a density-dependent reaction norm response would be predicted from these findings.

I would like to dedicate this dissertation to my parents for their confidence that I could and would get this done.

Acknowledgments

I would like to acknowledge the funding of this research from NIH grant AI-21884. I would like to thank my guidance committee for their help in accomplishing this dissertation. I would also like to acknowledge the assistance of Mike Kaufman and Tracy Smith for their technical knowledge and support.

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CHAPTER 1

Literature Review

Overview

Mosquitoes are responsible for transmitting the causative agents of some of the most widespread and prevalent infections of humans, including malaria, lymphatic filariasis, yellow fever, dengue fever, and the encephalitides. These relationships have been well reviewed elsewhere and will not be repeated here (e.g. Aultman et al. 2000). Although these diseases remain highly important causes of morbidity and mortality in tropical regions of the world, such as Africa, Asia, and South America, they historically occurred in temperate areas as well. In regions such as North America and Europe, mosquito-borne disease prevalence has dramatically declined due to indirect effects such as increased standards of living and improved medical care, and to direct effects such as vector control, habitat alterations, and intentional barriers blocking mosquito contact with humans (i.e., window screens, repellents). However, the United States, the European Economic Community, and other members of the 'First World' continue to be the centers and funders for research on arthropod-borne diseases, and for the development of anti-vector tools, medicines, and vaccines. The significance of mosquito-borne disease was dramatically imposed upon citizens of the United States during the recent outbreak of the West Nile virus in New York City and surrounding areas in 1999 and continuing into 2000. This virus, similar to St. Louis encephalitis and known to be vectored by species of

mosquitoes in the genus *Culex*, could spread beyond this region if measures are not taken to control the mosquito populations and/or the bird host populations harboring the virus.

In order to control populations of disease vectors and, in turn, control the disease agents they transmit, there must exist an extensive and thorough knowledge of the life cycle and ecology of these arthropods. The contrasting demands of a well-educated public to control medically-important and pest mosquitoes, on the one hand; while simultaneously protecting the environment from long-term effects of chemical insecticides, on the other hand, only reinforce the need for biological studies on vectors (Aultman et al. 2000).

There are approximately 2500 species of mosquito worldwide with roughly 150 species in the United States, including many species that participate in disease transmission cycles. The family *Culicidae* is biologically diverse, and indeed there is a rich history of investigations into the biology, ecology, physiology, biochemistry, molecular biology, systematics, and general life history of this important insect group (Clements 1992). Despite their diversity, the biology of mosquitoes can be distilled into basic components. The egg is laid by the mated female mosquito on top of water either in rafts or singly; or the eggs are laid in moist or dry soil or similar environments that will eventually be flooded with water. Tiny larvae, first instars, hatch from the eggs by bursting the top of the egg off using a specialized structure on the head capsule called an egg burster. These larvae then enter the water, swim about, and feed in the water column and along submerged surfaces using a highly complex mouth part

arrangement (Merritt et al. 1992). The larvae gain mass, and molt three times (passing through the second, third, and fourth instars), and then molt again to enter a motile, aquatic, and non-feeding stage called a pupa. An adult male or female then emerges from the pupa on top of the water, and after sclerotization, melanization, and wing stretching, it flies away. Males meet females for mating in a variety of ways, such as swarming or on substrates. Both males and females seek nectar for carbohydrate nutrient. Females of many species also seek vertebrate hosts for blood-feeding. The females utilize the blood physiologically to develop eggs. A single female mosquito may take many blood meals in her lifetime. Indeed, it is the serial process of blood-feeding that permits acquisition and transmission of disease agents. The above review is a generalization, and there are variations from it.

A variety of environmental factors - both biotic and abiotic - contribute to the growth and development of larval mosquitoes and to the consequent production of adults from individual larval habitats. These factors include physical and chemical water regime in the aquatic habitats, water temperature, quantity and quality of food available to larvae, and intensity of predation and parasitism (Clements 1992, Laird 1988). Larvae are thought to compete in a density dependent manner for food resources in many habitats, particularly in confined ones (see below). Hawley (1985) has proposed that a linkage exists between larval performance in habitats, adult production, the variation in adult female size that results from larval competition, and population dynamics. Physical features of adult mosquitoes resulting from competition and other factors in the larval

environment, leading to variation in adult body size, may contribute to their susceptibility to disease agents, their longevity, and consequently to their capacity as vectors (Hawley 1985, Clements 1992). As Washburn et al. (1988, 1989, 1991) discovered in studies involving protozoan and fungal parasites of *Aedes sierrensis*, the western treehole mosquito, the interaction of the mosquito with environmental parameters such as food availability, intraspecific competition for food, and even specific feeding behaviors profoundly influence the population's dynamics. Under specific conditions, natural enemies of larval mosquitoes may actually increase the fitness of certain adults by releasing these nonparasitized survivors from competition, and thereby increasing their body size and fecundity.

By examining the biology of mosquitoes from the viewpoint of interactions between mosquito populations and the ecosystems in which they live, we can gain a better understanding of the role that environmental factors play in larval development, adult mosquito production and fitness, and population dynamics. Examination of the food web dynamics involving larval mosquitoes and their microbial prey can provide insight into the relationships between larval mosquitoes and the natural food resources available to them. Increasing our knowledge of how manipulation of these environmental conditions can influence biological development will ultimately provide information as to an organism's ability to carry and transmit disease.

Mosquitoes in Container Habitats

Some 40% of all mosquito species have as their larval habitat small bodies of water formed in natural or artificial containers, such as leaf axils, bamboo internodes, treeholes, tires, rock holes, cemetery urns, and the like (Frank and Lounibos 1983). These habitats, called phytotelmata (from the Greek 'phyto' meaning plant, and 'telma' meaning pond or pool; Frank and Lounibos, 1983), contain a community of insects and microorganisms. Mosquitoes often figure prominently in the community living in phytotelmata. Because phytotelmata are small, discrete, often numerous, and can be sampled fairly easily, they have been viewed as excellent model systems for a variety of studies including community ecology, population ecology, and ecosystem modeling (e.g. Kitching (1983a, b, 1987). One of the most common and often investigated types of phytotelmata is treeholes. A generalized food web model of aquatic treeholes in temperate regions involves input of organic detritus in the form of senescent leaves (Kitching 1983a, b). Water enters the treeholes either as throughfall from the forest canopy or along the sides of the trees in the form of stemflow (Eaton et al. 1973). Stemflow washes inorganic ions into treeholes that may serve as nutrient resources for microorganisms associated with decomposing leaf litter (Kitching 1971, Carpenter 1982, Fish 1983, Fish and Carpenter 1982, Walker et al. 1988, Walker et al. 1991).

The leaf detritus-based conceptualization of treehole ecosystems has leant itself to a rigorous series of laboratory and field tests. Those studies conducted in the laboratory have involved plastic dishes designated as microcosms meant

to simulate natural treeholes, whereas studies in the field have used microcosms set up as treehole simulations, or have involved manipulations or sampling of natural treeholes. The bulk of studies have involved mosquitoes. Larval mosquito growth was found to be primarily a function of leaf litter ration available per larva across a range of larval densities (Fish and Carpenter 1982, Carpenter 1983), but variation in leaf quality also dramatically affected growth (Walker et al. 1997). Mosquito growth in microcosms with stemflow water was only slightly greater than that in microcosms with distilled water, suggesting that the influences of stemflow water on mosquito growth are minor compared to the influences of leaf detritus (Carpenter 1982). However, Walker et al. (1991) found that stemflow was stimulatory to growth, perhaps through a physical process of adding nutrients while diluting potential toxins such as ammonia and hydrogen sulfide. Field tests of the leaf detritus-based model showed strong differences in mosquito productivity between artificial treeholes with leaves compared to artificial treeholes with no leaves (Walker et al. 1991). In contrast, a field test found no difference in mosquito production between natural treeholes with an average amount of senescent leaf mass and treeholes from which leaves had been removed, indicating that other factors besides leaves impinge upon mosquito growth (Walker and Merritt 1988). More recently, Leonard and Juliano (1995) refuted these results with an intervention experiment involving caged mosquitoes that were provided or not provided a leaf substrate in real treeholes. The realism of caging mosquito larvae is certainly questionable.

Because of the small size of phytotelmata, the high densities of larvae in the habitats, and the fact that food is often in short supply, populations of mosquitoes inhabiting phytotelmata are thought to be regulated primarily by biotic factors such as density-dependent larval competition for food, facilitative density-dependent interactions among trophic levels in food webs, predation, and parasitism (Istock et al. 1975, 1976; Siefert 1984; Fish and Carpenter 1982; Livdahl 1982; Bradshaw and Holzapfel 1983; Chambers 1985; Frank et al. 1985; Hawley 1985; Lounibos 1985; Kitching 1987; Walker et al. 1987).

The Biology of *Aedes triseriatus*

Aedes triseriatus, the eastern treehole mosquito, is a container-breeding mosquito of North America. It is the subject of the research reported here. Because of its role as a vector of La Crosse encephalitis virus and dog heartworm, there is extensive literature on the basic biology of this species (Craig 1983), which is reviewed here. The geographic range of *A. triseriatus* extends from Texas to Manitoba, and eastward to New England, Ontario and Quebec (Darsie and Ward 1981). As with all mosquitoes, the life cycle of *A. triseriatus* involves an aquatic larval stage and a terrestrial adult stage. Gravid females lay eggs on the sides of water-filled containers in clusters at the air/water interface. Larvae of this species hatch from the eggs beginning in the spring, between March and April, in response to changes in photoperiod, temperature and a drop in oxygen concentration experienced when the eggs are flooded (Shroyer 1978, Gjullin et al. 1941, Borg & Horsfall 1953, Judson 1960). The hatch is asynchronous due to both a variation in response to the oxygen concentration

(Livdahl & Koenekoop 1985) as well as the influence of variable larval densities (Livdahl et al. 1984, Livdahl & Edgerly 1987). An asynchronous hatch provides a population structure with larvae of various developmental stages present at the same time.

Larvae have four instar developmental periods prior to pupation. The length of time for development through these stages depends on factors such as intraspecific competition for food (McCoombs 1979, Fish and Carpenter 1982) and temperature (Shelton 1973). Upon the fourth molt, the larvae become pupae. Adults emerge from tree holes in northern states from mid-June through September (Sinsko and Craig 1979, Scholl and DeFoliart 1978). There can be either one or two generations per year depending upon environmental conditions, in particular frequency of rainfall. Eggs of *A. triseriatus* may enter diapause beginning in August and delay hatching until the following year in a temperate climate (Shroyer & Craig 1980). In southern climates the late instar larvae may also enter diapause to overwinter (Simms 1982).

Male *A. triseriatus* adults emerge sooner than females, when eggs of both sexes are hatched at the same time. Females mate and search for a blood meal within 3 days post emergence (Walker et al. 1987). *Aedes triseriatus* females prefer mammalian hosts and have the unusual behavior of selecting chipmunk and squirrel hosts during the daytime. Development of eggs in a female's body ranges between 48 and 96 hours dependent on temperature, after which she deposits her eggs on the container edge above the water line. The number of

eggs laid can vary from less than fifty to more than two hundred depending upon the fecundity of the insect.

As mentioned above, treeholes are a type of phytotelmata and form the primary habitat for *A. triseriatus* larvae. However, this mosquito has also invaded artificial habitats, including discarded cans, buckets, rain barrels and junked tires. Treeholes of southern Michigan consist predominantly of rainwater accumulating in basins formed by living roots of deciduous trees. *Aedes triseriatus* larvae are found in pans or rot holes of deciduous trees in eastern North America (Jenkins & Carpenter 1946, Mitchell & Rockett 1981, and Bradshaw & Holzapfel 1983). Rot holes are formed at a wound, which penetrates the bark of the tree, and a pan is a tree hole with a complete bark lining.

Inhabitants of treehole ecosystems of southern Michigan and other areas where *A. triseriatus* occurs include larval mosquitoes, larvae of scirtid beetles, chironomid midges, ceratopogonids, syrphids (Jenkins and Carpenter 1946, Snow 1949, Kitching 1971) and a variety of microorganisms including both bacteria and protozoa (Lackey 1940, Walker and Merritt 1988, Walker et al. 1991). As discussed above, the treehole ecosystem is generally considered to be a heterotrophic system wherein all life is supported by inputs of senescent leaf material and other detritus, which decomposes through microbial processing (Kitching 1983). Episodic rainfall disturbs the water column, and results in inputs of water as throughfall or stemflow, and may variously increase or decrease anion and cation concentrations depending upon initial conditions (Walker et al.

1991). Microorganisms are found in treeholes both adhering to solid surfaces, especially leaves, and in the water column (Kaufman et al. 1999). The microbial lawn exposed on leaf surfaces can be rapidly grazed to depletion by mosquito larvae, while leaf surfaces not exposed to larval feeding are teeming with microbial growth (Fish and Carpenter 1982, Walker and Merritt 1991, Kaufman et al. 1999). *A. triseriatus* larvae consume particulate organic matter, including the biofilm generated by the interaction of microorganisms and leaf detritus (Walker et al. 1988).

An understanding of the interactions between these multiple biotic and abiotic factors influencing larval mosquitoes would greatly benefit the understanding of *A. triseriatus* growth and development. As Walker et al. (1991) have observed with reference to the biology of *A. triseriatus*: "Because treeholes are small, are discrete, contain a tractable community of organisms, and can be manipulated and simulated, they provide an ideal experimental situation for examining biotic interactions and processes within an ecosystem, as well as influences of abiotic and physical factors on an ecosystem." This theme is adopted here.

Microcosms

Interactions between microbes and their predators have been extensively studied and revealed using microcosm experiments (Coleman et al. 1983, Verhoef and Brussaard 1990, Verhoef 1996). The objective of utilizing microcosms as simplified model systems is to measure processes and population dynamics accurately in order to test ecological theories (Daehler and

Strong 1996, Mikola and Setala 1998). Microcosms are tools for testing ecological theories because the composition of the community can be strictly controlled and replicated (Kareiva 1989, Balciunas and Lawler 1995).

Food Webs and Trophic Interactions

A food web is a diagram depicting the complex interactions between all organisms in a habitat, which create, process, and decompose organic material. These food web models are useful in applied research by providing an understanding of community structure and population interactions. The organisms that create, process, and decompose organic material interact hierarchically at various trophic levels in what has come to be known as a "food web". Each step by which this energy stored in organic material is shuttled between organisms is a transition between trophic level and the interrelationships of various trophic levels constitutes such a web. Recently, great attention has been paid to food web dynamics, with special interest in aquatic ecosystems (Carpenter et al. 1985, Matsen & Hunter 1992, Matveev 1995, Armstrong 1994, Tavares-Comar 1996, Gaedke 1995). In its simplest conceptualization, a food web consists of top, intermediate and basal trophic species.

Trophic species is a term used by some ecologists (Cohen and Newman 1985, Yodzis 1988, Cohen and Briand 1984, Pimm 1982) to describe a collection of organisms in an ecosystem which share common prey and predators. Individuals comprising the top trophic level are not eaten by any other trophic species in the web, while at the bottom of the food web are basal species,

including both primary producers and detritus, which feed on no trophic species. Individuals occupying the intermediate levels then are utilized as a resource by at least one other trophic species while also exploiting an additional trophic species as a resource. Each trophic level within an ecosystem is influenced both qualitatively and quantitatively by factors including, but not limited to: 1) availability of nutrients, 2) competition for resource acquisition, 3) predation by higher trophic levels, and 4) external disturbances of the system. Although the concept of trophic level obviously has limitations, it can be utilized broadly as a means of classifying trophic interactions within the food web concept.

Trophic Cascades

Predictions of biological processes stemming from food web interactions must be based on the relationships between multiple trophic groups and feedback responses induced by these relationships. Hairston et al. (1960) first proposed the idea that regulation of any population is determined by the trophic level to which it belongs. The ideas presented in a trophic cascade reflect a set of interactions rather than a flow of energy. For example, in a terrestrial ecosystem with three trophic levels (plants, herbivores and carnivores), the plants are limited by resources such as water and nutrients, the herbivores are limited by the abundance of plant biomass, and herbivore prey density limits the carnivores. The theory of trophic cascades states that these interactions lead to a strong influence of top-down regulation with the condition of alternating regulatory processes. This idea of a "cascading" system beginning at the top of the web

and flowing down through multiple trophic levels has been further developed to include the regulation by upper levels on lower level productivity (Carpenter et al. 1985, McNaughton 1985, Carpenter and Kitchell 1988). These ideas can be graphically represented as outlined in Figure 1.1. These alternating patterns are reflective of direct feeding relationships of trophic levels following a set of food chain linkages. These relationships necessarily exclude omnivory, i.e., organisms feeding at multiple lower trophic levels. Correspondingly, it is a simple, qualitative, and somewhat unrealistic theoretical construct.

Top-down/bottom-up theory

The top-down:bottom-up theory of McQueen et al. (1986) considers the combined influences of predators and nutrient availability on ecosystem structure (i.e., food web relationships). It contrasts with the trophic cascade concept, above, in that it deals with the quantitative nature of trophic interactions and allows for omnivory. This theory predicts that although the maximum attainable biomass of a food web is determined by nutrient availability, the actual biomass is determined by the combined effects of bottom-up and top-down forces. This theory is concerned more with the magnitude of changes in trophic levels both above and below a perturbed level. Top-down:bottom-up theory also predicts that a reduction in resources or nutrients available to the lowest trophic level will result in decreased abundance, biomass and/or production of organisms in higher trophic levels, and that this effect will become proportionately weaker with each higher trophic level (Fig. 1.2). Similarly, the theory predicts that top down

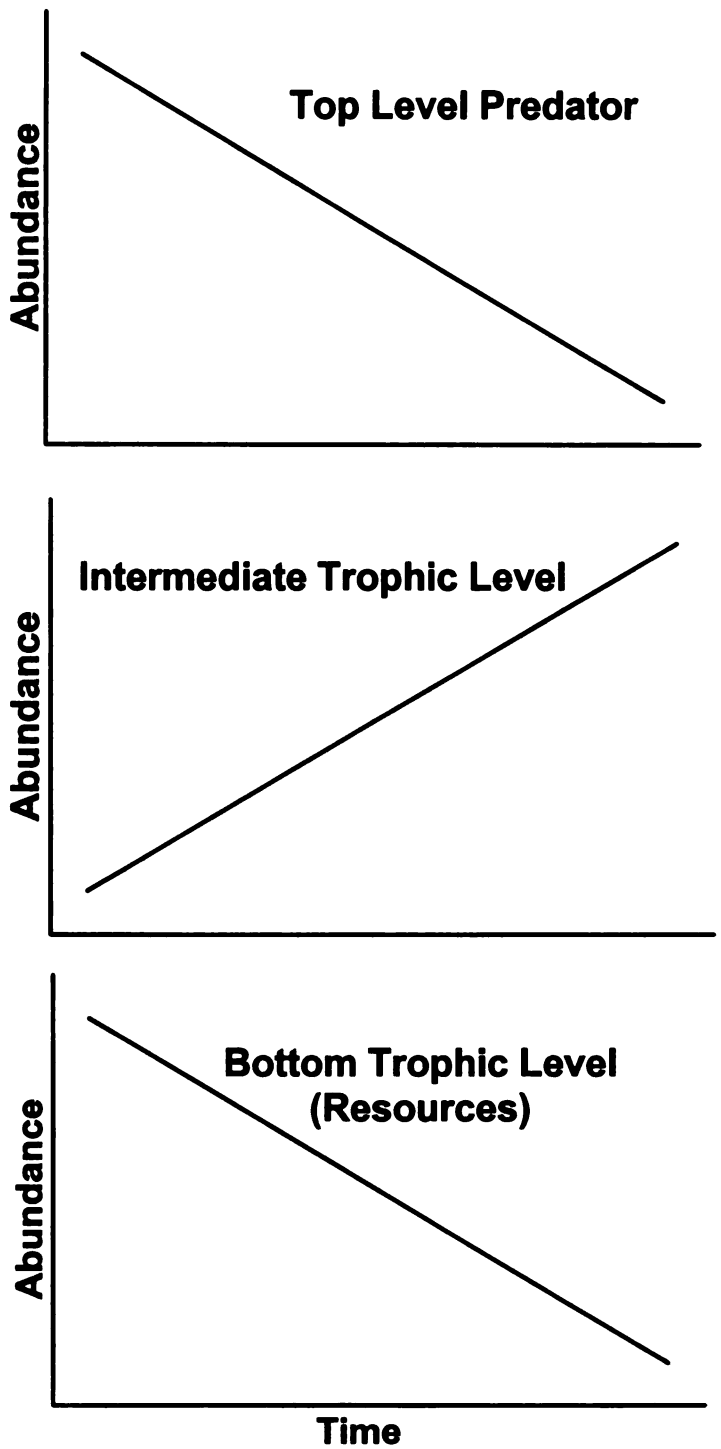

Figure 1.1 Descriptive example of a trophic cascade reaction to disturbance at the top trophic level.

forces will have their greatest effect at the highest trophic level but will dissipate as they reverberate through lower trophic levels.

The diminishing effect following top level disturbances appears to be substantiated with field observations in lake ecosystems by McQueen et al. (1989), as well as by analytical simulation by Herendeen (1995). Several aquatic studies also support the theory that "bottom-up" forces of increased productivity at basal trophic levels will result in increased abundance at all trophic levels (Abrams 1994, Mills & Shiavone 1982, Mittelbach et al. 1988, Leibold 1989). Although a dissipation of these effects of bottom-up forces to upper levels of the food chain has been observed by McQueen et al. (1989). Both Matveev (1995) and Persson et al. (1988) observed food webs in eutrophic lakes in which bottom-up impacts actually became stronger at higher trophic levels.

Theory of Trophic Interactions and the Tree Hole Ecosystem

The concepts inherent in the trophic cascade and top-down:bottom-up theories can be applied to a simplified model of *Aedes triseriatus* and the tree hole ecosystem of which it is a part. For the purposes of examination of *A. triseriatus* growth and development, the model food web in treeholes begins with organic detritus as the basal resource. The first trophic level contains the bacterial organisms that colonize the detritus, and/or subsist on the nutrients the detritus provides. The second, or intermediate trophic level is protozoans, because many protozoan species are predators of bacteria. At the top trophic level is the mosquito larvae which prey on microorganisms in the lower trophic

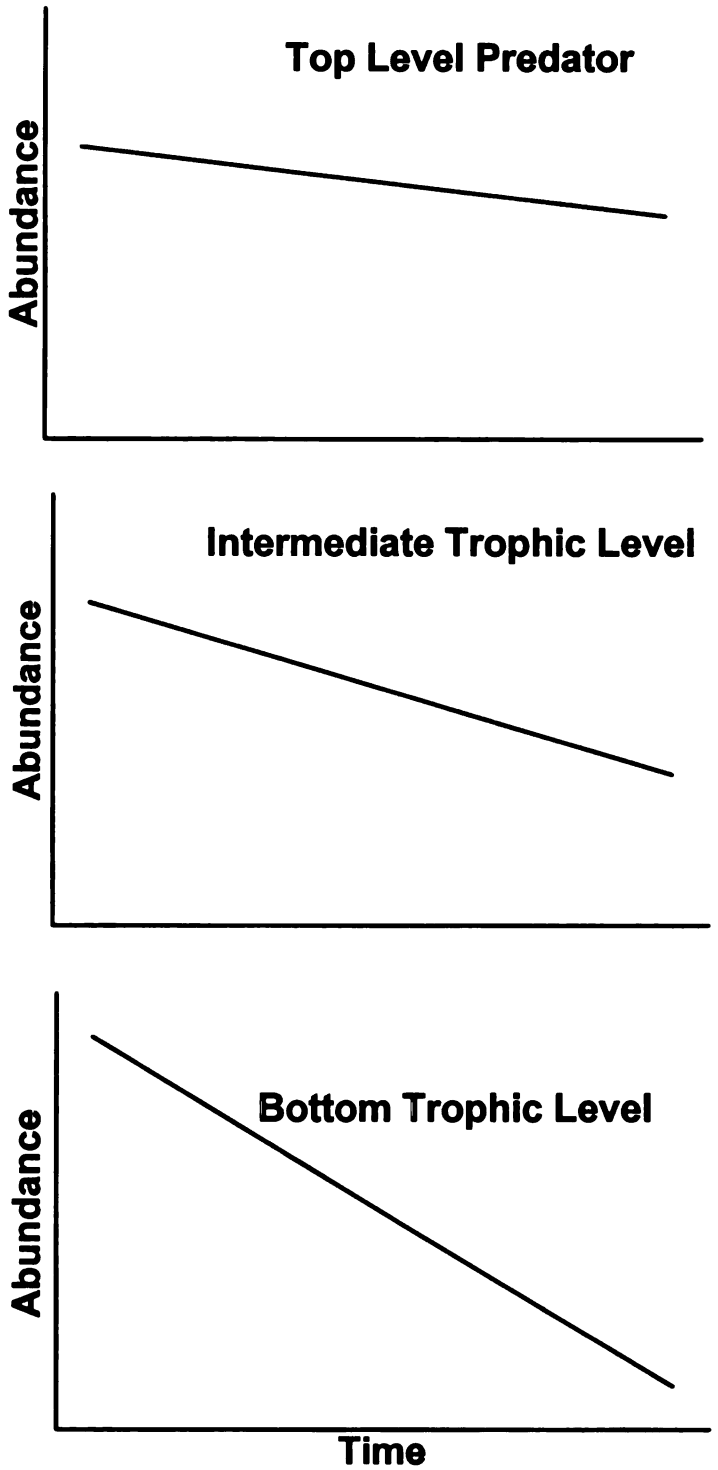

Figure 1.2 Descriptive example of diminishing bottom-up interactions as outlined by McQueen et al. 1989.

levels. This simplified model necessarily removes other invertebrate competitors or facilitators, but it allows close analysis of the relationship between mosquito larvae and their food resources. Michael G. Kaufman (unpublished) has constructed a food web diagram to depict these relationships based on current information about larval feeding habits, gut content analysis and microbial feeding strategies (Fig 1.3). The model presented diagrammatically in Fig. 1.3 is based on the generalized phenomenon of heterotrophic (vs. autotrophic) basis of ecosystem trophic dynamics. Indeed, many macroinvertebrates in freshwater ecosystems utilize allochthonous leaf detritus as food. Some invertebrates consume leaf detritus directly by shredding coarse particulate detritus or by gathering finer detritus (Berrie 1976, Slansky and Scriber 1985), while other invertebrates, including mosquito larvae, exploit the leaf detritus indirectly, by filtering, scraping, or browsing microorganisms in the biofilm on the leaf surface (Cummins and Klug 1979, Fish and Carpenter 1982, Walker and Merritt 1991, Merritt et al. 1992). Invertebrate growth on leaf detritus varies with the type of feeding mode, quantity of leaf detritus, chemical composition of leaf detritus, decomposition rate of leaves, and microbial conditioning of the leaf material (Kaushik and Hynes 1971, Berrie 1976, Anderson and Sedell 1979, Cummins and Klug 1979, Merritt et al. 1984, Walker et al. 1997).

As discussed above, there is some controversy over the exact role of leaf detritus in tree holes as a basal resource for *A. triseriatus* larvae. To review, detritus has been shown to be stimulatory to larval *A. triseriatus* growth (Walker et al. 1991, and references therein), but this effect was not repeated in natural

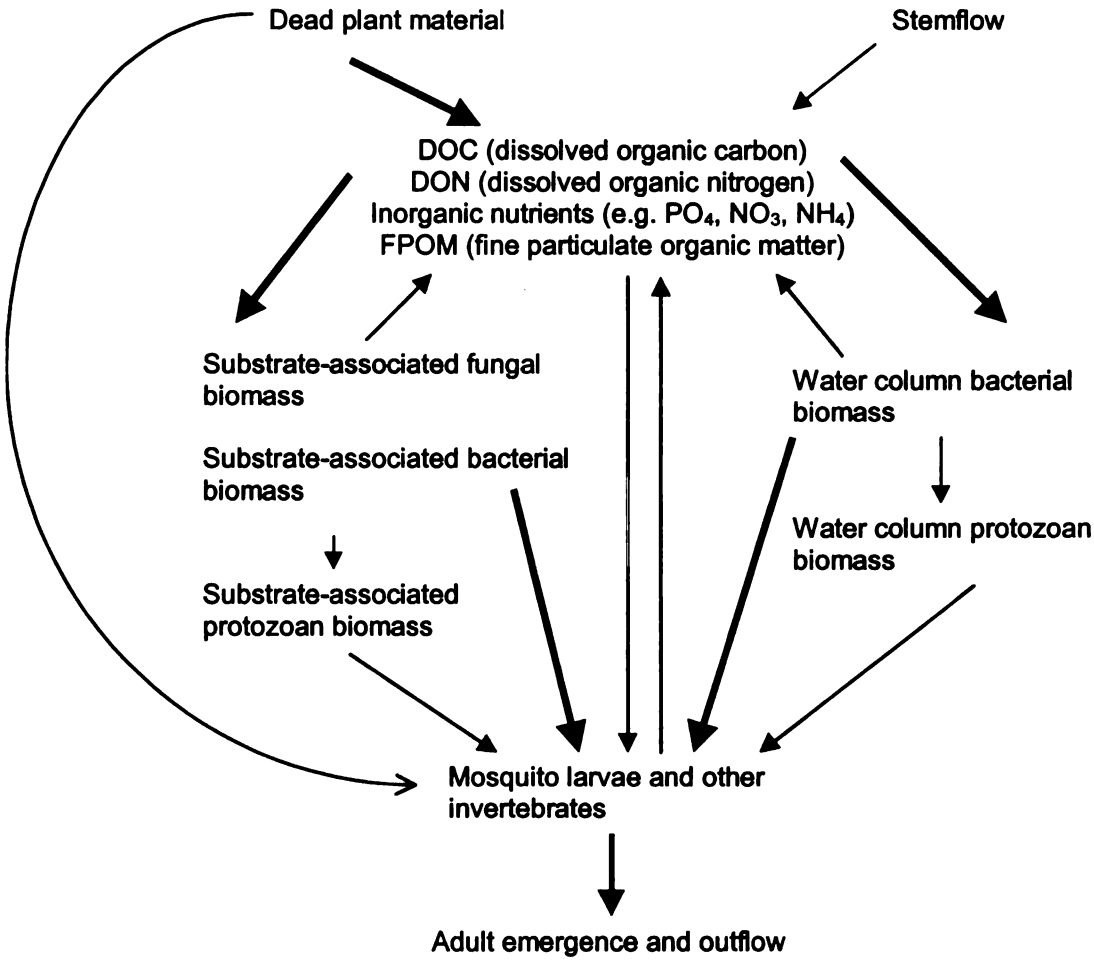

Figure 1.3 Food web diagram depicting relationships of *Aedes triseriatus* larval feeding habits as determined by gut content analysis and microbial feeding strategies. (M.G. Kaufman, unpublished)

treeholes (Walker & Merritt 1988). Leonard & Juliano (1995) did show that direct interactions with detritus enhanced larval growth and production of larvae in treeholes when larvae were confined within mesh cages that did, or did not, contain senescent leaves. However, those studies, similar to microcosm studies, eliminate contact with alternative organic surfaces, which could provide attachment sites and nutrient supplementation to microbial growth such as tree bark and sediment. The presence of a solid surface influences the number and activity of bacteria (ZoBell 1943, Sieburth 1976, Paerl 1980, Paerl & Merkel 1982). Nonetheless, microbial decomposition of organic carbon input, such as detritus, may provide a medium whereby nutrient liberation and microbial concentration both offer incentive for enhanced larval development.

It has been recognized that bacteria and protozoa are responsible for a large portion of the biomass, respiration, nutrient cycling, and productivity in aquatic ecosystems (Azam et al. 1983, Porter et al. 1985, Sherr and Sherr 1991). Thus, they are of clear importance in the overall trophic dynamics in ecosystems (Fenchel 1982, Pomeroy and Wiebe 1988, Sherr et al. 1988). Cochran-Stafira and von Ende (1998) have provided insight into the response of a basal-level bacterial community to predation in an aquatic microbial food web. Results of their experiments involving larvae of the pitcher plant mosquito, *Wyeomyia smithii*, in artificial pitcher plants, indicated that interactions among higher trophic levels could cascade down to the microbial level indicating the need to treat microbes as fully interacting members of a community.

In *A. triseriatus* habitats in northern climates, the absence of the predatory mosquito larvae *Toxorhynchites rutilus* makes *A. triseriatus* the predominant mosquito species in treeholes (Mitchell and Rockett 1981). In treeholes located in southern parts of the distribution of *A. triseriatus*, the co-occurrence of *Toxorhynchites rutilus* might reduce intraspecific competition among *A. triseriatus* larvae as it is becoming increasingly recognized that competition regulates the number of species in a community only when the members of the community actually compete, i.e., when they are at or near their carrying capacity (Menge & Sutherland 1976, Bradshaw & Holzapfel 1983). In northern climates however, field and laboratory observations have shown that reduced survival and pupal weight as well as extended development time can be traced to high larval densities which cause intraspecific competition (Moore & Fisher 1969, Mori 1979, Fish & Carpenter 1982, Broadie & Bradshaw 1991). In this situation, larval competition may be an important means of population regulation in this ecosystem. Thus, *A. triseriatus* larvae can be properly viewed as predators occupying the top trophic level in tree holes, with microorganisms in lower trophic levels comprising their prey.

Invertebrate studies have revealed that competitive interactions between larvae increase with increasing population density because food is a limiting factor (Lamberti et al. 1987, Wissinger 1988) and because food and density interact with one another (Hard et al. 1989). *Aedes triseriatus* larval densities specifically exhibit strong competition among larvae in response to manipulation of larval densities (Edgerly & Livdahl 1992). Presumably, the resource for which

these larvae are competing is food in the form of microorganisms. Gut content analysis of fourth instar larvae of multiple mosquito species revealed a predominance of bacteria and detritus (Walker et al. 1988, Merritt et al. 1990) with an estimated mean number of bacteria in the larval food bolus of *A. triseriatus* specifically being 2.2×10^6. Bacteria have been considered to be the most dominant of the microorganisms that comprise the diet of mosquito larvae (Laird 1988). Rozeboom (1935) showed that mosquito growth was possible on certain bacterial cultures alone. In addition, feeding by mosquito larvae has been shown to reduce the abundance of microorganisms in both field studies and microcosm experiments (Kurihara 1983, Walker et al. 1991). Larval feeding on microorganisms is not limited to bacteria, as gut content analysis also indicates the presence of protozoan organisms in the larval diet.

Omnivory is common in decomposer food webs (Polis 1991, Gunn and Cherrett 1993, Mikola and Setala 1998). When a predator utilizes two prey in the same ecological habitat, there is generally some interactive effect between these two prey species. Increases in the population density of one prey species can decrease a predator's influence on the opposing prey species, thereby allowing that population to increase in density (Murdoch 1969, Murdoch & Oaten 1975). An alternative situation arises when increases in the first prey species allow an increase in predator numbers which over time would result in decreases in the second population as well (Holt 1977, Holt & Lawton 1994). The fact that *A. triseriatus* larvae feed on both bacteria and protozoans indicates that the

relationships between trophic levels are not be clearly defined by these simple categories.

Growth Pattern Responses of Mosquito Larvae to Environmental Influences

In several insect species, a minimum weight must be achieved during the larval stage in order for development to continue (Nijhout & Williams 1974, Lounibos 1979, Safranek & Williams 1984). The mechanistic model of pupation presented by Gilpin & McClelland (1979) is based on the classic Bertalanffy growth equation (Bertalanffy 1960) and provides a model of an inverse relationship between pupal mass and development time. A generalized growth model of mosquito larvae, the pupation window model (Walker et al. 1997), predicts that larvae must attain both a minimum development time and a minimum mass prior to pupation according to the equation $(T-h_1)(W-h_2) = h_3$, where T= development time, h_1 = minimum development time, W = adult mass, = h_2 minimum adult mass, and h_3 indicates the curvature of the function. Faster growing larvae, not limited by resources, achieve the critical mass within the minimum development time and continue to grow to a maximum mass determined by phenotype (Figure 1.4). In the contrary situation, slower growing larvae pass the minimum development time without accruing sufficient mass to pupate; they either continue growing to the critical mass before they pupate (at a mass lower than the maximum possible); or they starve to death. Although the overall size of mosquito species is based in genetics, individual size can be

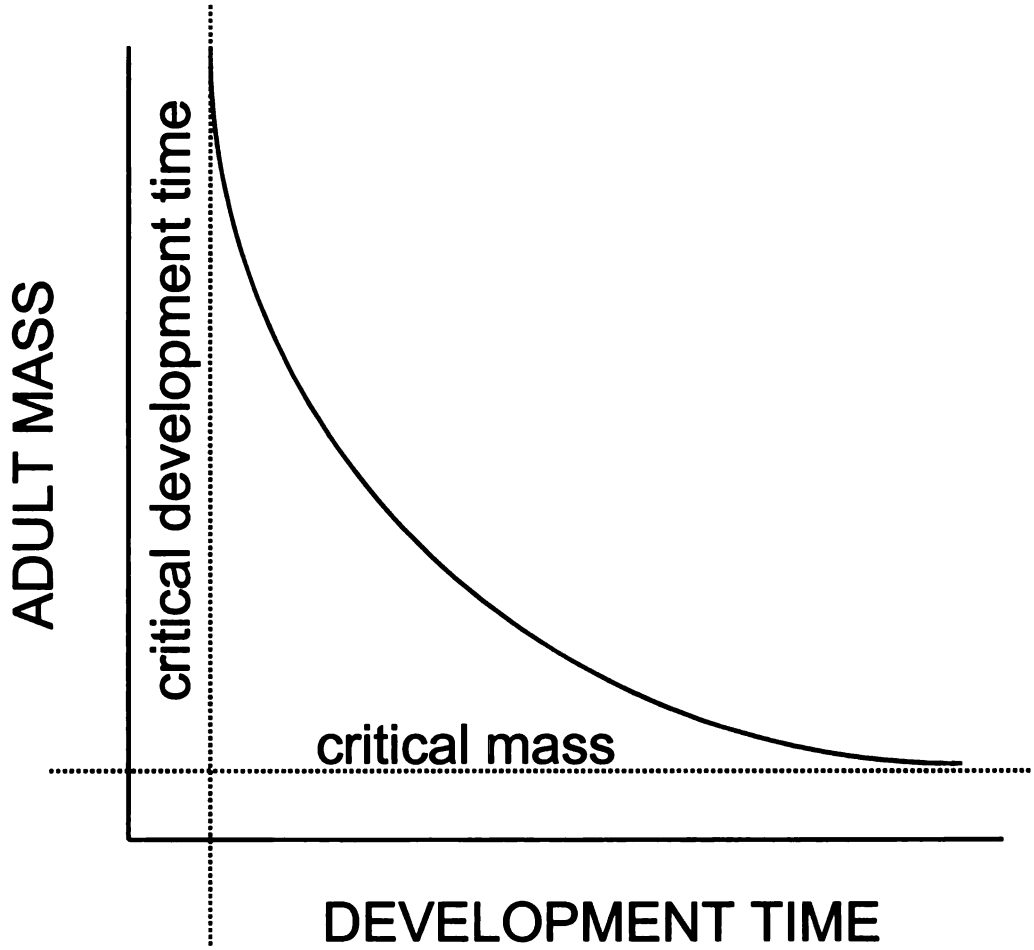

Figure 1.4 Pupation window model showing fit of curve reflecting development time and adult mass of females. The dashed horizontal line represents the critical (minimum) mass requirement for pupation and the dashed vertical line represents the critical length of development for pupation.

influenced by a number of environmental factors including nutrition, temperature, and larval density. These factors can also affect the shape and position of the curve depicting the pupation window model.

Reaction Norms

In variable environments, organisms will show covarying developmental responses that represent phenotypic plasticity overlaid on their fixed genotypes (Stearns & Koella 1986). The capability of organisms to adjust their responses to a variable environment has been termed phenotypic plasticity, while the expression of the response to any given set of environmental circumstances has been termed the "reaction norm" or the "norm of reaction." Schmalhousen (1949) originally defined the term within an evolutionary context as an expression of epigenetic effects on ontogenetic processes that allow organisms to adapt to variable environments. Schlicting & Pigliucci (1998) coined a new but closely related term, "developmental reaction norm," and re-defined it in their practical definition as follows: "The set of (multivariate) ontogenetic trajectories produced by a genotype (or sibship) in response to naturally occurring (or experimentally imposed) environmental variation."

Two of the most common and important environmental factors that affect the development of ectothermic animals are food supply and varying temperature. When these factors vary, they strongly affect development time to maturity (i.e., age) and size at maturity (i.e., size). However, they affect these two important developmental parameters in contrary ways (Sibly and Atkinson 1994). With

regard to temperature, age and size at maturity respond similarly, i.e., they both decrease with increasing temperature, thus the reaction norm (*sensu* Stearns and Koella 1986) for size-to-age at maturity has a positive slope. With regard to food supply, by contrast, size and age at maturity respond in opposite ways; development time decreases whereas size increases with increasing availability of food; the reaction norm has a negative slope. These contrary responses may result from the ways that metabolic rate and food acquisition rate respond differentially to variations in food supply and temperature, thus the latter two factors ought to interact significantly in affecting the reaction norm (Perrin 1995). These observations have stimulated a debate about the universality of these patterns among ectotherms, the physiological basis for these relationships, and whether there exists reciprocal selection for development time and body size at maturity (Berrigan and Charnov 1994, Perrin 1995).

For mosquito larvae, which are ectotherms, the literature supports the general observation that limitations in food supply result in an extended larval development time, smaller adult body size, and higher larval mortality (see review in Clements 1992). Temperature also affects development time and body mass of mosquitoes and other ectotherms during their development, but in ways contrary to changes in food availability. Decreased temperature results in increased development time, and also increased body mass (Clements 1992). Experimentation is necessary in order to predict how food availability and ambient temperature will interact to affect developmental processes of mosquitoes. Within the limits of thermal tolerance, the rate at which mosquito

larvae develop increases with temperature (Bar-Zeev 1957, Nayar 1968), whereas the resulting size of the adult varies inversely with temperature (van den Heuvel 1963, Nayar 1969, Hien 1975). Chambers and Klowden (1990) observed that the larvae of *Aedes aegypti* mosquito enter the pupal stage at a critical weight, which was lowered by raising the larval rearing temperatures. This supports the model for ectotherms which dictates that a decreased temperature reduces growth rate, and is manifested as extended development and larger size in 80% of 100 studies (Berrigan and Charnov, 1994).

Why is variation in mosquito body size important? It is now clear that body size is an ontogenetic trait that influences fitness through fecundity, and vectorial capacity, i.e. that combination of biological attributes that permits mosquitoes to transmit disease agents. Both in nature and in laboratory settings, there is wide variation in the size of adult mosquitoes within a given species (Fish 1985). In the laboratory, *Aedes aegypti* males exhibit 40-fold and females 50-fold variations in body mass (Christophers 1960). This variation in size is only partly explained by genetic predisposition, as environmental influences including temperature, food availability and population density also contribute to determination of adult size at emergence. Larger adult mosquitoes are more fecund (Briegel, 1999; Steinwascher, 1982; Washburn et al., 1989; Lyimo & Takken, 1993; Clements, 1992) than their smaller female counterparts which produce fewer eggs per reproductive cycle (Barlow 1955, Bar-Zeev 1957, Steinwascher 1982, Hawley 1985). As larval densities increase and competition for food correspondingly increases, adult female size decreases and the number of eggs laid therefore

goes down. This relationship has been incorporated into several mosquito population models with the idea that this kind of exponential dampening represents a strong control on population density (Hawley 1985). Although research has been inconclusive regarding the relationship of adult size and longevity (Haramis, 1985; Nasci, 1987; Landry et al., 1988; Mori, 1979; Walker et al., 1987), *Aedes* mosquitoes have shown a direct correlation between large size and success of blood-feeding (Nasci, 1990). Variation in body size also affects parameters contributing to the vectorial capacity of mosquitoes for pathogenic microorganisms (discussed in Walker et al. 1997), such as the fact that adult mosquito size can also determine their susceptibility to infection (Baqar et al., 1980; Grimstad & Haramis, 1984; Kitthawee et al., 1990).

Summary

Because mosquitoes are important ecological organisms as both pest species and transmitters of disease, it is important to examine their patterns of growth and development for clues to possible means of control. Growth and development ultimately determine important adult features directly related to the organism's ability to harbor and transmit pathogens. Female mosquito size is directly related to vectorial capacity and in return size is dependent on a variety of environmental factors. Food resources in microcosms can be simulated as leaf detritus, but an understanding of the relationship between basal resources and other trophic prey such as microorganisms present in natural treeholes gives a more accurate picture of the natural food resources contributing to larval

development patterns. Temperature, food resource availability and population density all contribute to the organism's size and the size contributes to the organism's ability to transmit disease. These relationships have encouraged research to analyze the how these influential parameter interact to determine larval growth and development patterns.

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CHAPTER 2

Trophic interactions in treehole ecosystems; importance of top-down and bottom-up forces.

Abstract

Larval mosquitoes of the tree hole species, *Aedes triseriatus*, are potential keystone predators on microorganisms in treehole ecosystems. These ecosystems are detritus-based food webs which can be simplified to three trophic levels interacting with *Aedes triseriatus* feeding habits; mosquito larvae as top predator, protozoa as prey of mosquitoes and predator of bacteria, and heterotrophic bacteria as transformers of detritus and as prey of protozoans and mosquitoes. Food web theory suggests that a trophic cascade imposed by feeding on lower trophic levels should have an alternating effect, where protozoan density would be reduced, predation pressure on bacteria would be lessened, and bacterial density would increase. Food web theory alternatively suggests that both top-down effects of predation, and bottom-up effects of nutrient inputs, will have effects on the trophic levels as well, and that the effects should diminish with higher order trophic level. Four experiments, two in laboratory microcosms and two in treeholes were designed to test these predictions. Results showed that the omnivorous feeding habits of the larval mosquitoes dampened any cascading influence of this keystone predator. Organic nutrient inputs had strong effects on bacterial and protozoan densities, but inorganic nutrients did not.

Introduction

The interactions of top-down (predation) and bottom-up (nutrient) effects have been closely examined in a variety of ecosystems in recent years. These studies have fueled a debate regarding whether the abundance of organisms in a given trophic level is more likely controlled by predation or by resource availability (Hunter and Price 1992, Menge 1992, Power 1992, Strong 1992, Hairston and Hairston 1993, Pace and Cole 1994). In aquatic ecosystems, studies support the hypothesis that bottom-up forces (i.e., inputs of nutrients into the most basal trophic level) increased productivity at all trophic levels (Abrams 1994, Mills & Shiavone 1982, Mittelbach et al. 1988, Leibold 1989) while other studies have shown bottom-up factors to predominate over the role of predators as regulatory factors of abundance or biomass (Persson et al. 1988, 1992). Contrasting research has indicated that predatory influences masked or decreased nutrient input effects thereby structuring the community (Leibold and Wilbur 1992, Oksanan 1988, 1991, Hairston and Hairston 1993). And finally, studies by Carpenter et al. (1991) showed that bottom-up and top-down forces act jointly and equally in their lake systems. The common determination by many ecologists is that bottom-up and top-down forces often operate in concert (Hunter and Price 1992, Power 1992, Rosemond et al. 1993, Balciunas and Lawler 1995). How these forces interact is a major question facing researchers. The tree hole ecosystem inhabited by *Aedes triseriatus*, the eastern treehole

mosquito in eastern North America , is amenable to imitation and manipulation in field and laboratory settings, and is an ideal ecosystem to study these processes.

The natural habitat of *A. triseriatus* is water-filled treeholes, a type of phytotelmata (Frank and Lounibos, 1983) consisting predominantly of rainwater accumulating in basins formed by living roots of deciduous trees. The container-breeding mosquito larvae are found in pans or rot holes of deciduous trees in eastern North America (Jenkins & Carpenter 1946, Mitchell & Rockett 1981, and Bradshaw & Holzapfel 1985). Both organic and inorganic nutrients enter this ecosystem naturally from external sources. Autumn shed leaves can enter treeholes to provide a source of particulate organic material; in fact prior studies have suggested that leaf detritus may be the major contributor of organic carbon for the growth of treehole invertebrate fauna, particularly mosquito larvae (Fish & Carpenter 1982, Carpenter 1983, Kitching 1983, Walker et al. 1997). Throughfall moving down the bark of these trees, referred to as stemflow (Eaton et al. 1973), carries with it inorganic nutrients into the treeholes. Heavy rainfalls are episodic and are capable of causing a disturbance to the ecosystem resulting in increases in nitrate and sulfate levels while concurrently diluting ammonium and phosphate in the system (Walker et al. 1991). Because treeholes are small, are discrete, contain a tractable community of organisms, and can be manipulated and simulated, they provide an ideal experimental situation for examining biotic interactions and processes within an ecosystem. *Aedes triseriatus* larvae can be viewed as a keystone predator (Paine 1969). upon microorganisms in this system.

The studies that follow were designed to examine trophic interactions using both microcosm and field studies to explore how the abundance of individuals in different trophic levels are affected by resources and by predation. Specifically, it manipulated the top level predator, *Aedes triseriatus* larvae, and levels of both organic and inorganic nutrient input in order to determine their effects on bacteria and protozoa. Our findings show that nutrient resource availability interacts with predation pressure in this particular ecosystem. In fact, predation effects may be diminished visibly in the presence of strong nutrient input.

Materials and Methods

Mosquitoes

Larvae of the mosquito *Aedes triseriatus* (Say) occur in pans (tree holes with a complete bark lining) and rot holes (tree holes formed at a wound penetrating the bark) in decidous trees of eastern North America (Jenkins and Carpenter 1946, Mitchell and Rockett 1981, Bradshaw and Holzapfel 1985). An understanding of the basic biology of this mosquito is important medically due to its ability to vector the La Crosse encephalitis virus (Turell and LeDuc 1983). Larvae begin hatching in northern treeholes in March and April and grow through four larval instars prior to pupation. Adults first emerge in late spring or early summer, mate, and females blood-feed and then lay eggs on the bark along the water line of treeholes. There are 1-2 generations per year in northern climates. If the eggs are flooded by rising water levels, the first-instar larvae may hatch the

same season; if not, the eggs enter diapause beginning in mid-August and do not hatch until the following year (Shroyer and Craig 1980).

Microcosm Preparation

Microcosms were made of PVC (polyvinylchloride) pipe with and inner diameter of 7.6 cm fitted with a fiberglass disc on the bottom end. Each microcosm was filled with 600 ml of sterile water (capacity, 700 ml). Senescent beech leaves [American beech (*Fagus grandifolia* Ehrh.)] served as a source of organic nutrient. Leaves were dried 48 hours at 40^0C, their petioles removed, leaf packs were weighed, and then added to the microcosms. Fifty four microcosms were assembled in the following treatments: 18 with no beech leaves, 18 with one g of leaves and 18 with 3 g of leaves. A mixture of natural tree hole contents including water, leaves and sediment was collected, combined in a blender and added to each of the microcosms in 10 ml aliquots. An incubation period of one week allowed establishment of the microbial community within the microcosms from this inoculation.

Microcosm Treatments

The effects of predation on the microbial community were studied by either adding or removing fourth instar larvae. To test the effects of removing a keystone predator, six microcosms of each leaf ration (0, 1 or 3 g) initially contained fifty fourth instar *A. triseriatus* larvae. Following the one week incubation period and a time zero sampling, these larvae were removed. To test

the effects of adding a predator, fifty fourth instar larvae were added to six microcosms of each leaf ration following the time zero sampling. Due to the varying leaf rations, this experiment also tested effects of organic nutrient input simultaneous with the predator treatments.

In order to study solely the influence of organic and inorganic nutrients on the microbial community, an experiment was conducted where no larvae were used. Organic nutrients were the beech leaves added to microcosms as described above. Inorganic nutrients were supplemented as a cocktail containing both nitrate and sulfate in proportions similar to those found in naturally occurring stemflow (Walker et al. 1991). Potassium nitrate and potassium sulfate were added at two different levels, 250 μM and 300 μM respectively for a low level addition and 2.5 M and 3 M respectively for a high level addition. Inorganic additions were made to six microcosms of each leaf ration twice during the experiment.

Tree Hole Experiments

Field experiments were also conducted in treeholes in mature American beech trees (*Fagus grandifolia*) located in Toumey wood lot on the campus of Michigan State University (East Lansing, Michigan). Predator removal experiments were conducted in two different years (1995 and 1997), during the spring and summer, to study predation effects on community densities. Two different removal methods were utilized: (1) removal of predators (the mosquito eggs) with a boiling water treatment, and (2) by removal of larvae through

selective additions of an asporogenic, liquid formulation of the bacterial-based mosquito larvicide Vectobac 12AS (Abbott Laboratories, Chicago, IL).

In January 1995, prior to larval hatch, water was removed from each of twenty seven treeholes and measured to determine their volumetric capacity. The empty treeholes were subsequently flooded to capacity with boiling water to kill diapausing eggs of *A. triseriatus* mosquitoes. Following this treatment, the original water that had been removed was returned to the treehole pans, along with detritus and other materials. On April 12 and again on June 9, first instar *A. triseriatus* larvae were restocked into twenty one of the prepared treeholes at a concentration of 1 larva per 5 ml of treehole volume. The remaining six prepared treeholes were left void of mosquito larvae. All treeholes involved in the study were enclosed with mesh screen fitted with a plastic neck and cap for sampling purposes.

During the first field experiment, the effects of basal nutrient supplementation on the bacterial community were studied with additions of colloidal suspensions of mixed lipids or proteins. The lipid mixture was prepared with cholesterol (Dadd and Kleinjan, 1976) and purified cod liver oil (a source of polyunsaturated fatty acids, purified with multiple Bligh and Dyer extractions). Eight restocked tree holes received additions of this lipid suspension to a final concentration of 10 ppm cholesterol and 10 ppm oil extract. The protein mixture, prepared by dissolving casein in water by elevating to pH 12 and then precipitated by lowering to pH 6 was added to final concentration of 50 ppm into five tree holes. As a negative control, seven tree hole sites received a corresponding volume of

deionized water. Nutrient additions were made on May 18 and 29, June 13, and July 10 and 20, 1995.

Treeholes in the same wood lots were utilized for the second experiment. In this series of experiments larvae were removed with exposure to Vectobac 12AS. A total of twenty-five treeholes were screened off and half were treated with the larvicide on May 27, 1997. Inspection of the treeholes indicated that larvae died as a consequence of this treatment. Sampling of treeholes began on June 6 and water column samples were collected from all treeholes each week for four weeks. Samples were preserved and processed as described below.

Microbial Enumeration

Samples (2 ml) from the water column of microcosms or treeholes were taken aseptically using sterile pipettes, and preserved in clean glass tubes by adding 2 ml of cold 8% paraformaldehyde for a final concentration of 4%. All water column samples were stored at 4^0C until enumeration. Direct microbial counts were conducted using the DNA binding fluorochrome, DAPI, and a slight modification of the procedures of Hobbie et. al., (1977) and Porter & Feig (1980). DAPI, 4'6-diamidino-2-phenylindole, is a fluorochromatic stain which fluoresces blue when bound to DNA and exposed to light at 365 nm (Wittekind, 1972). To determine bacterial densities, 250 μl of water samples were mixed with 75 μl of DAPI (400 μg/ml) and incubated for 20 minutes in reduced light conditions at 4 °C. The samples were then filtered unto 0.22 μm, black Nuclepore polycarbonate filters using a hand-pumped vacuum and a 13 mm (internal

diameter) glass chimney and filtering apparatus. Protozoan densities were determined using 1 ml of water sample mixed with 100 μl of DAPI (400 mg/ml) filtered unto 0.4 μm Nuclepore polycarbonate filters. The slides were stored in the dark at 4^0C until they could be enumerated. Visualization and enumeration was done with epiflourescence microscopy using a Jenalumar microscope model A/D. Counts were converted to densities using a standard formula.

Statistical analysis

Direct count data of bacterial and protozoan densities were compiled into databases using Microsoft Excel. The data were transformed with the common logarithmic transformation $\log_{10}(x + 1)$. Data were then subjected to repeated-measures analysis of variance using the PROC GLM procedure of SAS (1985) and following guidelines presented in Von Ende (1993).

Results

Microcosm experiments

Variation in organic nutrient addition to microcosms resulted in a highly significant effect on both bacterial and protozoan densities, under conditions where mosquito larvae were completely absent (Table 2.1). Treatments with 1 or 3 g of leaf material had higher densities of protozoans and bacteria than did treatments without leaf material (Figure 2.1). In contrast, there was no effect of inorganic nutrient additions on either bacterial or protozoan densities,

Table 2.1. Summary of ANOVAs testing for effects of organic and inorganic nutrients on microbial population densities.

Response variable	Error Mean square	DF	F statistic: Organic Nutrients	F statistic: Inorganic Nutrients	F statistic: Organic x Inorganic
Bacterial densities	0.37	2	27.88***	0.10	1.55
Over time	0.04	9	3.12***	0.47	0.81
Protozoan densities	0.96	2	98.44***	1.27	1.67
Over time	0.57	9	1.06	0.74	0.57

Note: *P < 0.05, **P < 0.01, ***P < 0.001

Table 2.2. Summary of ANOVAs testing for effects of organic nutrients and predation on microbial population densities.

Response variable	Error Mean square	DF	F statistic: Organic Nutrients	F statistic: Predation	F statistic: Organic x Predation
Bacterial densities	0.16	2	92.65***	1.28	2.70*
Over time	0.04	8	13.43***	2.22*	0.96
Protozoan densities	0.82	2	46.14***	6.68**	7.57***
Over time	0.62	8	4.29***	1.56	1.31

Note: *P < 0.05, **P < 0.01, ***P < 0.001

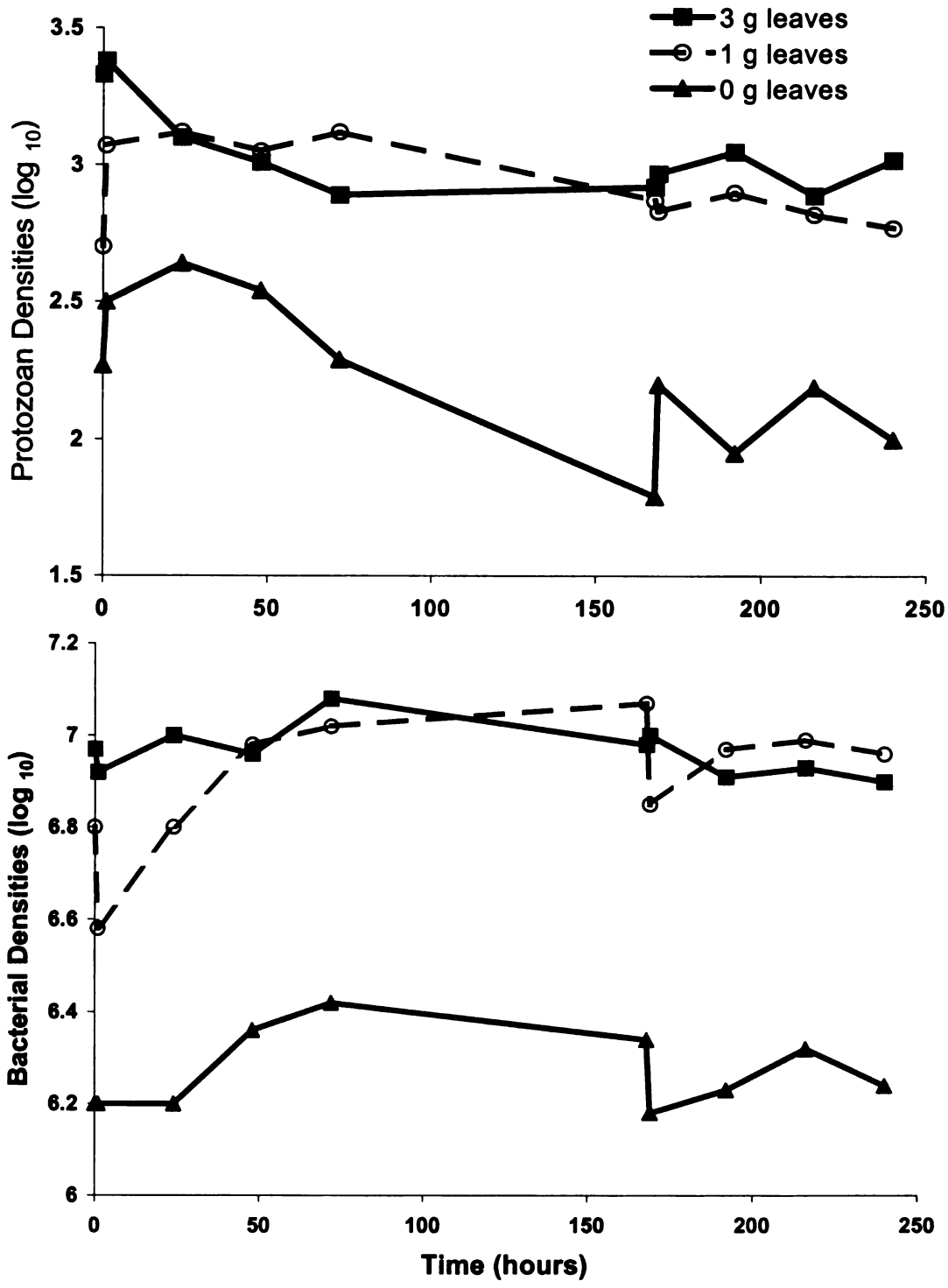

Figure 2.1 Bacterial and protozoan responses to organic nutrient input. Density values are represented as $\log_{10}$ values averaged over microcosms within each treatment group.

although there was a trend toward increased bacterial numbers in response to inorganic additions when no organic material was present (Figure 2.2).

Addition and removal of the *A. triseriatus* larvae in the microcosms resulted in differential responses of bacterial and protozoan densities. Predation had a highly significant effect on protozoan densities. Larval removal was followed by an increase in protozoan density and larval addition was followed by a decrease in protozoan density (Table 2.2, Figure 2.3). In contrast, there was no significant influence of larval addition or removal on bacterial density (Table 2.2, Figure 2.3). The varied levels of senescent beech leaves provisioned into microcosms interacted significantly with the predation treatments on protozoan and bacterial densities (Table 2.2). In order to examine this result further, the data for bacterial and protozoan densities in the absence of organic nutrients (i.e., 0 g of beech leaf litter per microcosm) were separated from the other data, and analyzed using repeated measures analysis of variance. There was a significant effect of predation on bacterial densities ($F = 4.06$, $df = 2,11$, $P < 0.05$) as well as on protozoan densities ($F = 8.75$, $df = 2,11$, $P < 0.01$) (Figure 2.4). In the absence of nutrient input, the densities of both of these groups were reduced by the addition of predators. The densities of protozoans and bacteria tended to increase immediately following physical manipulation of the microcosms, including the control microcosms, suggesting that movement and mixing released microorganisms associated with detritus or other substrates into the water column.

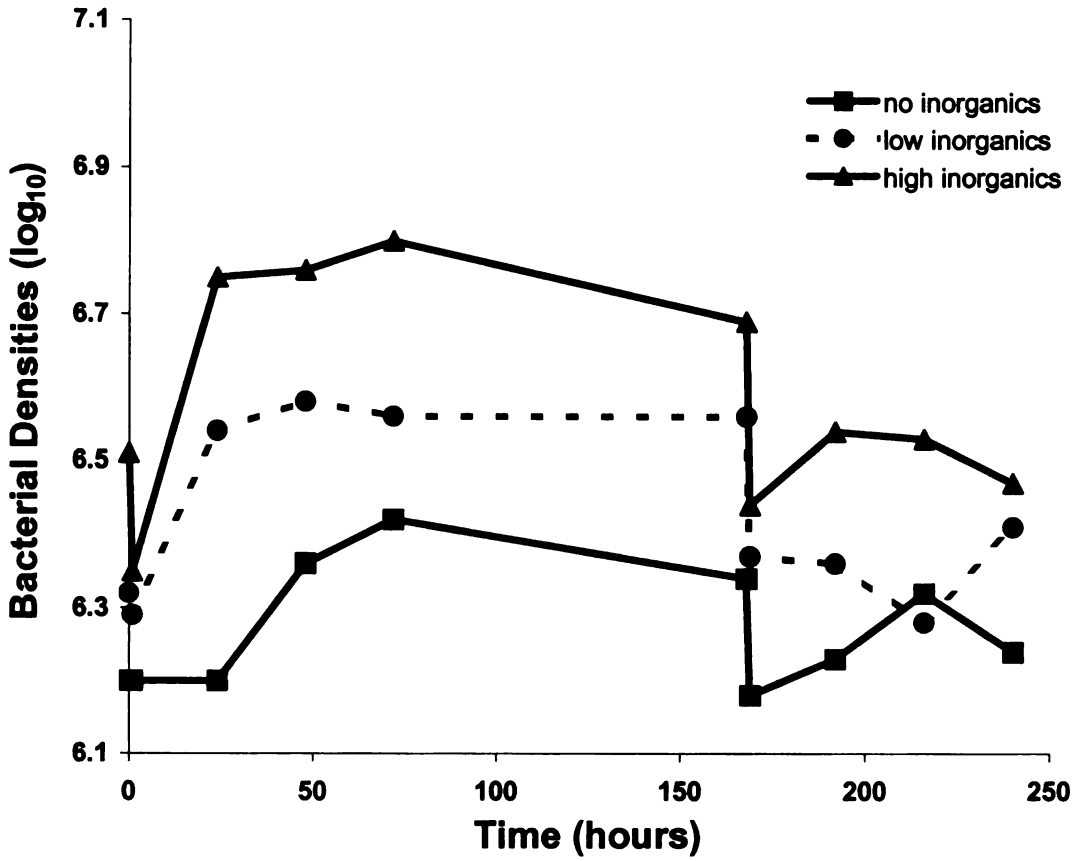

Figure 2.2 Response of bacterial densities to inorganic nutrient input in the absence of organic material.

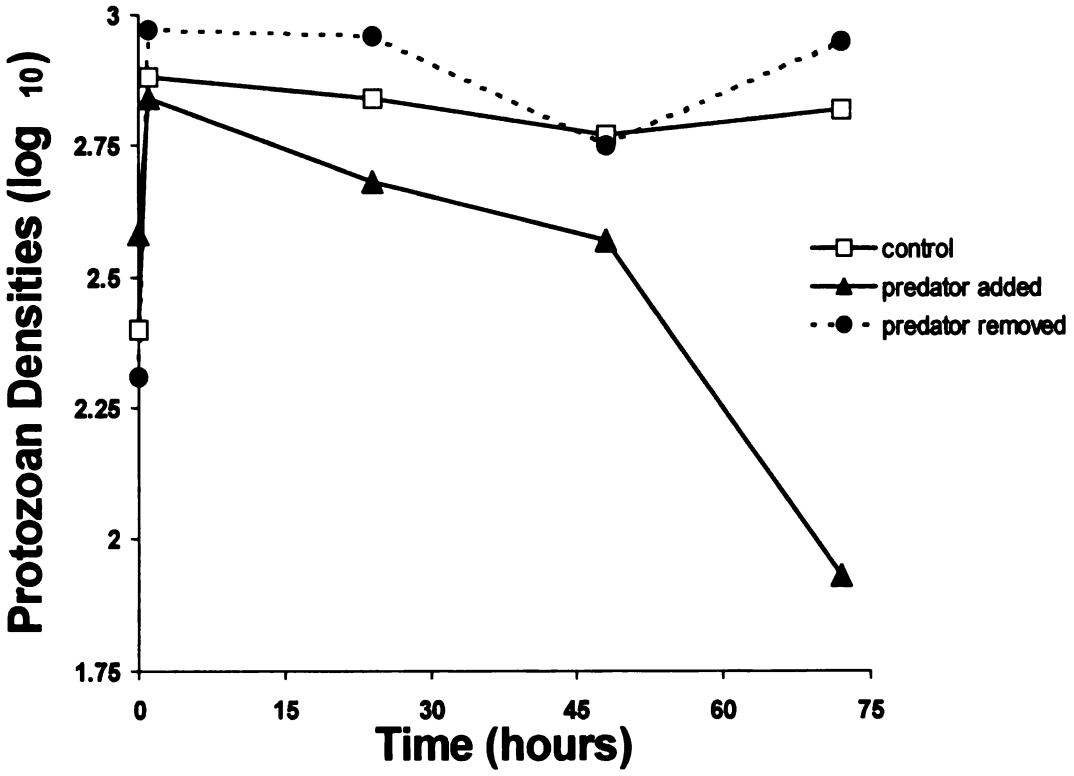

Figure 2.3 Population density of protozoa averaged across three levels of organic nutrient input.

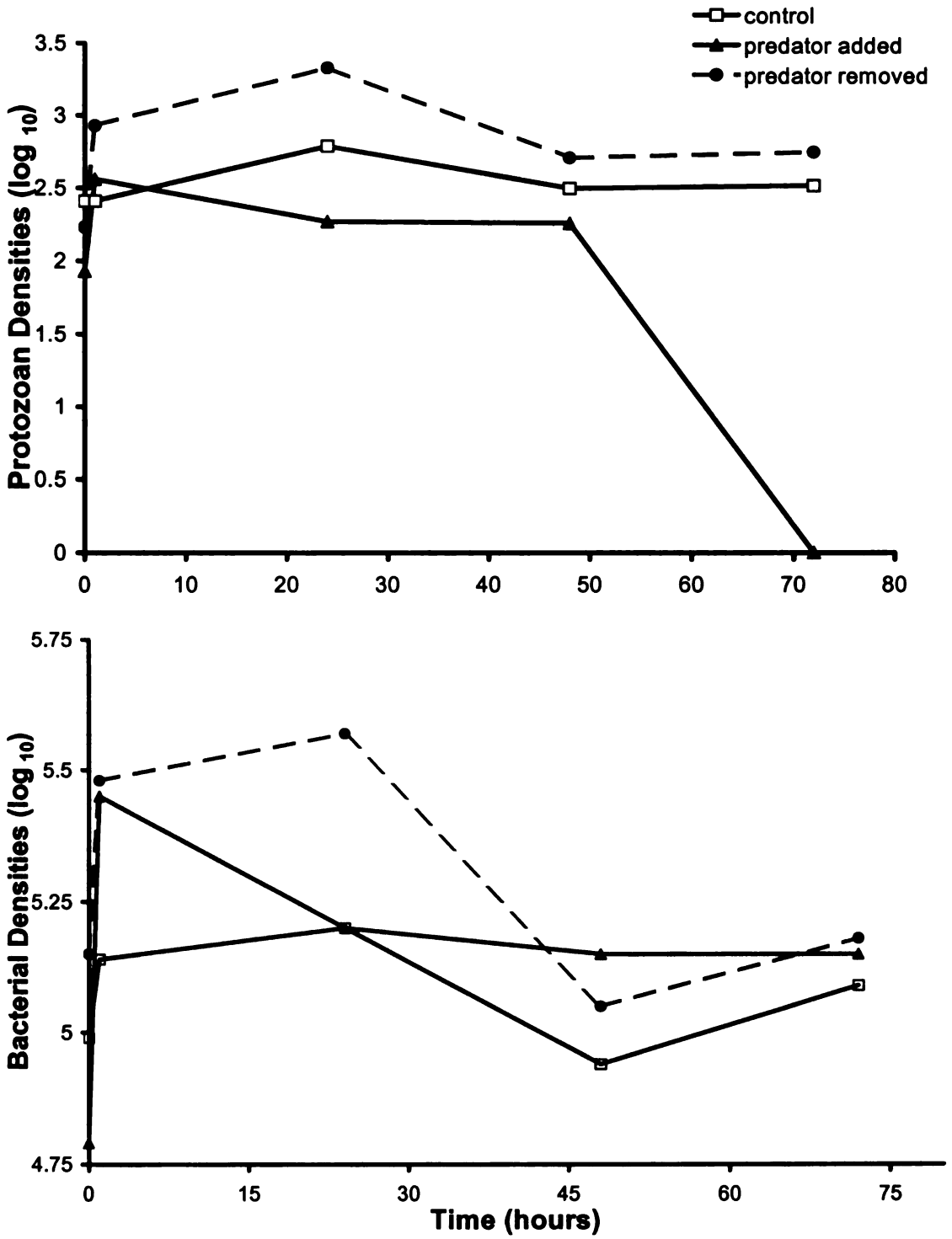

Figure 2.4 Bacterial and protozoan population density response to predator addition and removal in the absence of any organic material.

Tree hole experiments

In the first treehole experiment, the presence or absence of *A. triseriatus* larvae significantly affected bacterial densities. In treeholes treated with boiling water and later restocked with larvae, there was a trend for a reduction in bacterial densities compared to treeholes in which there were no larvae ($F = 20.10$, $df = 1$, $P < 0.01$) (Figure 2.5). Although in both treatment groups, those with larvae present and with larvae absent, bacterial densities increased over time as the experiment proceeded, the trend for those treeholes with larvae present was a steeper line and appeared to change in concert with the timing of emergence of adult mosquitoes (Figure 2.6).

In the second field experiment, where larvae were removed with Vectobac 12AS, results were similar to the first treehole experiment. Bacterial densities were significantly lower in treeholes with larvae, and conversely were higher in treeholes from which larvae had been eliminated (Figure 2.7; repeated measures ANOVA, $F= 6.03$, $df=1$, $P<0.05$).

Discussion

The results of the microcosm and tree hole experiments reported here indicated that *A. triseriatus* larvae function as a "keystone" predator or "top level predator" (*sensu* Paine 1969) in the tree hole ecosystem. Removal or elimination of larvae resulted in statistically significant changes in densities of protozoans and bacteria in the water column. Results were not entirely consistent among experiments, because in microcosms bacteria responded less dramatically and

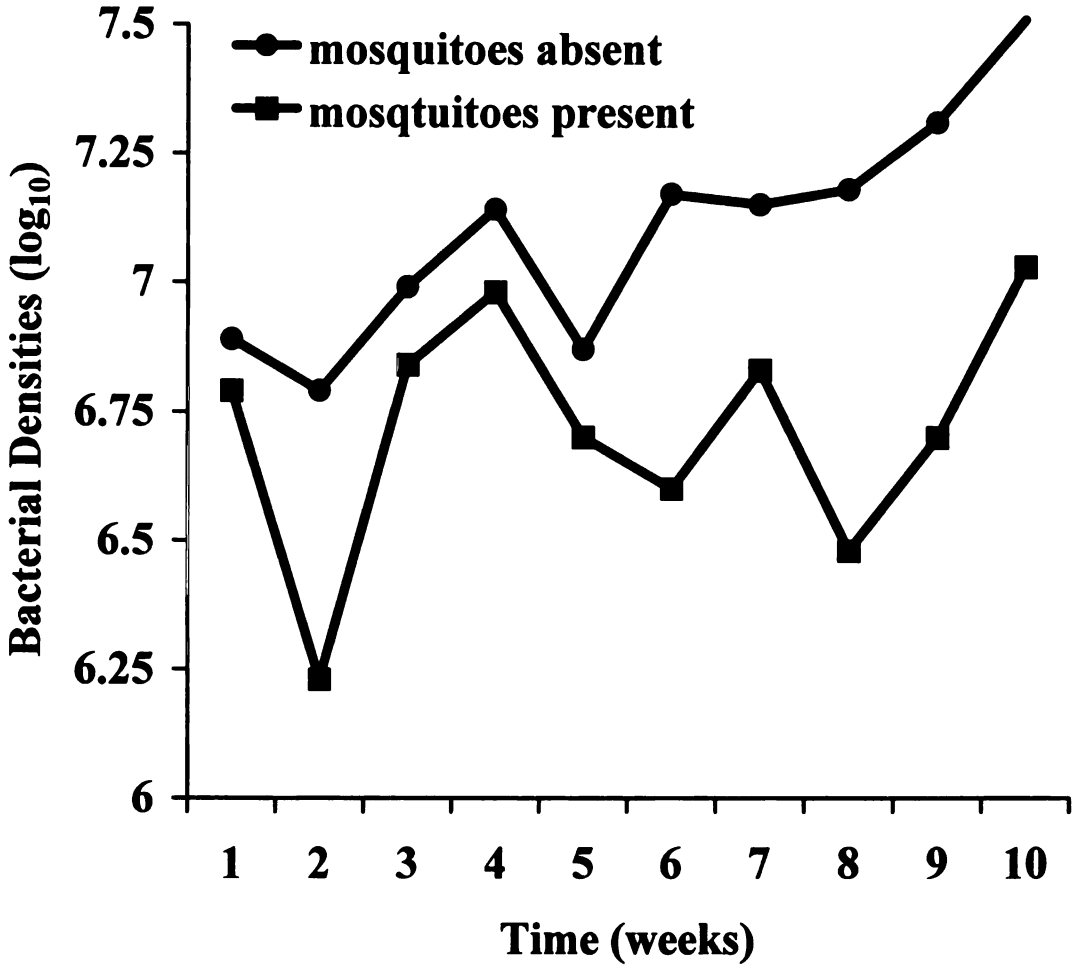

Figure 2.5 Bacterial population densities comparing treeholes either with mosquito larvae present or absent. Those with no mosquito larvae had larvae removed by exposure to boiling water. Those with larvae present were treated with boiling water and then restocked with first instar *Aedes triseriatus* larvae.

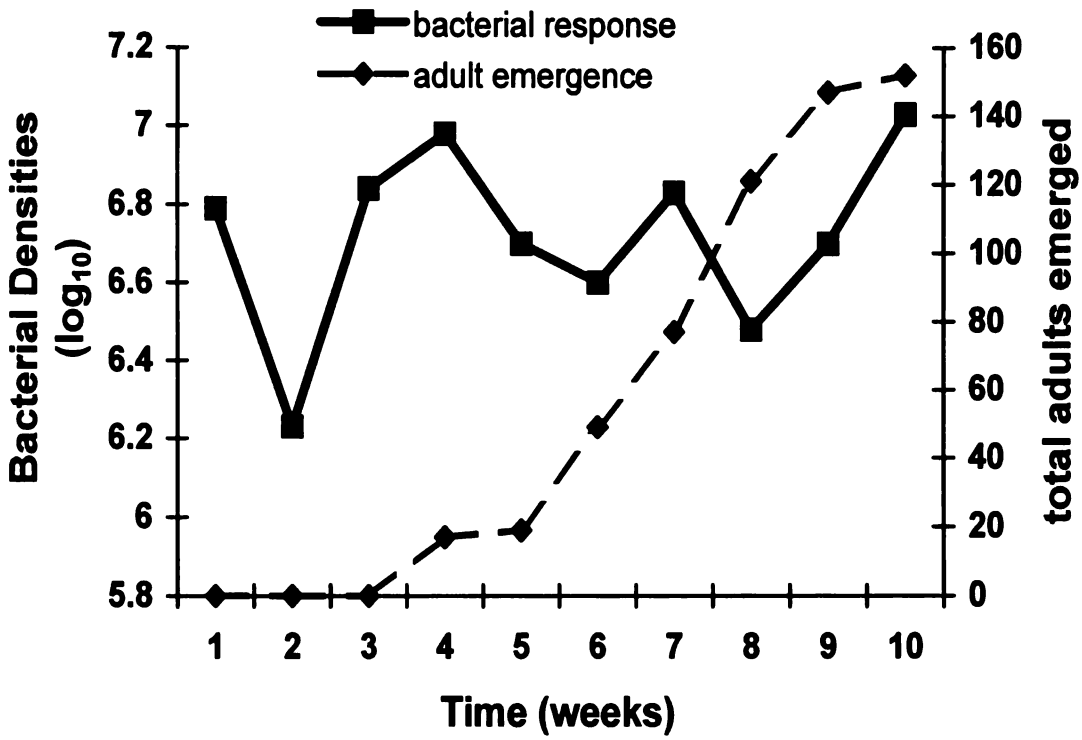

Figure 2.6 Relationship of adult emergence and bacterial population response. Solid line represents bacterial population densities over time and the dashed line represents the cumulative emergence of mosquitoes as adults. This line corresponds to removal of the larval predator through its natural life cycle. As this keystone predator exits the natural ecosystem the bacterial population responds by increasing in numbers.

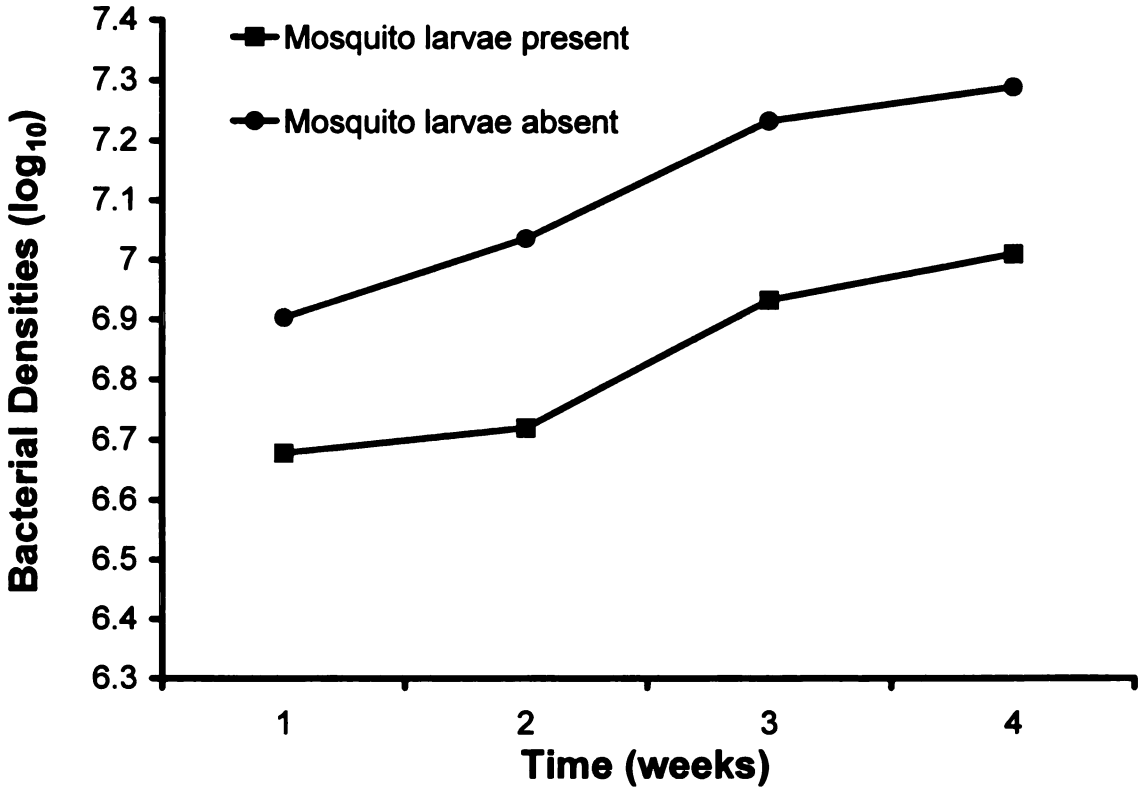

Figure 2.7 Bacterial population densities comparing treeholes either with mosquito larvae present or absent in the second treehole experiment, where larval mosquitoes were removed by addition of the bacterial-based liquid larvicide, Vectobac 12AS.

not in a statistically significant manner, to predator manipulations compared to the treehole experiments. The responses of protozoan densities were much more pronounced. Although the removal of this top level predator from the system results in drastic alterations of the remaining trophic levels, the relationship between the trophic levels of the treehole ecosystem do not follow the prescribed patterns of food web interactions. Previous studies involving three or more interacting trophic levels have provided evidence for strong top-down regulation involving microbes, microbivorous nematodes, and predatory arthropods (Santos et al. 1981, Parker et al. 1984), but no trophic cascade could be observed for productivity or biomass in a soil food web (Mikola and Setala, 1998). The same appears to be true for the treehole ecosystem. According to the trophic cascade theory of Carpenter et al. (1985), a reduction in the protozoan population density should be observed following predation by *A. triseriatus* larvae. This, in turn, should provide an increase in bacterial population densities by relieving their predation pressure. This specific interaction was observed in the pitcher plant ecosystem studied by Cochran-Stafira and von Ende (1998). There was an increase in bacterial densities in response to grazing by *Wyeomyia smithii* larvae. They proposed that the discrepancies between the pitcher plant studies and treehole studies could be caused by differences in larval densities found in each ecosystem.

However, *Aedes* larvae are omnivorous due to their indiscriminate filter feeding making the responses of lower trophic levels more complex. Omnivores feed on several trophic levels making the limiting processes in neighboring levels

less clear (Leibold 1989, Hunter and Price 1992, Abrams 1993, Osenberg and Mittelbach 1996, Persson et al. 1996). In addition to omnivory, other factors have been proposed for the uncoupling of trophic cascades at the microbial level. Studies have suggested that heterogeneity (Hunter and Price 1992) in prey edibility may increase the number of inedible organisms at intermediate trophic levels (Leibold 1989, Abrams 1993, Mikola and Setala 1998). Other studies (Balciunas and Lawler 1995, Hairston and Hairston 1993) suggest that resources can affect prey vulnerability and thereby limit trophic cascades.

McQueen et al. (1986) proposed the idea of top-down, bottom-up regulation in plant-based freshwater systems. The theory predicts that top-down forces act strongest at the top of the food web and weaken as they progress toward lower levels; in addition to this it also predicts the reverse for forces acting at the bottom levels of the food web. In other words, nutrient input or bottom-up forces are strongest acting on the lowest levels of the food web and weaken as they progress to higher levels. This study clearly showed regulation of upper level trophic species, protozoa, by predation and regulation of lowest level trophic species, bacteria, by organic nutrient input. Within a 72 hour time period the protozoan population was decreased one full log unit by predation by larval mosquitoes, the majority of this reduction in numbers occurred 48 hours after the larvae were added to the microcosms. As the protozoan population was decreasing in numbers the bacterial population was increasing along the same time frame. The actual numbers of bacteria do not show as large a change in number as protozoa, but this is most likely due to the turn over rate of bacteria

vs. the predation rate of larvae. When the predator is removed from the system the bacterial density plummets, but apparently not in response to increases in protozoan densities as is predicted by the trophic cascade model. In relationship to untreated microcosms neither protozoan populations nor bacterial populations appear to have any response to removal of predation pressure within a 72 hour period. Perhaps the protozoan population requires longer than three days to recover to a point that it is capable of making an impact on the bacterial population. The bacterial population in the meantime could be mediated by factors beyond the scope of this study such as interaction with virus to maintain a plateau population.

Organic nutrient input appears to be the main driving force influencing the structure of these small, isolated ecosystems. Hunter and Price (1992) point out the obvious, which is too often overlooked, when they observe that removal of higher trophic levels leaves lower levels present, while removal of primary producers leaves no system at all. This clearly indicates that nutrient input into any system ultimately drives the community structure. The effects of predation are greatly reduced in the presence of high levels of organic nutrient input into this particular ecosystem. Inorganic nutrients do not alter treehole community structure indicating that they are not the limiting resources for bottom-up influences.

Although these laboratory experiments do not indicate that a trophic cascade takes place in the treehole ecosystem there are other considerations which may be influencing this result. For example, bacterial turnover time is much faster

than that for protozoa so the limited time measured here may not be fully indicative of the true relationship between these two trophic levels. In addition, these measurements of bacterial and protozoan densities were made only from the water column of the microcosms, not directly from leaf surfaces. Many microbes adhere to this substrate and larval grazing takes place at leaf surfaces as well as in the water column. The increase in number of microorganisms in all microcosms following physical manipulation show that the total population density within the ecosystem is not limited to those in the water column. It is possible that critical interactions between different trophic levels occur exactly at the point of interaction of substrate, microorganisms and grazing. Due to the high impact of nutrient regulation and the apparent relationship between treehole ecosystems and other microbial systems (Mikola and Setala 1998, McQueen and Post 1988) it appears that regulation of specific food webs is more accurately explained by top-down; bottom-up theory as opposed to trophic cascades. However, we cannot rule out the possibility that changes in the species comprising the bacterial trophic level influenced the regulatory factors acting on this ecosystem as they have in others (Hunter and Price 1992, Strong 1992). These results imply a rebounding of bacterial population numbers in response to decreased numbers of this top-level predator for the treehole ecosystem.

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CHAPTER 3

Age and size at maturity in response to variable food and temperature regimes for larvae of the mosquito, *Aedes triseriatus*: Effects of differential mortality

Abstract

The development of larvae of the mosquito *Aedes triseriatus*, consisting of both gain of mass to the time of metamorphosis and the duration of this period of time, is influenced greatly by external environmental factors, in particular food availability and temperature. Although the interactions of these factors in affecting development has been studied previously in other invertebrate (including mosquito) systems, the experiments reported here directly tested their effects and any interactions between them as well as the influence of experiment-wise mortality on key growth parameters (namely, development time to and mass at pupation). Both variations in temperature and food availability strongly affected growth responses in a series of experiments. In a cohort experiment, temperature and food availability did not interact significantly. This unexpected result may have been a consequence of differential mortality in cohorts held at low and high temperatures, as cumulative mortality was substantially greater at lower temperatures. Alterations in population densities due to both decreased temperatures and decreased food rations likely restructured the population such that resources per individual increased, allowing for increased mass at lower temperatures. Parameter estimates from fits of

development time and mass at pupation to a pupation window model showed differences depending upon whether larvae were reared as cohorts or as individuals. In the former case, the estimates showed a significantly higher body mass and extended development time in larvae held at low temperatures. In the latter case, the estimates showed an extended development time but no change in body mass. These results suggest that differential mortality during cohort development indeed influences the growth outcomes of individual larvae. The general observation that individual mosquitoes, and other ectotherms, develop through a long development time to a larger body size at low temperatures compared to high temperatures, may be a consequence of changing food ration as larval density decreases and food becomes more readily available to survivors. This observation challenges the long-standing dogma that invertebrates experiencing low temperatures are larger at maturity compared to those reared at higher temperatures, as some direct physiological consequence of the temperature regime.

Introduction

Variations in food supply and temperature affect development time to maturity (age) and mass at metamorphosis (size at maturity) in contrary ways in ectothermic animals (Sibly and Atkinson 1994). With regard to temperature, age and size at maturity respond similarly, i.e., they both decrease with increasing temperature, thus the developmental reaction norm (*sensu* Stearns and Koella 1986, Schlichting and Piggliuci 1998) for size-to-age at maturity has a positive

slope. With regard to food supply, by contrast, size and age at maturity respond in opposite ways. Development time decreases whereas size increases with increasing availability of food, and the reaction norm has a negative slope. These contrary responses may result from the ways that metabolic rate and food acquisition rate respond differently to the same variations in food supply and temperature, thus the latter two factors ought to interact significantly in affecting the reaction norm (Perrin 1995). These observations have stimulated a debate about the universality of these patterns among ectotherms, the physiological and evolutionary bases for these relationships, and whether there exists reciprocal selection between development time and body size at maturity (Berrigan and Charnov 1994, Perrin 1995, Atkinson 1994).

Patterns of mortality during growth may also vary as temperature and food supply interact, and thus may affect the relationship between development time and body mass (Sibly and Atkinson 1994, Perrin 1995). For example, development times are extended at lower temperatures, and body sizes are predicted to be larger at lower temperatures, but mortality might be greater at lower temperatures than at higher ones, thus increasing the *per capita* food supply of survivors and therefore their growth rate. Interestingly, Sibly and Atkinson (1994) proposed the opposite model –that mortality would be higher at higher temperatures—to explain patterns of body size variation in ectotherms using a optimality modeling approach within the context of a tradeoff between development time and optimal body size (and therefore, fecundity, inasmuch as body size and fecundity are typically positively correlated). Specifically, they

noted: "discounted juvenile mortality correlates positively with rearing temperature" and that "If discounted juvenile mortality rate does not increase at higher temperatures, some of [our] predictions are difficult to reconcile with the observed effects of rearing temperature on adult body size because . . . most ectotherms respond to higher rearing temperature by maturing at a smaller size." Although there has been no experimental test of the hypothesis that body size could be mediated by variation in mortality during development, it could provide a mechanistic explanation for the patterns observed in laboratory and field experiments (Perrin 1995).

The food supply of many aquatic macroinvertebrates in freshwater ecosystems is allochthonous organic detritus, particularly shed, senescent leaves (Anderson and Sedell 1979, Cummins and Klug 1979). Some of these invertebrates shred and consume leaf detritus directly for food (e.g., crane fly larvae, Lawson et al. 1984), while others browse the microorganisms comprising the biofilm on the surface of the detritus and thus utilize the detritus resource indirectly (e.g., mosquito larvae, Merritt et al. 1992). For invertebrates with either feeding mode, their growth and metamorphosis depend upon the quantity and quality of leaf detritus available to them and the extent of microbial conditioning of the detritus (Berrie 1976, Ward and Cummins 1979, Fish and Carpenter 1982, Lawson et al. 1984, Slansky and Scriber 1985, Walker et al. 1997). Larvae of the treehole mosquito *Aedes triseriatus* (Say) utilize indirectly the leaf detritus that falls into treeholes (Fish and Carpenter 1982, Carpenter 1983, Walker and Merritt 1988, Lounibos et al. 1993, Leonard and Juliano 1995, Walker et al. 1997).

Craig (1983) reviewed the biology of this mosquito. Larval growth of *A. triseriatus* is largely dependent upon mass or ration of leaf detritus available per individual larva (Carpenter 1983, Leonard and Juliano 1995), although variation in quality of leaves also affects larval growth (Fish and Carpenter 1982, Lounibos et al. 1993, Walker et al. 1997, Strand and Merritt 1999). A microbial lawn develops on the leaf surfaces, which the larvae browse intensely (Fish and Carpenter 1982, Walker and Merritt 1991, Kaufman et al. 1999). The larvae also actively feed in the water column (Walker and Merritt 1991) and the bacterial and protozoan populations in the water column respond quantitatively and qualitatively to this feeding pressure (Walker et al. 1991, Kaufman et al. 1999, Merritt et al. in preparation). Thus, microorganisms associated with leaf surfaces and the water column serve as larval food.

The growth of *A. triseriatus* subsisting on decomposing senescent leaves is described well by a curvilinear relationship between development time to and mass at pupation called the 'pupation window model' (Gilpin and McClelland 1979, Carpenter 1984, Walker et al. 1997). The model predicts that larvae will pupate at or beyond some critical, minimum mass after having passed through a minimum developmental period. This concept was first proposed as a generalizable growth model for insects by Nijhout (1975). The pupation model describes an inverse, nonlinear relationship between development time and mass at pupation. Larvae develop faster and pupate at a greater mass above the critical mass when food is in ready supply, but develop slower and pupate at a lower mass (or do not pupate at all, but rather discontinue growth, lose mass,

and starve to death) when food is in short supply. The mathematical expression of growth is derived from fitting the growth trajectories of individual insects' development time and body mass at maturity, or mean development time and body mass in the case of cohorts, in mass and time space according to the following equation:

$$W_t = W_0 \exp\{(ak/m - k)(B_0/N_0)[\exp((m - k)t) - 1]\}$$

as described by Gilpin and McClelland (1979), Dye (1982), and Carpenter (1984), where W_t = the mass of an individual at day t, W_0= the mass of a newly-hatched larva, a= the efficiency of conversion of leaf mass into mosquito tissue, k = decomposition rate of leaves, m = larval mortality rate, B_0 = initial mass of leaf detritus, and N_0 = initial number of mosquito larvae. The endpoint of a successful development to adult emergence is then represented by coordinates (x,y) corresponding to development time and mass. These data are fitted to the following hyperbolic equation from Carpenter (1984):

$$h_3 = (time - h_1)(mass - h_2)$$

where h_3 represents the sharpness of the bend in the hyperbola, h_1 is a development time minimum, h_2 is a body mass minimum, and *time* and *mass* are parameters estimated from experimental data.

Temperature affects rate of growth of larval mosquitoes and body size at maturity, as it does all ectotherms (Bertalanffy 1960, Clements 1992, Atkinson 1994). Within thermal limits of tolerance, the rate at which mosquito larvae develop increases with temperature (Bar-Zeev 1958, Nayar 1968), whereas the resulting size of the pupa or emergent adult varies inversely with temperature

(van den Heuvel 1963, Nayar 1969, Hien 1975). Chambers and Klowden (1990) observed that larvae of the mosquito *Aedes aegypti* (L.) pupated at a lower mass when larval rearing temperature was increased, and that mass at pupation correlated positively with accumulation of nutritional reserves in the last larval instar, just prior to pupation, as food supply (a laboratory diet) was increased. Thus, temperature influences not only rate of growth, but also the efficiency with which nutrients are acquired and assimilated.

In the present study, experiments on the growth of an indirect detritivore, larvae of the mosquito *Aedes triseriatus*, were conducted to measure the interactive effects of variable food availability and temperature on age and size at maturity. Because mortality of larvae may vary with these factors as well, experiments were conducted to evaluate the effects of mortality among cohorts of larvae on the responses of development time and mass. The pupation window model was used as a tool to estimate body mass at and development time to maturity to compare responses under different experimental conditions.

Materials and Methods

Four laboratory microcosm experiments were conducted in order to examine the effects of temperature, food resource availability and density on larval *A. triseriatus* growth, development and mortality (or conversely, survival to the adult stage). In all experiments, senescent beech leaves [American beech (*Fagus grandifolia* Ehrh.)] served as the source of organic nutrients (*cf.* Walker et al. 1997). The leaves were dried for 48 hours at 40^0C, their petioles were removed

to eliminate refractile material, leaf pieces were weighed, and then were added to the microcosms as individual pieces or in packs. The first experiment was designed to examine the possibility of interactive effects of temperature and food availability on larval mosquito growth and development, and to measure mortality of larval cohorts at different temperatures. Microcosms for this experiment were plastic dishes, 15.5 cm in diameter x 6.5 cm deep with friction-fitting lids. Each microcosm was filled with 400 ml of stemflow collected from an American beech tree in nearby woods as described by Walker et. al. (1991). Twenty newly hatched first instar *A. triseriatus* larvae were then added. Leaf packs, prepared as described previously, were added in amounts of 0.5, 1.0 or 2.0 g, creating leaf rations of 25, 50 and 100 mg/larva. The microcosms were then arranged randomly with respect to treatment in constant-temperature incubators at 15, 20 or 25^{0}C, resulting in a 3 x 3 factorial design. Each treatment combination was replicated with six microcosms. It was not possible to replicate at the level of incubator owing to limitations in availability of equipment, however, incubators were of the same make and any differences in results were unlikely to have been affected by them. The microcosms were examined daily and newly emerged, adult mosquitoes were collected and killed by freezing. Following completion of the experiment, adults were dried for 48 hours at 40^{0}C and weighed to the nearest 0.001 mg on a Cahn electrobalance. Male and female emergence (total number per microcosm), mean development time (days from the beginning of the experiment until emergence) and mean body mass were calculated. A two-way analysis of variance (ANOVA) was used to test for significant treatment effects

on the responses. Data of mean development time and mean female mass were fitted to the pupation window model using least squares, nonlinear regression with the NLIN procedure of SAS (1985), following the methods described in Walker et al. (1997). Initial estimates used as input for parameters h_1 and h_2 were derived from plots of development time and mass, whereas initial estimates for h_3 ranged between 0.1 and 0.5.

In the second laboratory experiment, a constant temperature was maintained, but food level and larval density were varied. Thus, this experiment was designed to examine whether larval density within an experimental cohort *per se* will affect survivorship of larvae. Microcosms for this study consisted of polyvinylchloride pipe fitted with a fiberglass disc on the bottom (inner diameter, 7.6 cm; capacity of 700 ml). Microcosms were filled with 600 ml of sterile water and then inoculated with 1 ml of a blended slurry created from natural treehole contents (leaves, water and sediments) collected locally. In addition, leaf packs were prepared as previously described and distributed into the microcosms in 1.0 and 3.0 g amounts. The microcosms were covered with nylon screen, fitted with a foam plug, and allowed to incubate for one week prior to the start of the experiment. The experiment commenced after this time, when either 20 or 90 newly hatched, first instar *A. triseriatus* larvae were added to microcosms. In addition to varying food level and larval density, this experiment also examined the effects of disturbance on these closed systems. Previously, disturbance with stemflow was found to enhance larval survival to the adult stage (Walker et al. 1991). Because larval survival is hypothesized here to be a factor affecting

development time and body size outcomes, this kind of disturbance was simulated experimentally here. It was done by pouring sterile water or stemflow water through a funnel containing a mesh filter (to remove large particulate matter), followed by dripping the water through an 18 gauge needle at a rate of approximately 15 ml/min. A set of microcosms was not subjected to this treatment, as an experimental control group. The microcosms were disturbed in this way once per week for the five weeks. The design of this experiment resulted in two larval densities, three disturbance factors and two food levels for a 2 x 3 x 2 factorial design. The experiment was replicated four times for each treatment combination. Newly emerged, adult mosquitoes were collected and experimental responses were recorded as described previously. Three way ANOVA was used to test for significant treatment effects on growth responses and survival.

The third experiment was similar to the first in that it was designed to test for effects of variable food resources and varied temperature regime on larval *A. triseriatus* development. However, this experiment eliminated within-cohort, larval mortality and any effects of density because larvae were held singly. Microcosms were glass, liquid scintillation vials. Each vial was filled with 20 ml of stemflow, and one newly hatched *A. triseriatus* larva was added. Leaf packs were formed in the range of 25 to 100 mg, and were added. There were a total of 120 microcosms. The same three temperature regimes were utilized as in experiment 1, i.e., 15, 20 and 25 ^{0}C, with forty microcosms containing variable leaf masses at each temperature. Microcosms were monitored daily for

emergence of adults, and adults were treated and data responses recorded as in previous experiments. The response data were analyzed using analysis of covariance (ANCOVA) where leaf mass was held as the covariate, and coefficients from linear regression using leaf mass as the independent variable were estimated to determine the effect of variation of leaf mass among different temperatures on growth responses. Data of development time and female mass were fitted to the pupation window model as with experiment 1.

The fourth experiment combined effects of variation in food resource availability, temperature regime and larval density on larval growth, metamorphosis, and mortality of *A. triseriatus*. Microcosms were scintillation vials provided with 20 ml of collected stemflow as in experiment 3. The three temperature regimes utilized in previous experiments were used again, i.e., 15, 20 and 25 ^{0}C. Larval densities were 1, 5 or 10 larvae per microcosm. Forty microcosms were used for each of the combinations of temperature and density, containing a varying amount of leaf mass created by addition of leaf packs weighing from 25 to 100 mg each resulting in a total of 360 microcosms. Adults and adult data were collected as described above. For microcosms with 5 or 10 larvae, mean adult male or female mass and mean development time were calculated and used as the response variables. An ANCOVA with leaf mass as covariate and density and temperature as fixed effects was used to test for effects of these treatments on the response variables.

Results

Experiment 1: larvae reared at densities of 20/microcosm

In the first experiment, both temperature and food regime affected the development time, metamorphosis, and survival of male and female *A. triseriatus* larvae. Overall, male and female development times were longest at the lowest temperature and the lowest food level, and both significantly decreased with increasing temperature and food level ($F = 155.12$, $df = 2$, $P < .001$ and $F = 165.10$, $df = 2$, $P < .001$ respectively) (Figure 3.1). Body mass was significantly influenced by food level for both males and females ($F = 63.67$, $df = 2$, $P < .001$ and $F = 26.23$, $df = 2$, $P < .001$), and temperature significantly affected body mass of females ($F = 13.91$, $df = 2$, $P < .001$) and males ($F = 3.80$, $df = 2$, $P < .05$). As shown in Figure 3.2, male and female body mass increased with increasing food level but only female mass increased in a definitive pattern as temperature decreased. The survival rate of both males and females was significantly and directly related to food level ($F = 13.66$, $df = 2$, $P < .001$ and $F = 8.12$, $df = 2$, $P < .001$) with the lowest survival rate corresponding to the lowest food level (Figure 3.3). Male survival was not influenced by temperature, but female survival was significantly affected ($F = 13.741$, $df = 2$, $P < .001$) and was lowest at the lowest temperature (15 ^{0}C). There were no significant interactions between food level and temperature for any of the growth responses.

Pupation window model fits to response data, constructed as plots of development time and adult mass, are shown in Figure 3.4. Estimates of parameters for the model are shown in Table 3.1. For 20 and 25 °C, the data fit

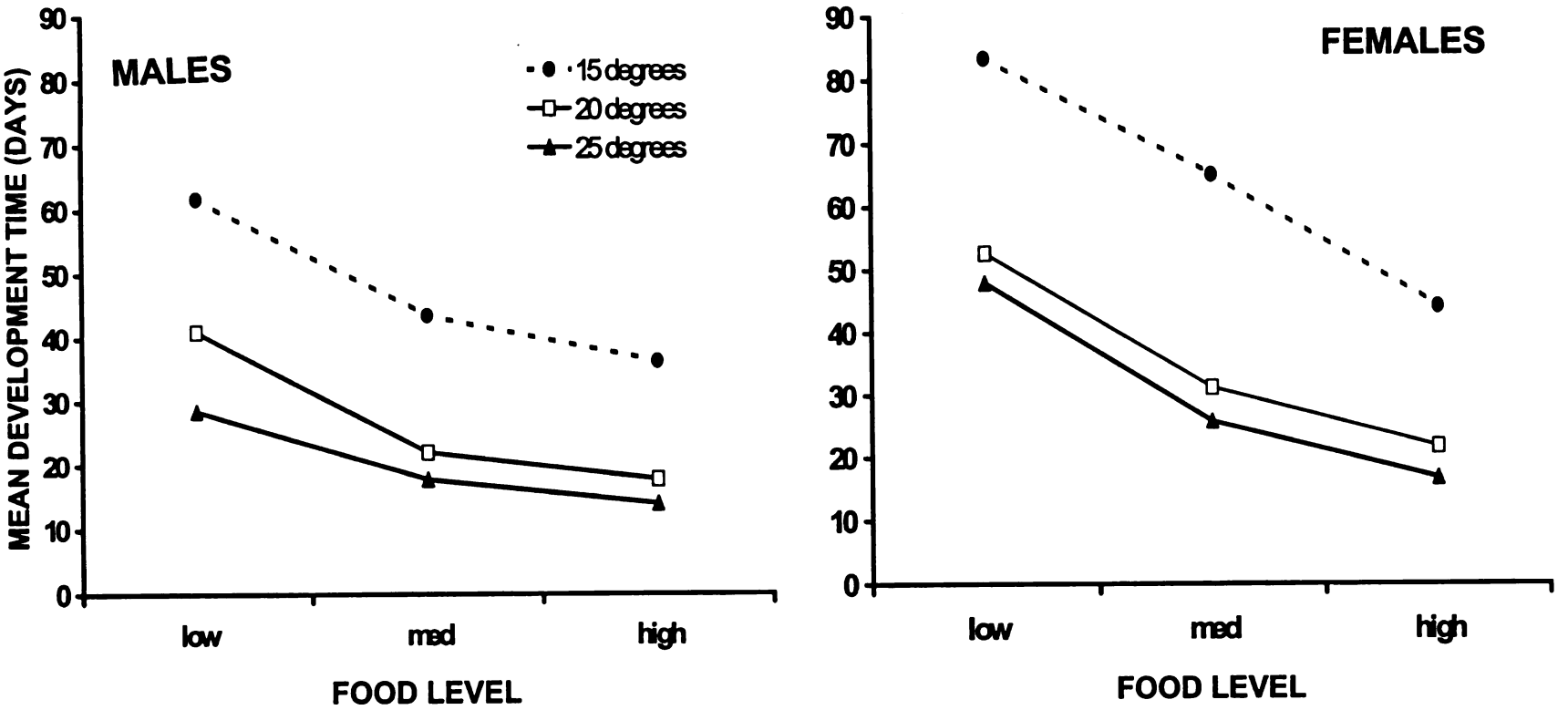

Figure 3.1 Male and female development time for three temperatures and three food rations.

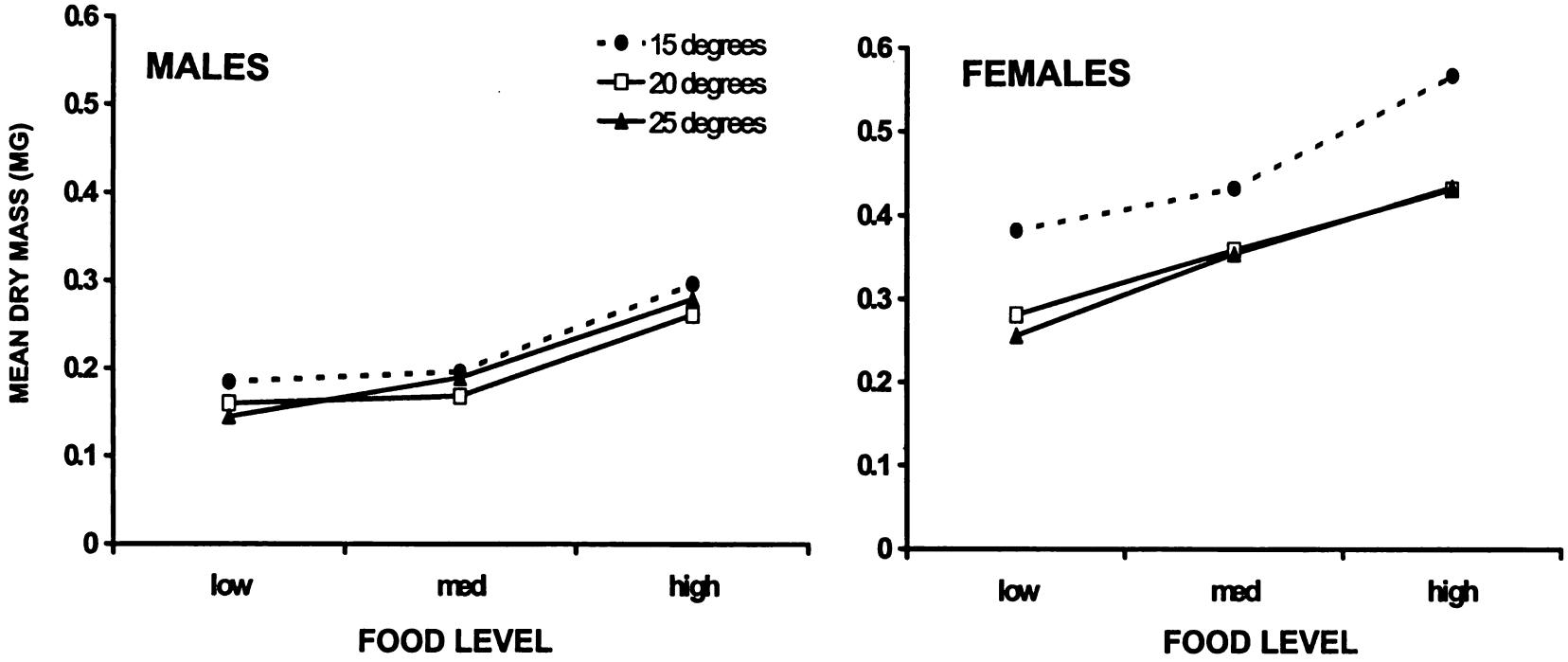

Figure 3.2 Male and female mass for three temperatures and three food rations.

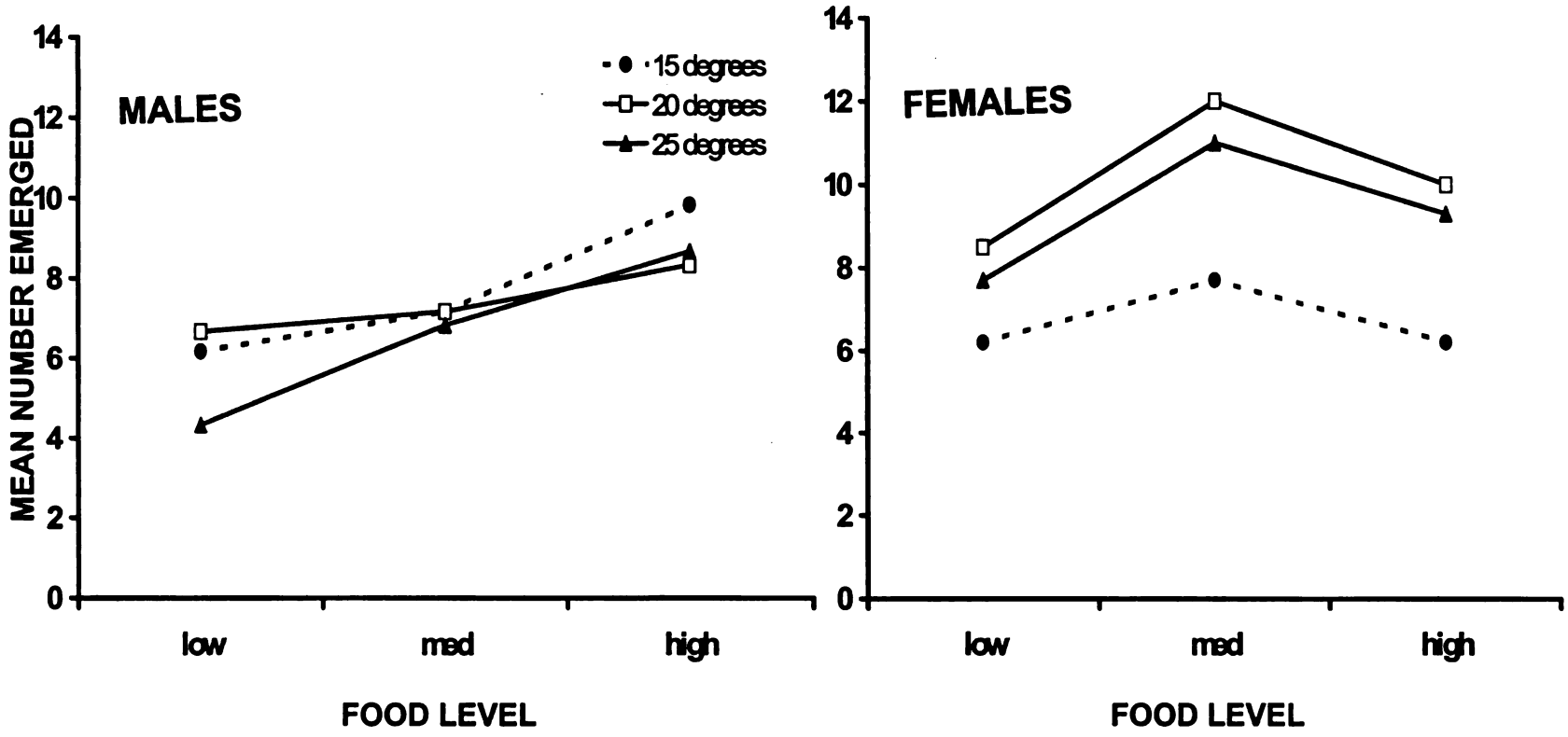

Figure 3.3 Male and female survival rates for three temperatures and three food rations.

the model well and conformed to the prediction that body mass and development time would be negatively correlated under conditions of variable food availability. However, at the lowest temperature, there were fewer females produced per microcosm, and in some microcosms no females emerged. Under these conditions, the data fit the pupation window model poorly and the parameter estimates had high variability (Table 3.1). The estimates of body mass (h_2) and development time (h_1) parameters showed that the mass at pupation was lowest at the highest temperature, highest at the lowest temperature, and intermediate at the intermediate temperature.

Experiment 2: larvae reared at densities of 20 or 90/microcosm

Disturbance had no significant statistical effect on any growth and development parameters measured for this experiment and replicates were therefore pooled for further analysis. Summary data are shown in Table 3.2. Food level significantly affected both male and female development time as well as female mass ($F = 16.68$, $df = 1$, $P < .001$ and $F = 55.15$, $df = 1$, $P < .05$ respectively). Male mass was not influenced by food level in this experiment. For females, body mass increased and development time decreased as food ration increased ($F = 41.38$, $df = 1$, $P < .001$) (Table 3.2). Survival of larvae also increased with increasing food levels. The effects of density on larval growth parameters were also examined in this experiment. Densities of 90 larvae per microcosm with 1 gram of leaf material resulted in a food ration of approximately 11 mg/larva. Under these conditions, few females pupated, and survival was

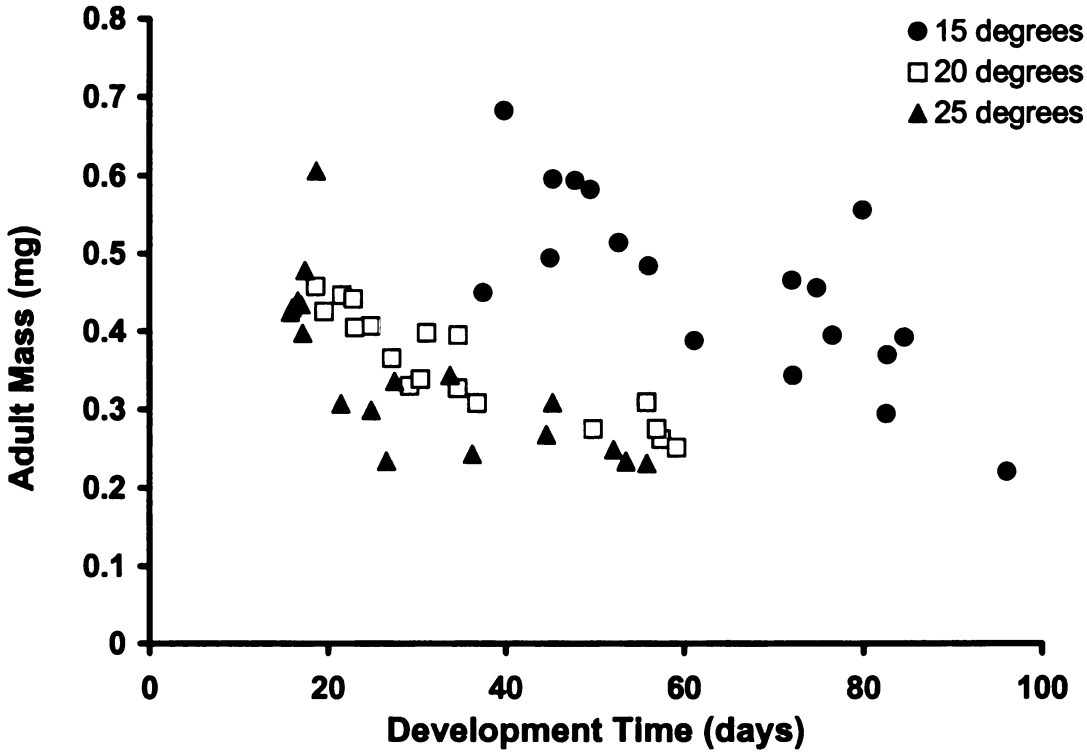

Figure 3.4 Scatter plot of pupation window data obtained from larvae reared in populations of twenty.

Table 3.1 Estimates of pupation window parameters for results of experiment 1 (larvae reared in populations of twenty), plus upper and lower asymmetric 95% confidence limits are given in parenthesis.

Temperature	h_1	h_2	h_3
15	39 (38.0 – 41.9)	0.44 (0.38 – 0.50)	0.25 (-0.02 – 0.70)
20	22 (21.1 – 23.7)	0.35 (0.32 – 0.38)	-0.03 (-0.16 – 0.08)
25	19.5 (19.1 – 19.9)	0.31 (0.27 – 0.34)	-0.16 [-0.27 – (-0.060)]

Table 3.2 Survival, development time and adult mass at low and high leaf rations. Values are given as the mean ± SEM (N = 6 per group)

		20 larvae		90 larvae	
		1 g leaves	3 g leaves	1 g leaves	3 g leaves
% Survival					
	Total	8.67 + 2.50	14.33 + 3.34	.250 + 0.45	12.67 + 3.60
Development Time					
	Males	22.55 + 2.67	20.58 + 1.39	30.00 + 5.00	21.57 + 3.31
	Females	26.95 + 3.44	23.1 + 2.33	0	24.75 + 5.32
Adult Mass					
	Males	.2013 + .056	.406 + .078	.105 + .015	.132 + .037
	Females	.350 + .129	.611 + .148	0	.205 + .049

significantly affected by density (F = 320.51, df = 1, P < .001). The higher density also significantly reduced female mass (F = 226.91, df = 1, P < .001) and resulted in a general trend of decreased size and increased development time for both males and females in response to increased densities (Table 3.2).

Experiment 3: larvae reared individually

This experiment eliminated any effects of density by rearing larvae individually. Figure 3.5 shows that there were positive correlations between starting leaf mass and the resulting adult mass of both males and females. For females, the relationship tended to be linear to 100 mg of leaf material/larva continues while for males the relationship appear to plateau at about 75 mg. The regression equations are shown in Table 3.3. Male and female development times were significantly influenced by temperature (F = 686.65, df = 2, P < .001 and F = 82.83, df = 2, P < .001 respectively) as evidenced by extended development times with decreasing temperatures. Development time for females was significantly slowed by decreasing food levels (F = 14.1, df = 1, P < .001) while male development time was not effected. Adult masses of males and females were significantly affected by food level (F= 4.3, df = 1, P< .05 and F= 109.1, df = 1, P < .001 respectively) however, male mass was not affected by temperature and the female mass was significantly affected to (F = 4.30, df = 2, P < .05) for this experiment. In addition, the greatest mass for females was at 20^0C (mean mass = 0.53 mg) and not at the highest (0.46 mg) or lowest (0.48 mg) temperature. A Least Squares multiple comparisons test based upon the

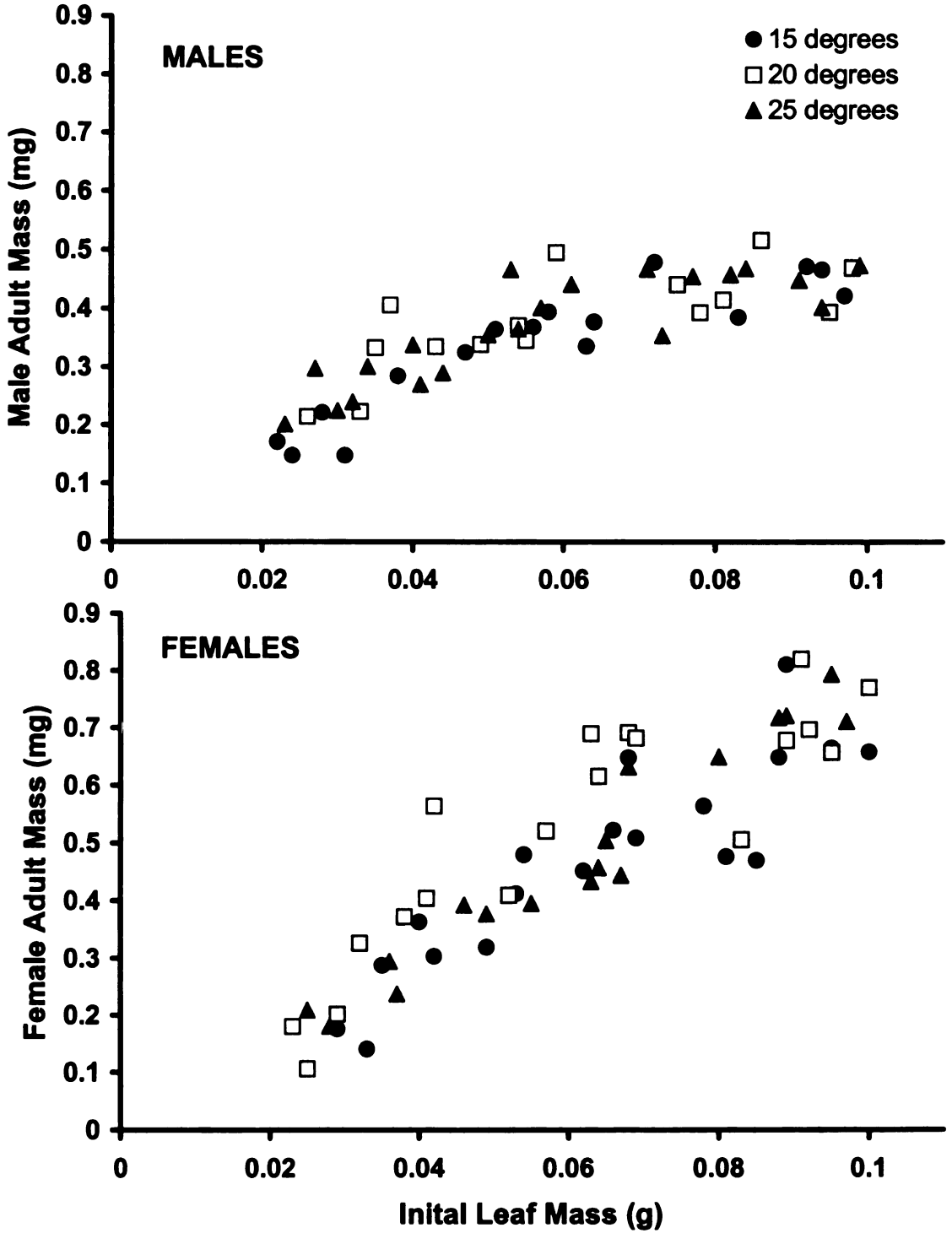

Figure 3.5 Scatter plot of data for male and female adult mass against the starting leaf mass available for development.

Tukey-Kramer adjustment showed that the female mean mass at 20 °C was significantly greater that the mass at 15 °C, but equal to that at 25 °C. Examination of the pupation window curves for individually reared larvae (Figure 3.6) indicates that in fact decreasing temperature extends development time without significantly increasing the mass required for pupation. Estimates of parameters for the model are shown in Table 3.4.

Experiment 4: variable densities

There was a significant effect of temperature on female mass ($F = 3.34$, $df = 2$, $P < 0.05$) but not male mass under varied larval densities. Although survival was reduced at high densities and low temperatures, the average mass for female larvae reared individually was highest at the intermediate temperature (20 ^{0}C) but for larvae reared in groups of 5 or 10 the average female mass was highest for the lowest temperature (15 ^{0}C). Similar to previous experiments both male and female development times were influenced by temperature ($F = 55.23$, $df = 2$, $P < .001$ and $F = 55.56$, $df = 2$, $P < .001$ respectively). Food levels slightly influenced male development times ($F = 4.219$, $df = 1$, $P < .05$) but not female development times while adult masses for both males and females were affected by food levels ($F = 46.10$, $df = 1$, $P < .001$ and $F = 26.16$, $df = 1$, $P < .001$ respectively).

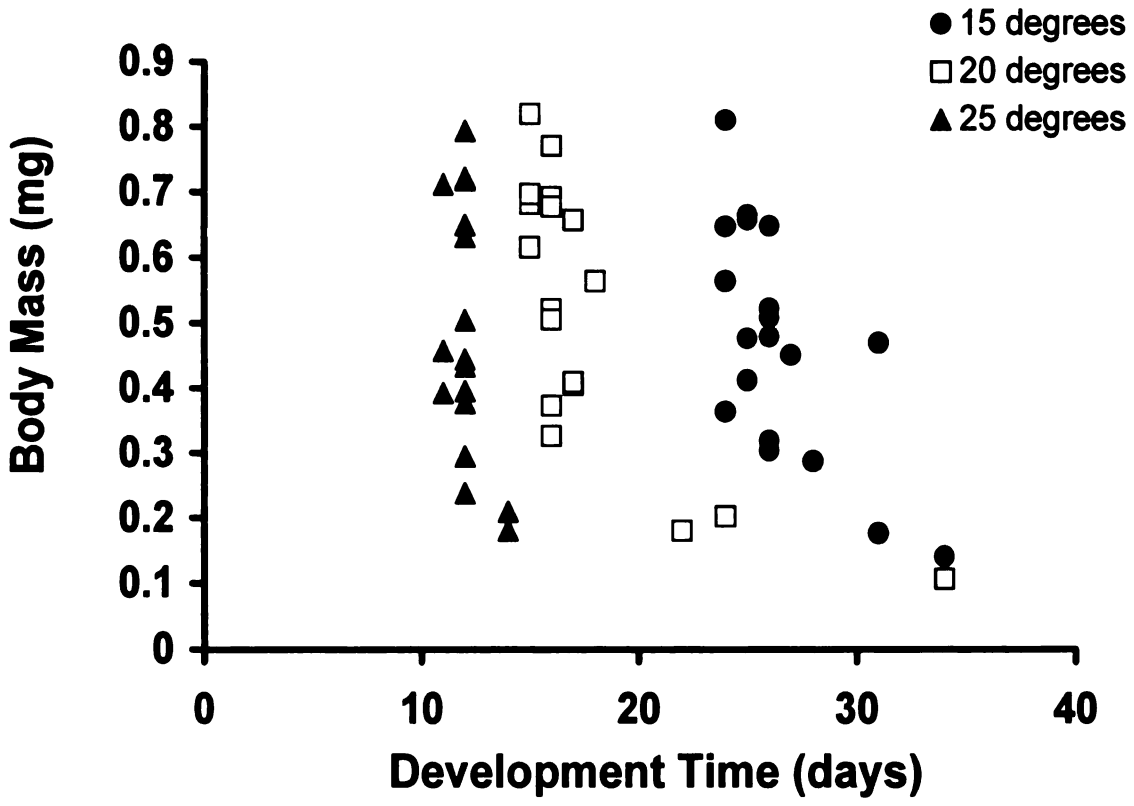

Figure 3.6 Scatter plot of pupation window data obtained from individually reared larvae.

Table 3.3 Linear regression equations for relationships between temperature, leaf ration and adult mass. Y = mosquito mass (mg), X = leaf mass (mg).

SEX	TEMPERATURE	EQUATION
Male	15	Y = 0.106 + 0.004X, r = 0.90
Male	20	Y = 0.217 + 0.003X, r = 0.74
Male	25	Y = 0.182 + 0.003X, r = 0.84
Female	15	Y = 0.020 + 0.007X, r = 0.92
Female	20	Y = 0.085 + 0.008X, r = 0.90
Female	25	Y = -0.029 + 0.008X, r = 0.97

Table 3.4 Estimates of pupation window parameters for results of experiment 3 (larvae reared individually), plus upper and lower asymmetric 95% confidence limits are given in parenthesis.

Temperature	h_1	h_2	h_3
15	30 (25.6 – 34.9)	0.46 (0.29 – 0.64)	-0.11 (-0.72 – 0.49)
20	20 (17.9 – 22.9)	0.44 (0.26 – 0.62)	-0.52 [-1.01 – (-0.05)]
25	14 (9.3 – 18.7)	0.53 (-0.18 – 1.24)	0.02 (-0.18 – 1.24)

Discussion

The effects of temperature and food ration level were similar among all experiments with regard to development time but these trends were not consistent for body size. Thus, the results reported here did not always support the existing models for ectotherm development for body size (Sibly and Atkinson 1994, Perrin 1995). Both male and female mass tended to respond positively to increases in leaf mass, while female (but not male) development time was generally shortened in response to increases in leaf mass. Male and female development time generally decreased with increased temperature. Body mass varied with temperature, but only when larvae were reared in cohorts as in experiment 1. This finding is consistent with the generalizations of Atkinson (1994). However, results from experiment 3, when larvae were held individually, did not support this generalization. Thus, the most important outcome of the experimental analyses reported here is that the experimental context can affect the outcomes regarding the mass and development time relationships that were under study. The effect was likely mediated through differential larval mortality.

In experiment 1, larvae were held in cohorts at initial densities of 20 per microcosm, provisioned with low, medium, or high food levels, and were held at one of three constant temperatures. Although the main treatment effects in this experiment were marked, the ANOVA showed that temperature and food level did not interact with each other. Significant interaction terms in ANOVA should occur if there were different responses to development time and mass for each

combination of food level and temperature in the first experiment. Further, feeding rate and assimilation rate respond differently to the same changes in temperature when food supply varies (Bertalanffy 1960, Perrin 1995), thus growth rate (an outcome of the combination of these two variables) should have responded differently to interactions of the main treatments. One possible explanation for this result relates to the differential larval mortality within treatment combinations during the course of the experiments. Thus any interactions between main treatments could have been masked by this uncontrolled covariate. Another possible explanation is that food supply was not actually constant for larval cohorts during the course of the experiment, because there would have been differential development of microorganisms on the surface of leaves as a function of temperature as well. Thus availability and acquisition of microbial food were also variables, but they were not measured.

The influence of temperature on mass varied with sex of the larvae, and with experimental conditions including cohort or individual rearing, and density of larvae in cohorts. Males and females responded differently to changes in temperature and food. Females tended to show clear experimental responses to variations in food and temperature, whereas males did not consistently respond. These results likely have their explanation in sexual polymorphism in development time and body size phenotypes among *Aedes* mosquitoes (Christophers 1960, Clements 1992). Males may have been selected not to grow to an optimally large body size but rather to grow rapidly and leave habitats early, before they dry or before some other catastrophic event occurs. Females, on the

other hand, may be selected to optimize body size during larval growth in order to maximize fitness, inasmuch as body size and fecundity are positively correlated in female mosquitoes (Clements 1992). Thus, it should not be surprising that male and female larvae would respond differently to combined effects of variation in food availability and temperature. influenced by temperature while females are significantly affected. When larvae were reared in large microcosms where a small number shared a common food resource, females reared at the lowest temperature (15 ^{0}C) attained the greatest mass but also had the highest level of mortality. In contrast, when larvae are reared individually, females in the intermediate temperature (20 ^{0}C) were at a greater mass than those reared at the lowest or highest temperatures (15 ^{0}C), but this difference was not extreme. In addition, when larvae were reared individually the resulting graphs of development time against adult mass (representing the pupation window model) showed a stunting effect on the development time axis of the curve (Figure 3.6).

Mortality, in general, tended to be higher at lower temperatures in all experiments. When density was controlled as a factor influencing larval development and the food supply or ration per larvae was also controlled, it altered the larval response to food supply. Larvae reared among cohorts resulted in a pupation window model exhibiting a negative correlation between body mass and development time under various conditions of food availability. Although this relationship between mass and development time was maintained within each of the three temperatures, decreasing temperature ultimately resulted

in extended development time but greater body masses. This result contradicts the prediction of the current pupation window model which specifically states that prolonged development time yields larvae that pupate at a low mass near to a fixed, critical mass. Results of this first experiment suggest that although body mass at metamorphosis and development time to metamorphosis covary with food availability, these growth responses do not covary with temperature. However, survival rate of larvae in the microcosms could have influenced the food ration per larvae resulting in the increased body size at the lower temperatures. In fact, when population density was isolated as an influencing factor in development, the responses in terms of development time and mass parameters similarly changed. When larvae were reared individually, temperature regime appeared to have a reduced influence on adult mass. For females, it is important to note that although temperature did have a significant effect on adult mass, the greatest mass was found for larvae reared at the intermediate temperature (20 ^{0}C) and not for larvae reared at the lowest temperature (15 ^{0}C) as was found when larvae were reared together. These results indicate that interactions between larvae play an important role in female growth patterns.

The relationship between development time and body mass for *Aedes triseriatus* appears to be impacted by patterns of mortality. The predictions of Sibly and Atkinson (1994) indicate increased mortality with increased temperature, which they implicate as a possible evolutionary agent directing reduced adult body size as a tradeoff for fecundity at high temperatures. This

prediction is disputed by the findings presented here that *A. triseriatus* mortality is highest with the reduction of temperature. This relationship excludes the theory of a tradeoff between development time and optimal body size. In contrast these results indicate that the reaction norm as influenced by mortality acts in response to food supply, creating larger body size at lower temperatures when food availability was altered through juvenile mortality. Removal of the mortality variable resulted in elimination of the expected body size reaction norm to temperature (Atkinson 1994, Perrin 1995).

The pupation window model has been used to describe the relationships between larval development time and mass at pupation. This model predicts that pupation will occur at some minimum mass after a minimum developmental period. The results of this study suggest that the critical point of pupation can be altered by varying the food supply available, so that increased food allows for increased mass and decreased food results in increased development time. Larval *Aedes triseriatus* do follow the general patterns set forth by the pupation window model, however, the pupation window model does not allow for variation in other external environmental factors acting on the developing larvae. Temperature also is a major influence for invertebrate development parameters and both food resources and temperature can influence the potential for an individual to survive. By combining different combinations of various temperatures, food levels and densities, these experiments help to better explain the relationships between these factors and their influence on *Aedes triseriatus* growth and development.

In conclusion, temperature, food availability and population density all play a great part in modeling the developmental patterns of larval *Aedes triseriatus*. Although these experiments did not show a direct interaction between the influencing forces of temperature and food level, they did indicate their relatedness through the factor of mortality. Larval mortality is significantly influenced by both reduced food resources and decreased temperatures. When mortality reduces the population density it redistributes the ration of food available among the remaining cohorts, this alteration in food availability directly alters the possibility of maximum mass possible for these larvae. Although the exact relationship between these three influential growth parameters has yet to be determined, it is clear that future models of larval mosquito growth and development must include mortality.

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CHAPTER 4

Summary and Synthesis

The intentional control of mosquito populations can be a complex activity. It must satisfy the demands of diverse groups of people. In order to control effectively a mosquito population without causing undue environmental harm, one must consider a range of biological attributes of the target population, and the community of which it is a part and the ecosystem in which it lives. The need for this kind of knowledge in order to provide the necessary biological data to develop and implement new control of management methods of mosquito populations is the justification for the research reported here. The studies presented apply not only to *Aedes triseriatus per se*, but to other species that dwell in similar types of habitats, i.e., water-filled containers. Aside from these practical or applied considerations, the research reported here addresses basic scientific issues: how do trophic interactions affect the structure of biological communities, and how do variations in environmental factors affect ontogenetic parameters of organisms.

Currently in the United States, control of mosquito populations is conducted in an integrated fashion, involving approaches directed at both the aquatic and terrestrial life stages. Entomopathogenic bacteria have an increasingly important role in control of larval mosquitoes due to their selectivity, their low environmental impact, and experimental evidence that evolution of resistance to Cry11A from *Bacillus thuringiensis* serovariety *israelensis* and the binary toxin in *Bacillus*

sphaericus can be averted by simultaneous exposure to the cytolytic toxin Cyt1a (Wirth et al. 1997, Wirth et al. 2000). This strategy of vector control could be enhanced by improvement of current strains of these bacteria through incorporation of combinations of toxins, or by bioengineering new larvicidal strains selected from resident bacteria in larval mosquito habitats (Thanabalu et al. 1992, Porter et al. 1993, Thiery et al. 1993, Smith et al. 1998). For any of these approaches, there must be a thorough knowledge of the dynamics of microbial communities in larval habitats, coupled with an understanding of the population dynamics of the larvae themselves. This dissertation addressed aspects of these interrelated topics. The treehole ecosystem in which *A. triseriatus* is a dominant species, given its role (as shown here) as a keystone predator, appears to be an ideal model system for studying them.

The dynamics of larval feeding and their interaction with the microbial compartment of the tree hole ecosystem was developed here as a major theme. Two theoretical constructs central to trophic relationships in ecological systems were used as a format for design of experiments: the trophic cascade theory, and the top:down/bottom:up theory (see Chapter 2). Studies of larval feeding have previously shown that larvae feed both in the water column as well as on leaf surfaces, and that the microbial community in the treeholes is affected by feeding (Fish & Carpenter 1982, Walker & Merritt 1991, Walker et al. 1991, Kaufman et al. 1999). The studies presented here show that under certain circumstances, larval feeding reduces the microbial density in the water column, including bacteria. However, protozoans and bacteria responded differently to feeding, as

bacteria remained at high densities even when larval feeding was intense. In contrast, protozoans were grazed to low levels. Interpretation of these findings within the context of food web theory suggests the absence of a trophic cascade, indicating that relationship did not exclusively pass from bacteria to larvae through the protozoan trophic level. A top-down effect was most apparent, as the pressure of larval feeding altered the densities of protozoans and to lesser extent bacteria. These findings reinforce the idea that trophic levels are an artificial construct when predation can span taxa as different as bacteria and protozoa. The presence of a clear bottom-up effect with relation to presence of organic detritus merely reinforces the fact that heterotrophic, organic inputs are necessary in this ecosystem to support production of microbial and insect biomass (Walker et al. 1997). More interesting was that there was no detectable response of the microbial populations under study to supplementation of inorganic nutrients. Consequently, it cannot be concluded that there were bottom-up effects of inorganic nutrient additions to the treehole ecosystem. Apparently, other factors are governing bacterial dynamics, and the data here suggest that inorganic nutrients (within the confines of the experimental design) are not limiting.

These findings also have general relevance to the idea that bacteria native to a larval habitat could be selected and engineered to host combinations of genes encoding larvicidal toxins. Notably, bacteria remained at high densities even in the presence of actively feeding larvae. Thus, recombinant bacteria might persist under conditions when feeding is intense. Further studies involving intentional

releases of recombinants into microcosms under experimental conditions of the type described here and in Kaufman et al. (1999) would help to elucidate the fate of such organisms when they are mixed with natural populations of bacteria. Release of any genetically altered microorganisms could impact the ecosystem, and ultimately mosquito production, in ways that are indirect. For example, the studies on growth and development reported in Chapter 3 clearly indicated that increased population densities impacted the population by increasing mortality rate, and in turn the increased mortality rate produced fewer but larger adult females. By the same process, reduction of the population through use of biological control agents could increase the overall size of surviving individuals. Increasing the size of the females, in turn, could effect dynamics of transmission of disease agents, and population dynamics. Larger adult female mosquitoes are more fecund, producing a larger number of eggs per reproductive cycle (Barlow 1955, Bar-Zeev 1957, Steinwascher 1982, Hawley 1985). In some *Aedes* mosquito species, there is a positive correlation between body size and success of blood-feeding (Nasci, 1990). Variation in body size also affects parameters contributing to the vectorial capacity of mosquitoes for pathogenic microorganisms (discussed in Walker et al. 1987), such as the fact that adult mosquito size also can determine their susceptibility to infection (Baqar et al., 1980; Grimstad & Haramis, 1984; Kitthawee et al., 1990). Biological control by genetically engineered microbes or other entomopathogenic microbes could cause undesirable effects; research into this possibility is needed.

Although the experimental studies on trophic relationships presented here represent offer certain firm conclusions, there are still open questions. Further studies could proceed along several lines. For example, the microcosm approach here simplified the treehole community, when in fact there is considerable species packing in it. Additional studies could expand the number of species of insects that occur in natural tree holes, such as beetle larvae, midge larvae, and so forth, to examine the trophic interactions among them and *Aedes triseriatus* larvae. Another line of inquiry would be to examine the bacterial component of the food web not as an entire trophic level, but as multiple trophic compartments. Kaufman et al. (1999) have determined that qualitative shifts in the bacterial community can occur under feeding pressure from mosquito larvae. These shifts suggest variations in response to predation that may have to do with variation in digestibility of individual cells, or other factors. Finally, the plateau of bacterial density observed even in the absence of predatory pressure indicates some other controlling force is dampening bacterial growth, perhaps suggesting a carrying capacity. I would propose an investigation that examines minute resource acquisition and interaction of bacteria with viruses. Although these studies have generated much information regarding feeding relationships in container habitats, there are still important questions that remain unanswered.

Variation in size of adult ectotherms was studied here in regard mainly to development responses, however there are evolutionary implications. In *Drosophila*, multigenerational selection under different thermal regimes resulted

in selection for larger body size in populations maintained at lower temperatures (Partridge & French 1996). This trend, termed "Bergmann's rule" (Bergmann, 1847), mirrors the generalized trend currently accepted for developmental effects on size. As pointed out by Atkinson (1996), the temperatures influencing the evolution of ectothermic organisms may have the same qualitative effects on physical traits as the temperatures influencing the development of an organism. The developmental effect of temperature on size is the result of phenotypic plasticity within the individuals of a single generation reared under different environmental conditions.

Sibly and Atkinson (1994) observed that, intuitively, the optimal approach for organisms should be for adult body size to increase with temperature due to fecundity advantages associated with larger size. However, current reviews of growth studies by Atkinson (1994) indicate that of the 109 studies examined, 83.5% resulted in size reduction with increased temperature. It is interesting to note that the largest number of studies involved arthropods and within this phylum, the number studies indicating that size is reduced with increased temperature is somewhat lower (78.75%). Atkinson (1996) reviewed some hypotheses regarding the trend toward decreased size with increased temperature including von Bertalanffy's dichotomous explanation of catabolic versus anabolic processes (von Bertalanffy 1960). Von Bertalanffy (1960) suggested that the chemical dynamics of catabolic processes are more severely influenced by temperature than the physical processes of anabolism. Therefore in his growth equation, the rate of growth is expressed as the difference between

anabolism and catabolism: $dw/dt = aw^m - bw^n$. In the equation, w is weight, t is time, and a, b, m and n are indices particular to genotype and environment. The coefficient a affects anabolism and is not influenced by temperature while the coefficient b relates to catabolism which increases with temperature.

Following these principles, as temperature increases the coefficient b would increase resulting in increased growth rate and reduced final size. The puzzle becomes more complex with the consideration of food resources and juvenile mortality rates on growth reaction norms. Both reduced food and reduced temperature do in fact slow the growth rate, and predictions indicate that lowered growth rate should delay maturity and decrease size (Stearns and Koella 1986, Perrin and Rubin 1990), which are the opposite of the predictions discussed previously (Atksinson 1994). The findings of the experiments involving *Aedes triseriatus*, presented here in Chapter 3, indicate that when reared individually, they do correspond to the simple principles of growth rate reduction with limiting external influences of temperature and food. The complex interaction of temperature and food appear to be linked with another critical factor, juvenile mortality. For certain species, Stearns and Koella (1986), found that as the growth rate decreased juvenile mortality increased rapidly. Sibly and Atkinson (1994) stated that increased juvenile mortality rate selects for smaller adult body size under conditions of spatial and temporal heterogeneity. In contrast to previous studies reviewed by Atkinson (1994) in which he could find no consistent correlation between temperature and mortality rate, our data indicate that for *Aedes triseriatus* there is a positive correlation between reduced

temperature and increased larval mortality. The reaction norm for this species appears to interact with temperature, food resource and juvenile mortality. When larvae of *Aedes triseriatus* were reared as cohorts, they followed essentially the principles set forth by von Bertalanffy (1960), i.e., when temperatures are reduced the larvae grow to a larger size at a decreased rate. In other words, when population density factor is held to be constant in the experimental design, the experiments become less realistic. However, population density is in fact a variable, because of larval mortality. Consequently, the food ration increases for those survivors, and adults are consequently larger.

Because there was a strong effect of juvenile mortality on growth rate (mediated through changes in food ration through time), it was surprising that temperature and food did not interact on the growth responses. The lack of these statistically significant interactions may be explained by the dynamics of the food resources utilized by *Aedes triseriatus* larvae. Temperature would not only have interacted with growth rates of the larvae, but also with microbial growth rates, recolonization processes on grazed leaf surfaces, and on microbial conditioning and mineralization of the leaf material itself. If leaf surfaces were heavily grazed at all temperatures, then the rates of microbial processes on leaf litter (at different temperatures) might simply have been masked by grazing intensity.

The growth and development studies also have not ultimately determined the relationships of external or environmental factors on larval growth and development. Future studies could examine the relationship of temperature to

food resource availability, the effects of decreased temperature on both leaf decomposition and on bacterial turn-over rate. Additionally, future studies could examine the genetic basis for the range of growth parameters observed here. These studies concentrated on environmental influences, but there is little known about genetic control of adult mosquito size. The ultimate size of any individual organism is a combined effort of both genetic interactions and environmental influences.

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APPENDIX 1

Entomology Voucher Specimens

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2000-04

Title of thesis or dissertation (or other research projects):

Aedes triseriatus and treeholes; trophic interactions and factors influencing larval growth.

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)

Jennifer Ruth Penrod

Date June 5, 2000

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Aedes triseriatus	Michigan, Ingham County, East Lansing Michigan State Univ., ex. Lab culture					5	5		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Jennifer Ruth Penrod

Date 5-Jun-00

Voucher No. 2000-04
Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 24 Aug 2000
Date