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7-2007

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Direct and Indirect Effects of Animal Detritus on Growth, Survival, and Mass of Invasive Container Mosquito *Aedes albopictus* (Diptera: Culicidae)

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ABSTRACT   Compared with plant detritus, animal detritus yields higher growth rates, survival, adult mass, and population growth of container-dwelling mosquitoes. It is unclear whether the benefit from animal detritus to larvae results from greater microorganism growth, direct ingestion of animal detritus by larvae, or some other mechanism. We tested alternative mechanisms by which animal detritus may benefit the invasive container-dwelling mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae). In the laboratory, larvae were reared under three conditions with access to 1) detritus, but where microorganisms in the water column were reduced through periodic flushing; 2) water column microorganisms, but larvae had no direct access to detritus; or 3) both water column microorganisms and detritus. Access treatments were conducted for three masses of animal detritus: 0.005, 0.010, and 0.020 g. Water column bacterial productivity (measured via incorporation of [^{3}H]leucine) decreased significantly with flushing and with larval presence. Removing microorganisms through flushing significantly reduced mass of adult mosquitoes (both sexes), and it significantly prolonged developmental times of females compared with treatments where water column microorganisms or microorganisms and detritus were available. Survival to adulthood was greatest when larvae had access to both water column microorganisms and 0.020 g of detritus, but it declined when only water column microorganisms were available or when 0.005 g of detritus was used. These findings indicate both direct (as a food source) and indirect (assisting with decomposition of detritus) roles of microorganisms in producing the benefit of animal detritus to container mosquito larvae.

KEY WORDS   adult mass, bacteria, container mosquito, survival

Understanding vector ecology is a crucial challenge to our understanding of vector-borne disease dynamics. Container mosquitoes, which are vectors of a variety of diseases, have recently received attention, specifically in relation to how invading species alter native host–pathogen interactions (Juliano and Lounibos 2005) and how competitive interactions affect virus–host dynamics (Alto et al. 2005). Containers (e.g., tree holes, discarded automobile tires, and bamboo stumps) receive inputs of organic detritus, which have profound effects on community and population dynamics of mosquitoes (Merritt et al. 1992, Kitching 2000). Major types of detritus inputs include plant (e.g., leaves, fruit, and seeds), animal (e.g., dead invertebrates), and stem flow (i.e., organic-rich water that flows down tree surfaces and enters tree holes during precipitation events) (Yee and Juliano 2006). Container mosquitoes do not typically feed directly on detritus; instead, they feed on heterotrophic bacteria, protozoa, and fungi, which themselves subsist on de-

trital inputs (Walker et al. 1991, Merritt et al. 1992, Sota and Kato 1994). Generally, container mosquito larvae use their mouthparts to browse on hard surfaces and to filter particles and microorganisms from the water column (Merritt et al. 1992).

Although considerable progress has been made in understanding the effects of plant detritus (Fish and Carpenter 1982; Walker et al. 1997; Kaufman et al. 1999, 2002) and stem flow (Kitching 1971, Carpenter 1982, Walker et al. 1991) on container mosquito populations and species interactions, much less attention has been directed to understanding the effect of animal detritus on container species (Daugherty et al. 2000, Yee 2006, Yee and Juliano 2006) or the mechanism by which animal detritus benefits mosquitoes. Yee and Juliano (2006) demonstrated that when the eastern tree hole mosquito, *Ochlerotatus triseriatus* (Say), was reared with animal detritus, adults were heavier, survival was greater, and populations grew more rapidly than those that were reared only on leaf detritus. Daugherty et al. (2000) showed that animal detritus additions could increase the likelihood of coexistence between competing container mosquitoes *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse)

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(Diptera: Culicidae), whereas leaf-only treatments led to the competitive exclusion of *Ae. aegypti* by *Ae. albopictus.* Barrera (1996) found that *Ae. albopictus* was the superior competitor to *Ae. aegypti* when raised on leaf detritus, but competitive asymmetry was reversed when animal detritus (liver powder) was used. Yee (2006) monitored inputs of leaf and animal detritus into 16 tree holes in Illinois over four 24-h periods. Of the 21 observations where some detritus was collected, eight had inputs of animal detritus that were similar to or exceeded inputs of leaf detritus. No previous work on container systems has determined whether the benefit from animal detritus to larvae results from greater microorganism growth or direct ingestion of animal detritus by larvae.

We selected *Ae. albopictus* as our study organism because of its importance as an invasive species (Juliano and Lounibos 2005) and its status as a vector of arboviruses, such as dengue, La Crosse encephalitis, eastern equine encephalitis, and West Nile encephalitis (Mitchell et al. 1992; Ibanez-Bernal et al. 1997; Gerhardt et al. 2001; Turell et al. 2001, 2005). Since its introduction into the United States in the mid-1980s from Asia (Hawley et al. 1987), *Ae. albopictus* has become established throughout most of the southeastern United States (O'Meara et al. 1995). *Ae. albopictus* has been shown repeatedly to be the superior competitor to many resident container-dwelling mosquitoes (Daugherty et al. 2000, Teng and Apperson 2000, Aliabadi and Juliano 2002, Costanzo et al. 2005, Juliano and Lounibos 2005).

We report here a laboratory investigation of the mechanism(s) by which animal detritus benefits container mosquito performance. We manipulated the abilities of larvae to ingest detritus, to ingest water column microorganisms that subsist on detritus, or a combination of these food resources to determine which resource type was more important to mosquito survival, developmental time, and adult mass. We made a series of measurements of water column bacteria productivity (indirect measure of all microorganisms) via ^{3}H leucine incorporation rates to quantify the food environment for larvae and to understand the effect of mosquito feeding on bacteria. Based on past observations, we hypothesized that larvae would do the best in high detritus environments with access to detritus and water column microorganisms (Yee and Juliano 2006, Yee et al. 2004, Kesavaraju et al. 2007), and that larval feeding would reduce water column microorganism productivity (Kaufman et al. 2001).

Materials and Methods

Experimental microcosms consisted of 100-ml plastic beakers filled with 100 ml of deionized (DI) water and 50 μl of microorganism inoculum obtained from 21 abandoned automobile tires in Springfield, IL. Tire water contained protozoans, fungi, and bacteria based on microscopic examination. DI water was added as needed to maintain water levels during the experiment. Microcosms were placed in an incubator set on

a photoperiod of 14:10 (L:D) h at 27°C (approximate late spring to early summer conditions in Illinois; D.A.Y., unpublished data). We randomly assigned six to eight microcosms to eight trays within the incubator. Trays were loosely covered to reduce evaporation and light penetration. To minimize effects of variation in environmental conditions within the incubator, trays were rearranged every other day.

Three levels of detritus were used: 0.005, 0.010, and 0.020 g of dry mass of dead adult fruit flies [*Drosophila melanogaster* (Meigen)]. These masses correspond to ≈20, 40, and 80 fruit flies, respectively, and they are similar to levels used by Yee and Juliano (2006) to compare the effect of plant and animal detritus on the performance of the mosquito *Oc. triseriatus.* Because we wanted to create conditions of intraspecific competition, we chose levels of animal detritus for which survivorship of mosquito larvae would be <100% (Yee and Juliano 2006, Yee 2006). Adult fruit flies were acquired from colonies within the Department of Biological Sciences, Illinois State University, and they were cold-killed and then oven-dried at 60°C for 48 h before being used in microcosms. Animal detritus was placed into a submerged 10-ml plastic beaker within each 100-ml microcosm.

To test the mechanism by which animal detritus benefits mosquito growth and survival, three treatments were established by manipulating larval access to water column microorganisms or animal detritus: reduced microorganism access (RMA), no detritus access (NDA), and full access (FA). For the RMA treatment, the entire water column was removed and replaced with new DI water every other day to reduce water column microorganisms. This flushing should reduce water column food availability for larvae, but it would allow direct larval feeding on the detritus or other surfaces. For the NDA treatment, a 100-μm piece of mesh was glued over each 10-ml beaker containing the detritus. This barrier prevented mosquito larvae from having direct access to the animal detritus for feeding, but it allowed nutrients to leach from the detritus into the water column. Water was not removed in the FA treatment, nor was access to the detritus restricted, so larvae were free to feed in the water column and on detritus surfaces. A partial 100-μm mesh cover was glued to the 10-ml plastic beaker holding the animal detritus in the RMA and FA treatments, but the top remained open to allow mosquito larvae access to the detritus. A sham flush was preformed for the NDA and FA treatments, where water was poured out and back into the beaker at the same times as the flushing in the RMA treatment. Six replicates were established of all detritus amounts for each of the three detritus access treatments, yielding 54 experimental units. To determine how larval feeding affected water column bacteria productivity, two additional replicates of each detritus amount were established (constructed in the same manner as the NDA treatment). These no larvae (NL) replicates were treated the same way as the NDA treatment except they did not receive larvae. Microcosms were

incubated for 4 d before addition of mosquito larvae and flushed or sham-flushed on day 2.

Ae. albopictus were collected as larvae from tree holes along Indrio Road, Fort Pierce, FL (27° 31′ 14 N, 80° 23′ 39 W) to establish a laboratory colony from which we generated F_1 eggs used for this experiment. Field-collected larvae were raised to adults on bovine liver powder (ICN Biochemicals, Cleveland, OH) and housed in 0.1- by 0.1-m cages where females were bloodfed on anesthetized laboratory mice (IACUC protocol 01-2005). Larvae for this experiment were hatched in a solution of 0.33 g nutrient broth per 750 ml of DI water. Twenty-four hours after the initiation of the hatch, larvae were rinsed to remove nutrient solution, and then five larvae were added to each microcosm (except NL microcosms). The experiment ended 31 d after mosquito addition (approximately twice the amount of time for a well-fed *Ae. albopictus* to complete development; Livdahl and Willey 1991). Each day, we removed and isolated pupae, and we collected newly eclosed adults. Larvae that died were left in microcosms. Adults were sexed, dried at 60°C for 48 h, and weighed to the nearest 0.0001 mg by using a Cahn microbalance.

To quantify differences in microorganisms among our treatments, we measured the production of new bacterial biomass via quantification of tritiated L-leucine (4,5-^{3}H, 50 Ci mmol^{-1}) incorporation rates from two replicates from each treatment combination. The replicates used were the same across all measured times. Bacterial productivity served as an overall indication of microorganism productivity. This technique is specific to bacteria in aquatic systems (Riemann and Azam 1992), and it has been used to quantify bacterial productivity in container mosquito experiments (Kaufman et al. 2001, Yee 2006). We measured water column bacterial productivity following procedures outlined by Kirchman (1993) and refined by Kaufman et al. (2001) for container systems. Specifically, into two replicate 1-ml fluid samples from each microcosm we added [^{3}H]leucine at a concentration of 25 nM, and then we incubated for 30 min at 27°C and quantified [^{3}H]leucine incorporation (nanomoles per milliliter per hour) into protein as a measure of new bacterial biomass production. The incubation was ended by the addition of trichloroacetate (5% final concentration). Quantification of the amount of labeled protein in precipitates was conducted using standard liquid scintillation counting techniques (LS-6500 scintillation counter, Beckman Coulter, Fullerton, CA). Means of replicate values from each microcosm were the bacterial productivity values for each container. Bacterial productivity was quantified on day 4 (before adding mosquitoes, before flushing) for all samples (except NL) to define initial differences among treatments and detritus amounts. Bacterial productivity was again quantified on day 8 to compare RMA, NDA, FA, and NL treatments. Bacterial productivity was quantified on day 8, after flushing or sham flushing all microcosms. Finally, bacterial productivity was measured on day 12, 13, and 14 (day 8–10 postlarval addition) to determine the effect of flushing on bacterial productivity and to understand long-term effects of mosquito foraging on water-column bacteria. These measurements took place immediately after the flush (0 h), and again 24 and 48 h postflush.

Statistical Analyses. Differences in bacterial productivity on day 4 (prelarvae addition) and day 8 (4 d postlarval addition) were assessed using one-way analysis of variance (ANOVA) (PROC GLM, SAS Institute 2004) with combinations of treatment (RMA, NDA, FA, and NL for day 8) and detritus amount (0.005, 0.010, and 0.020 g) as the independent variable (hereafter detritus access–mass combinations). Tukey's honestly significant difference (HSD) tests (Sokal and Rohlf 1995) were used to resolve pairwise differences among means. Bacterial productivity measured on day four ($\log_{10}x$) and day 8 ($x^{0.2}$) was transformed to meet ANOVA assumptions of normality and homoscedasticity.

Repeated measure multivariate ANOVA (MANOVA, PROC GLM, SAS Institute 2004) was used to assess differences among the three measurement periods (0, 24, and 48 h postflushing) for bacterial productivity values among the nine access–mass combinations. Profile analysis was used to determine whether values for bacterial productivity changed between 0 and 24 h and between 24 and 48 h. Values for bacterial productivity on all days were transformed ($\log_{10}x$) to meet MANOVA assumptions of normality and homoscedasticity.

We analyzed mosquito mass and developmental time for each sex, separately, by using MANOVA. Significant MANOVA effects were interpreted using standardized canonical coefficients (Scheiner 2001), which quantify the magnitude of the contributions of the individual dependent variables in producing significant multivariate differences. Access–mass combinations that failed to produce adults were excluded from analyses. When necessary, significant effects were analyzed further using multivariate pairwise contrasts (Scheiner 2001) with a Bonferroni adjustment to control for experimentwise error rate. For contrasts, we compared dependent variables among treatments (RMA, NDA, and FA) within each detritus amount. Differences in survival to adulthood were assessed using one-way ANOVA with access–mass combinations as independent variables, and Tukey's HSD tests used to resolve pairwise differences among means.

Differences among the treatments (RMA, NDA, FA, and NL) in the percentage of detritus remaining from the start of the experiment were analyzed using one-way ANOVA. Tukey's HSD tests were used to resolve pairwise differences among means.

Results

Before mosquito larvae addition, bacterial productivity differed among detritus access–mass combinations ($F_{8, 17} = 26.54$; $P < 0.001$). Bacterial productivity values were significantly lower in the RMA treatment than in either the NDA or FA treatments in the 0.005- and 0.010-g detritus masses (Fig. 1a). Bacterial productivity increased with increasing detritus mass for

a. Pre-Larvae Addition
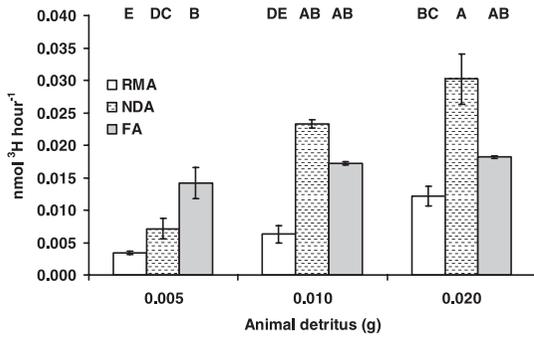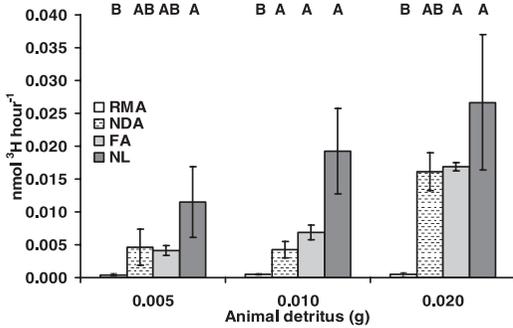

b. Post-Larvae Addition (4 days)

Fig. 1. Bacterial productivity (based on [³H]leucine incorporation rates; means ± SE; $n = 2$) in microcosms for four different treatments (RMA, reduced microorganism access, water removed every other day; NDA, no detritus access, larvae with no direct access to detritus; FA, full access, larvae given access to detritus and detritus-derived microorganisms; and NL, no larvae added) across three different amounts of animal detritus (0.005, 0.010, and 0.020 g). (a) Measurements taken before addition of five first instars of *Ae. albopictus*. (b) Measurements taken 4 d after larvae introduction. The same letters shared by means indicate no significant differences after correcting for multiple comparisons.

the RMA ($0.005 = 0.010 < 0.020$ g) and NDA ($0.005 < 0.010 = 0.020$ g) treatments, whereas there were no significant differences in bacterial productivity values for the FA treatment across detritus masses (Fig. 1a).

Four days after the addition of larvae, we detected significant differences among the detritus access–mass combinations ($F_{11, 23} = 12.64; P < 0.001$). There were no detectable differences in bacterial productivity between the NL and FA or NDA treatments across all detritus masses, although the RMA treatment was significantly lower than all other detritus access–mass combinations (Fig. 1b).

There was a significant access-mass combination effect ($F_{11, 12} = 48.87; P < 0.001$), time effect (Pillai's Trace$_{2, 11} = 0.688; P = 0.002$) and a time by access–mass combination interaction (Pillai's Trace$_{22, 24} = 1.696; P < 0.001$) for bacterial productivity for days 8 through 10 after larvae addition. In addition, profile analysis detected differences in bacterial productivity between 0 and 24 h ($F_{11, 12} = 20.54; P < 0.001$) and 24

and 48 h ($F_{11, 12} = 4.06; P = 0.012$). Values for bacterial productivity declined across the two time periods (Fig. 2). Immediately after flushing (Fig. 2a), there was significantly greater bacterial productivity for containers without mosquitoes for the 0.010- and 0.020-g detritus masses compared with those treatments with larvae. In addition, bacterial productivity in RMA microcosms was significantly lower than either NDA or FA treatments regardless of detritus mass. One day after flushing (Fig. 2b), differences among access-mass combinations were less obvious, although in general high (0.020 g) and medium (0.010 g) detritus amounts for NL and NDA had higher bacterial productivity compared with the other treatments (Fig. 2b). Some recovery of bacterial productivity had occurred by 24 h, with no detectable differences among RMA and other treatments for many of the detritus amounts (Fig. 2b). Differences among the access–mass combinations for the 48 h postflushing period were similar to the 0-h measurements (Fig. 2c). Specifically, bacterial productivity for RMA was significantly lower than for NL; mean bacterial productivity values for NDA and FA were intermediate in most cases (Fig. 2c).

Adult female mass and time to pupation differed significantly among detritus access–mass combinations (Pillai's Trace$_{12, 54} = 1.33; P < 0.001$). The standardized canonical coefficients for developmental time were large (2.270) relative to those for adult mass (0.174), indicating that developmental time contributed more to the significant multivariate effect. In high detritus FA microcosms, female mass was almost double that for females in the high detritus RMA treatments, whereas mean female mass in NDA microcosms was intermediate (Fig. 3a). Female mass from medium amounts of detritus did not vary among access treatments. Developmental time differed among high detritus microcosms, with shorter times for females in the FA and NDA microcosms compared with the RMA microcosms (Fig. 3a). Developmental time also was shorter in the medium detritus amounts for females in the FA treatment compared with the other treatments (Fig. 3a).

Male mass and developmental time also differed among detritus access–mass combinations (Pillai's Trace$_{14, 54} = 1.13; P < 0.001$). The standardized canonical coefficient for time to pupation was small (0.102) compared with that for adult mass (1.520), indicating a large role for mass in contributing to the significant multivariate effect. Males were significantly larger in FA 0.020-g microcosms compared with other high detritus microcosms, but there were no differences among access treatments in the medium detritus amount (Fig. 3b). Developmental times did not differ among males in the high or low detritus amounts among the three treatments (Fig. 3b).

Overall, 127 of the initial 225 larvae survived to reach adulthood, with an additional 33 individuals still alive as larvae after 31 d. Survival of mosquitoes differed significantly among the detritus access-mass combinations ($F_{8, 53} = 13.23; P < 0.001$). There were significant differences in survival between the FA and

a. 0 hours post flush
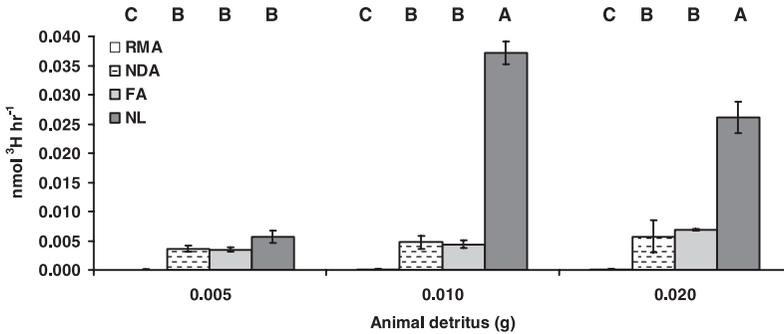
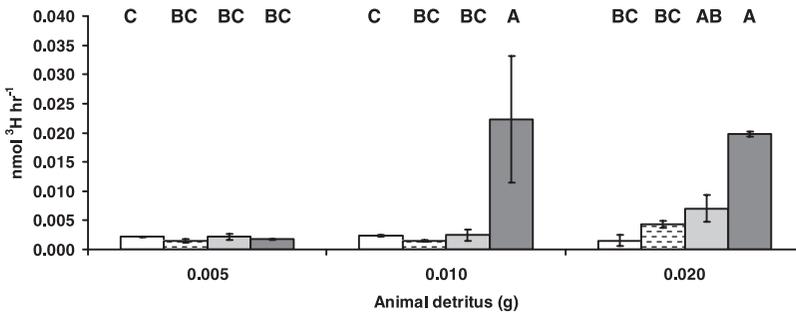### b. 24 hours post flush
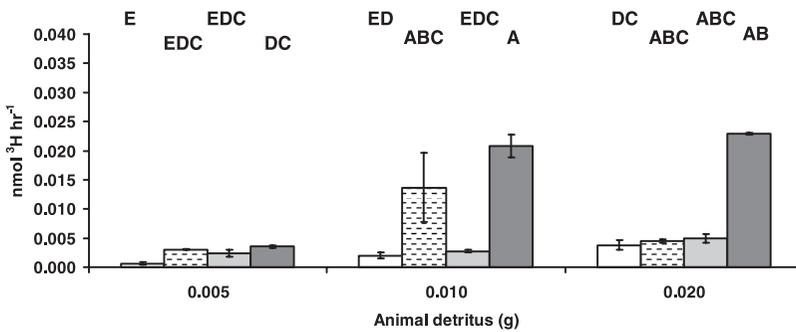
c. 48 hours post flush

Fig. 2. Bacterial productivity (based on [^{3}H] leucine incorporation rates; means $\pm$ SE; $n = 2$) in microcosms for four different treatments (RMA, reduced microorganism access, water removed every other day; NDA, no detritus access, larvae with no direct access to detritus; FA, full access, larvae given access to detritus and detritus-derived microorganisms; and NL, no larvae added) across three different amounts of animal detritus (0.005, 0.010, and 0.020 g) for days 8 to 10 postlarvae addition. Measurements were taken immediately (a), 24 h (b), and 48 h (c) after flushing RMA and sham flushing NDA, FA, and NL treatments. The same letters shared by means indicate no significant differences after correcting for multiple comparisons.

NDA treatments in high detritus, with the RMA treatment intermediate (Fig. 4). There were no significant differences among the treatments for medium detritus amounts. No larvae survived in the low detritus RMA treatment, and on average only one of the five larvae reached the adult stage in either FA or NDA low detritus microcosms (Fig. 4).

There were significant differences among the treatments in the amount of detritus remaining in a sample of containers at the end of the experiment ($F_{3,29}$ =

3.10; $P = 0.042$). Significantly more detritus remained for NDA microcosms (mean percentage of detritus left $\pm$ SE = 19.4 $\pm$ 1.76) than for RMA microcosms (7.7 $\pm$ 1.80), whereas NL (11.75 $\pm$ 1.25) and FA (12 0.91 $\pm$ 3.37) treatments were intermediate.

Discussion

The role of animal detritus in container mosquito systems is well studied for pitcher plants (Bradshaw

a. Females

Fig. 4.   Mean survival ± SE for *Ae. albopictus* adults in microcosms for three different treatments (RMA, reduced microorganism access, water removed every other day; NDA, no detritus access, larvae with no direct access to detritus; and FA, full access, larvae given access to detritus and detritus-derived microorganisms) across three different amounts of animal detritus (0.005, 0.010, and 0.020 g). The same letters shared by means indicate no significant differences after correcting for multiple comparisons.
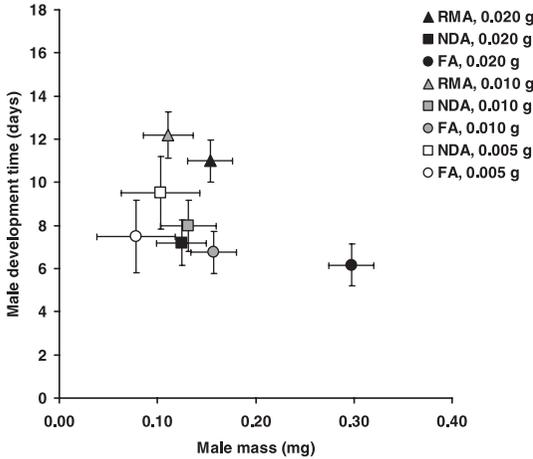
b. Males

Fig. 3.   Bivariate means ± SE for mass (milligrams) and developmental time to adult eclosion (d) for female (a) and male (b) *Ae. albopictus* in microcosms for three different treatments (RMA, reduced microorganism access, water removed every other day; NDA, no detritus access, larvae with no direct access to detritus; and FA, full access, larvae given access to detritus and detritus-derived microorganisms) across three different amounts of animal detritus (0.005, 0.010, and 0.020 g).

and Holzapfel 1986), but it is a relatively new topic for tree holes and tires (Daugherty et al. 2000, Yee and Juliano 2006, Yee 2006, Kesavaraju et al. 2007). The mechanism by which this type of detritus benefits mosquitoes is unknown. We have shown that survival, developmental rate, and adult mass of *Ae. albopictus* increased directly with detritus mass. More importantly, water column microorganisms seem to be the key to mosquito growth and development. When water column microorganisms were reduced through flushing, mass of adult female and male *Ae. albopictus* were significantly lower and developmental times for
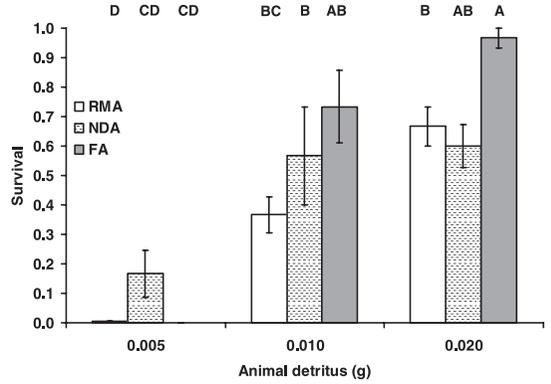
females were almost twice as long compared with treatments with water column microorganisms. That the amount of detritus remaining at the end of the experiment in reduced water column microorganism microcosms was the same as that in microcosms with water column microorganisms and detritus, but that mosquitoes faired poorly in reduced water column microorganism treatments, is further evidence of the importance of water column microorganisms to mosquito growth and development.

The value of bacteria to mosquito larval nutrition is well known (Merritt et al. 1992; Kaufman et al. 2001, 2006), and previous studies have shown that bacteria are an important trophic link between detritus and mosquitoes. For example, in container systems, leaf material is usually converted into microorganism biomass before it is useful as a food resource to larvae (Walker and Merritt 1988), as mosquitoes do not generally consume large (i.e., >50-μm) detritus particles (Merritt et al. 1992). Animal detritus decomposes at a faster rate than does plant material (Swift et al. 1979, Begon et al. 1990, Yee and Juliano 2006); therefore, the nutrients in animal detritus may be more quickly available to microorganisms and ultimately to larvae. Yee and Juliano (2006) showed that the percent of detritus lost in animal-based microcosms in the absence of mosquito larvae was nearly 80%, but only 30% of leaves of equal amount were lost after 30 d. Besides decomposing faster, animal detritus can be ingested directly by larvae (Daugherty et al. 2000, Yee and Juliano 2006). Direct ingestion would seem to be a more efficient means for larvae to obtain nutrients from animal detritus (Yee and Juliano 2006), although in this experiment mosquitoes with only access to detritus did not perform well when water column microorganisms were significantly decreased through flushing. This fact may point to the combined nutritional

value of microbial biomass and detritus to larvae. When we calculated the percentage of detritus lost, represented as the total biomass of adults in high detritus microcosms, we found that adults mass in microcosms with access to microorganisms and detritus (i.e., FA) represented a greater share of the lost detritus ($7.32 \pm 0.58\%$; $n = 5$) than either RMA ($2.66 \pm 0.40\%$; $n = 3$) or NDA ($3.39 \pm 0.78\%$; $n = 5$) microcosms. This additive effect of microorganisms and detritus on adult mass is most likely to be important in natural containers, where larvae would have access to both types of resources. Curiously, survival in high detritus microcosms was significantly lower in NDA treatments compared with FA (Fig. 4), although male and female mass and developmental time did not differ between these two treatments (Fig. 3). One possible explanation for the differences in survival but not mass could be related to dead larvae that were left in containers. Low survival in NDA treatments means that animal detritus, in the form of dead larvae, would have been available as food for surviving larvae. As was true in the FA and RMA treatments, direct feeding on detritus by larvae was important for maximizing adult mass (Fig. 3).

Behavior of larvae also seems to be an important determinant of the benefits of animal over plant detritus. *Ae. albopictus* have been observed to carry animal detritus, but not plant material, to the surface using their mouthparts (Daugherty et al. 2000, Kesavaraju et al. 2007, this study). This indicates that larvae may perceive animal detritus as a high-quality resource and direct greater foraging effort at that resource. In microcosms where larvae could only access detritus (RMA), the amount of detritus remaining at the end of the experiment was the lowest. Increased feeding of larvae on detritus may have increased fragmentation of detritus, causing more to be lost during flushing events. That larvae of *Ae. albopictus* also spend more time feeding on animal versus plant detritus when offered a choice (Kesavaraju et al. 2007) is further evidence that they perceive animal detritus as a high-quality resource. High rates of feeding on animal detritus and movement of animal detritus around the container may further accelerate detritus breakdown or affect microorganism communities. Other aquatic detritivores have been shown to enhance microorganism abundance or activity on biofilms through grazing (Lopez et al. 1977, Smith et al. 1982). Thus, the interaction between larvae and microorganisms is likely to be complementary, with microorganisms causing breakdown of detritus and making detritus available to larvae for direct ingestion, whereas larval feeding may stimulate microorganism activity and thereby intensify microorganism breakdown of animal tissue.

Mosquito larvae presence decreased significantly bacterial productivity in the water column, a result also obtained by Kaufman et al. (2001). Although we could not detect effects of feeding on bacterial productivity when larvae were small (Fig. 1a), we identified significant negative effects on bacterial productivity when larvae were larger (i.e., third or fourth instars) (Fig. 3). In addition, mean bacterial productivity rates were depressed in all treatments even after 4 d of larval presence (Fig. 1a versus b), with an overall decrease in bacterial productivity of 46.2 and 54.7% in FA and NDA treatments, respectively. Surface associated bacteria were not measured because the procedure for quantifying surface bacterial productivity is destructive. The importance of surface associated bacterial productivity to larvae has been shown to be much higher than that for the water column (Kaufman et al. 2001), and mosquito foraging has a greater effect on bacterial productivity on surfaces (Kaufman et al. 2001, Kaufman and Walker 2006). We also did not measure standing stock of bacteria, although standing stock and bacterial productivity on surfaces have been shown to be negatively affected by the presence of larvae (Kaufman et al. 2001). Our flushing treatment seemed to affect more than water column bacteria, because overall production of mosquitoes was low in reduced microorganism containers. Although we made no effort to remove surface-associated bacteria, flushing also would likely result in the loss of fine and dissolved organic matter, which would likely have effects on surface-associated microorganisms. Reductions in soluble carbohydrates for microorganisms with less frequent flushing have been observed in a similar system (Kaufman and Walker 2006). Thus, the effect of flushing may alter water column and surface microorganisms, as well as dissolved nutrients, all of which seem to have significant negative effects on larvae.

Bacteria may provide larvae with resources for maintenance, whereas other microorganisms (e.g., fungi and protozoans) provide essential nutrients for growth (Kaufman et al. 2002). We did not measure other microorganisms in this study, so it is unknown how other groups responded to our treatments or to mosquito presence. Labile carbon released from leaf detritus is used by fungi that outcompete leaf-associated bacteria for leaf-derived resources (Gulis and Suberkropp 2003). It is unknown whether such interactions also occur on animal detritus or whether fast-degrading animal detritus yields fewer microorganism interactions compared with slower decaying leaves. Future work should focus on identifying microorganism compositional differences between plant and animal detritus, and on identifying the relative role(s) of different microorganism groups to the benefit of animal detritus to mosquito growth.

An interesting result of our study was that males and females responded differently to treatments (Fig. 4), with greater impacts of microorganisms and detritus on male mass, and on developmental time for females. This difference between the sexes has been noted for a related species, *Ochlerotatus sierrensis* (Ludlow) (formerly in the genus *Aedes;* Reinert 2000), in which females maximized mass by delaying pupation, whereas males minimized developmental time by pupating at a lower mass (Kleckner et al. 1995). Kleckner et al. (1995) suggested that this situation results from selection acting on different fitness components in each sex. The results presented here are consistent

with Kleckner et al. (1995), although mosquito species and detritus types differed between these studies (Kleckner et al. 1995), suggesting that intersexual differences in selection on components of fitness are similar regardless of the detritus type.

Acknowledgments

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