Consequences of detritus type in an aquatic microsystem: effects on water quality, micro-organisms and performance of the dominant consumer

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SUMMARY

1. Variation in detritus quality and quantity can have significant effects on aquatic invertebrate food webs. Allochthonous inputs of detritus are the principal energy source for organisms in aquatic tree hole microsystems. We compared the effects of two major detritus types found in tree holes, senescent leaves (Sugar Maple and White Oak) and invertebrate carcasses (dead adult fruit flies and crickets), on several water quality characteristics of laboratory microcosms as well as on mass, survival and population performance of the dominant tree hole consumer, *Ochlerotatus triseriatus* (Diptera: Culicidae). To date, no study has documented the effects of animal detritus in tree hole microsystems or on resident consumers.
2. Aquatic environments receiving invertebrate carcasses had significantly greater total nitrogen, total reactive phosphorus and higher pH, than leaf-based environments. Decay rate of invertebrate carcasses was greater compared to leaf material. Consumption of O_2 by micro-organisms increased with increasing detritus amounts, but we detected no difference between detritus types.
3. *Ochlerotatus triseriatus* larvae grew faster in animal-based treatments, and mean mass of larvae was significantly greater when more animal detritus was used. The effect of animal-based treatments on larvae translated into higher performance for adults, which were three times heavier than counterparts from plant-based containers. Survivorship and estimated population growth rates were significantly greater for *O. triseriatus* reared on animal-based versus plant-based detritus.
4. We hypothesise two mechanisms for the pronounced effect of invertebrate carcasses on mosquito performance relative to that associated with leaf detritus: (i) invertebrate carcasses decompose more quickly and release nutrients more effectively into the aquatic environment; or (ii) *O. triseriatus* larvae may directly ingest nutrient-rich components of invertebrate carcasses. Because even relatively small animal detritus additions can have strong effects on *O. triseriatus* populations, studies need to be conducted to explore the overall role of animal detritus in tree holes in nature.

Keywords: animal carcass, leaf litter, micro-organisms, mosquito larvae, tree hole

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Introduction

Detritus is a fundamental component of most food webs, affecting trophic dynamics, species interactions and ecosystem functioning (Moore *et al.*, 2004). Detritus-based food webs are an important area of research

as detritus represents the dominant energy base in many ecosystems (O'Neill & Reichle, 1980). Although terrestrial plant detritus has been well studied in aquatic systems (Cloe & Garman, 1996), most plant-derived detritus is only indirectly available to consumers, as it must first pass through decomposer trophic levels. Detritus from animal sources (e.g. terrestrial invertebrates) also can affect aquatic food webs but may be more immediately available to consumers who ingest it directly (Mason & MacDonald, 1982; Garman, 1991). More efficient transfer of animal- versus plant-derived energy has been suggested for top consumers (i.e. fish) from a variety of stream studies (Garman, 1991; Cloe & Garman, 1996; Nakano, Miyasaka & Kuhara, 1999; Henschel, Mahsberg & Stumpf, 2001).

Tree holes are good examples of detritus-based systems that receive plant and animal inputs. Tree holes are cavities in trees that collect water and detritus. They are colonised by a diverse community of aquatic macro-invertebrates (Kitching, 2000) and are widely distributed in temperate and tropical regions. Tree hole food webs receive energy almost exclusively from allochthonous inputs, as primary production is essentially absent (Carpenter, 1983). Detrital inputs affect populations and community composition of heterotrophic micro-organisms, which in turn have consequences for invertebrate consumers. The most common types of terrestrial detritus into tree holes are senescent plant parts (primarily leaves), stem flow (water flowing along tree trunks after precipitation events containing fine and dissolved organic matter) and terrestrial invertebrate carcasses (D. A. Yee, unpublished data). Although there have been many tests regarding the effects of leaf litter inputs (e.g. Lounibos, Nishimura & Escher, 1993; Léonard & Juliano, 1995; Walker *et al.*, 1997; Srivastava & Lawton, 1998) and stem flow (e.g. Kitching, 1971; Carpenter, 1982; Walker *et al.*, 1991) on populations and communities in tree holes, no study has examined the role of dead invertebrates as an energy source for aquatic inhabitants of tree holes. For species inhabiting man-made aquatic containers (e.g. discarded automobile tires), Daugherty, Alto & Juliano (2000) have shown that animal detritus additions could increase the likelihood of co-existence between competing mosquitoes *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), although leaf-only treatments resulted in exclusion

of *A. aegypti*. Furthermore, Barrera (1996) found that *A. albopictus* (Skuse) was superior in competition to *A. aegypti* when raised on leaves, but competitive asymmetry was reversed when the detritus was derived from animal tissue (liver powder). In another aquatic microsystem, pitcher plants, the effects of invertebrate carcasses are well known, as animal detritus is a major determinant of invertebrate consumer abundance and diversity (Istock, Vavra & Zimmer, 1976; Cresswell, 1998, 2000; Sota, Mogi & Kato, 1998). For instance, the abundance of the mosquito *Wyeomyia smithii* (Coquillett) has been shown to be positively related to abundance of animal detritus in pitcher plants, and negative effects of density dependence can be minimised by additions of animal detritus (Bradshaw & Holzapfel, 1986). Because tree holes may last for years or decades, usually contain more invertebrate species, and receive greater accumulation of multiple types of detritus over time than short-lived pitcher plants (Kitching, 2000), animal detritus inputs may play a larger role in affecting community and population dynamics in tree holes than in pitcher plants.

Mosquitoes inhabit a variety of standing water from tree holes and discarded automobile tires, to ponds and wetlands (Clements, 1992). Mosquito larvae in container habitats feed on heterotrophic micro-organisms (i.e. bacteria, fungi, protozoa) that grow on container and detritus surfaces or are suspended in water (Clements, 1992; Merritt, Dadd & Walker, 1992). Because mosquitoes may produce multiple generations throughout the active season and may reach substantial population densities, they may make significant contributions to aquatic and terrestrial food webs. Most tree hole microsystems contain mosquitoes, many of which are important vectors of animal and human diseases (Kitching, 2000). Perhaps the most common species in tree holes in the eastern United States is the Eastern Tree Hole mosquito, *Ochlerotatus triseriatus* (Say) (Bradshaw & Holzapfel, 1985). Growth and development of this species are strongly affected by leaf litter quality and quantity (Fish & Carpenter, 1982; Carpenter, 1983; Léonard & Juliano, 1995; Walker *et al.*, 1997), but no study has compared the effects of animal versus plant detritus on the performance of this or any other tree hole consumer.

We tested three hypotheses concerning the effects of detritus type on aquatic microcosms: (i) water chem-

istry of tree hole microcosms differs based on detritus type; (ii) micro-organism metabolic rates (MR) are affected by detritus type; and (iii) performance of *O. triseriatus* larvae and adults are differentially affected by the inputs of animal versus plant detritus. We evaluated these hypotheses in a series of laboratory experiments. In the first study, we determined how several water chemistry variables were affected by detritus type and amount in laboratory microcosms. In our second experiment, we measured the response of micro-organism community respiration rates to different detritus types and to the presence of *O. triseriatus* foraging and the effects of detritus type and amount on growth and development of mosquito larvae. In the third experiment, we quantified the effect of animal and leaf detritus on adult mass, survival to adulthood, and estimated population growth of *O. triseriatus*.

Methods

Origin of detritus, mosquitoes and experimental microcosm design

Larvae of *O. triseriatus* were collected from tree holes at Parklands Preserve (Lexington, IL, U.S.A.) and raised to adults on bovine liver powder (ICN Biochemicals, Cleveland, OH, U.S.A.). Resulting adults were housed in cages where females were blood fed on anaesthetised laboratory mice to obtain eggs. Mosquito eggs were placed in 20 mL glass tubes in a solution of 0.33 g nutrient broth per 750 mL deionised (DI) water to induce hatching. After 24 h, hatched larvae were rinsed to remove nutrient solution and transferred to appropriate experimental microcosms.

Leaves of two tree species [White Oak (*Quercus alba* L.) and Sugar Maple (*Acer saccharum* Marsh)] commonly found in tree holes were collected in the fall from Parklands Preserve and stored dry at room temperature. Leaves were cut into approximately 1.25 cm² pieces after the petioles were removed. Decorated crickets (*Gryllodes sigillatus* Walker) and adult fruit flies (*Drosophila melanogaster* Meigen) were obtained from colonies within the Department of Biological Sciences, ISU. Cricket parts (abdomens, legs) were apportioned equally among treatments. We chose these animal types to represent large-bodied terrestrial (crickets) and small-bodied flying

invertebrate (fruit flies) forms that might be encountered in natural tree holes. Animals were cold-killed and dried at 50 °C for 48 h before being used in microcosms.

All experiments were conducted in 100 mL plastic beakers to which we added 100 mL of DI water and 100 μL of micro-organism inoculum collected from natural tree holes. Samples of inoculum were collected from 10 tree holes at Parklands Preserve and homogenised before adding to experimental microcosms. Detritus treatments consisted of eight combinations of detritus type and quantity: High Leaf (0.25 g Oak or Maple), Medium Leaf (0.05 g Oak or Maple), Medium Animal (0.05 g cricket or *Drosophila*) and Low Animal (0.01 g cricket or *Drosophila*). We chose this unbalanced design because past experiments indicated that 0.01 g of leaf material is insufficient to support development of *O. triseriatus* to pupation, but 0.25 g of animal detritus would lead to fouling of the microcosms. Values for animal detritus are consistent with values of 0.075 and 0.030 g of dead *Drosophila melanogaster* used by Daugherty *et al.* (2000). These amounts were shown to have pronounced effects on the competitive outcome between *A. albopictus* and *A. aegypti*. For all experiments, microcosms were maintained in a 24 °C incubator on a 12 : 12 day : night cycle and DI water was added as needed to maintain water levels.

Experiment 1: detritus effects on water chemistry. We established 25 replicates of each treatment combination ($n = 200$). Every 5 days for 25 days, we destructively sampled five randomly selected replicates and measured pH, conductivity (μMHOS cm^{-1} at 24 °C), total N (TN) (mg L^{-1}), total reactive phosphorous (TP) (mg L^{-1}), tannin (mg L^{-1}) and per cent detritus decay. TN, TP and tannin were measured using a Hach D800 meter (Loveland, CO, U.S.A.) with appropriate test kits. Remaining detritus was filtered though a 10 μm mesh screen and dried at 50 °C for 48 h. The amount remaining was subtracted from the initial amount to obtain the per cent decay.

Experiment 2: micro-organism metabolic rates and mosquito feeding, growth, and development. We established 20 replicates of the eight treatment combinations ($n = 160$). Replicates were established over two successive days (80 day^{-1}). Six days after establishment, we

randomly and destructively selected four replicates of each treatment combination and determined micro-organism O_2 consumption, expressed in µL of O_2 h^{-1}, as a measure of micro-organism MR using a Gilson Differential Respirometer (GDR, Gilson Inc., Middleton, WI, U.S.A.). After stirring each container, we measured fluid MR from an 8 mL sample from each microcosm. In addition we measured MR from two 17-mm leaf punches placed in 8 mL DI water from treatments containing leaves. For all samples, the final hour of a 3-h measurement was used as MR data. After MR measurements on day 6, we added five newly hatched *O. triseriatus* larvae to eight of the 20 replicates of each treatment combination. On days 12 and 20, we measured MR for four replicates of each treatment combination both with and without mosquitoes. For day 12 samples, we removed all larvae from MR measurement containers, dried them at 60 °C for 48 h and weighed them individually to the nearest 0.0001 mg using a Cahn microbalance (Cahn Instruments, Cerritos, CA, U.S.A.) and determined mean dry mass (mg) and mean instar for each container. We did not collect larval data on samples from day 20 as few individuals remained among all treatments due to death and pupation.

Experiment 3: adult mosquito performance. Twenty replicates of the eight treatment combinations were established over three successive days ($n = 160$). Six days after establishment, we added five newly hatched *O. triseriatus* larvae to all containers. We checked for daily pupae and transferred them to individual vials until they eclosed. Adults were sexed, dried at 60 °C for 48 h and individually weighed to the nearest 0.0001 mg using Cahn microbalance. We ended the experiment 30 days after mosquito addition. For each microcosm we calculated a composite index of population performance (λ'). This index estimates a cohort's daily finite rate of increase (Juliano, 1998) and is based on r', which is an estimate of the realised per capita rate of population change (Livdahl 1982; 1984), and incorporates female mass, development time and survivorship. This index has been used previously to estimate population growth for mosquitoes (e.g. Lounibos *et al.*, 1993; Juliano, 1998; Daugherty *et al.*, 2000). As we were interested primarily in population growth, we do not present data on male *O. triseriatus* performance here, although results among treat-

ments for adult mass, development time and survival were similar for both sexes.

Statistical analyses

Experiment 1. Differences among the eight treatment combinations over the five sampling days were analysed using ANOVA (PROC GLM, SAS Institute Inc, 1990) for each of the water chemistry variables. Because testing each of the six water chemistry variables separately increases the probability of committing a Type I error, we adjusted the α level for testing each variable ($\alpha = 0.05/6 = 0.008$). To further resolve differences among treatments, we used *a posteriori* contrasts. To meet assumptions of normality and homoscedasticity, several variables were transformed: square root of conductivity, log of TN + 1 and per cent decay and 0.3 power of TP.

Experiment 2. ANOVAs (PROC GLM, SAS Institute Inc, 1990) were conducted to determine if differences were apparent among the eight treatment combinations. For all analyses, a block effect was used to account for temporal variation in the set up of experimental containers. Analyses were conducted on day 6 for MR for fluid and leaf-surface samples, separately. We performed *a posteriori* contrasts for fluid MR among combinations of resource type (animal or leaf) and amount. We reduced the significance threshold using a Bonferonni correction (i.e. $\alpha = 0.05/6 = 0.0083$). For leaf surfaces, we compared High Leaf versus Medium Leaf. Leaf-surface MR was log transformed to meet assumptions of homoscedasticity and normality.

We used ANOVA to determine if the presence of mosquito larvae affected MR. We used combinations of detritus and amount, day (12 or 20) and mosquito presence or absence as independent variables in one-way ANOVAs on leaf and fluid MR values, separately. Coding the data in this way produced 32 treatments representing combinations of detritus type and amount, day and mosquito presence (e.g. cricket low, day 12, mosquitoes absent). For each fluid sample representing a detritus–amount combination (e.g. cricket low), we tested for differences in the mean MR between containers with and without mosquitoes with a Bonferonni adjustment. We also tested for the effect of mosquitoes in a similar way for Medium Leaf and High Leaf amounts. For all days, log transforma-

tion of MR was needed to meet assumptions of homoscedasticity and normality.

Performance of mosquito larvae was analysed on day 12 using MANOVA with the eight detritus type by amount treatments as the independent variable, and mean instar and mean larval mass as dependent variables. Significant MANOVA effects were interpreted using standardised canonical coefficients (Scheiner, 2001), which quantify the contributions of the individual dependent variables to significant multivariate effects.

Experiment 3. Survival, adult female mass (mg) and λ' were analysed using separate one-way ANOVA with the eight treatment combinations as the independent variable, and a block effect to account for variation in the experimental containers set up over the 72-h period. We performed *a posteriori* comparisons to identify specific differences in means among treatments. Survival and female mass were arcsine square root and log transformed, respectively, to meet assumptions of homoscedasticity and normality.

Results

Experiment 1

Values for pH, conductivity, TN and tannin all showed significant day by treatment interactions (Table 1). Containers with animal detritus were more basic, regardless of amount, than leaf-based containers (Fig. 1a). In general, pH increased through time for all detritus types expect 0.01 g cricket. Conductivity was relatively stable across time, but was significantly higher in 0.05 g animal treatments and 0.25 g Maple than in other treatments (Fig. 1b). Low amounts of plant detritus (0.05 g) had the lowest conductivity values. TN (Fig. 1c) and TP (Fig. 2) also were significantly greater in animal versus plant containers,

although by day 30, TN had declined significantly in containers with 0.05 g of both animal detritus types. Low animal detritus (0.01 g) yielded TP levels that were indistinguishable from leaf microcosms containing 25 times more detritus (Fig. 2). Throughout the experiment, both TN and TP amounts were below detectable limits in low amounts of leaf material. Tannin levels for leaf detritus microcosms were significantly higher than those for animal-based microcosms (Fig. 1d), but declined over time. Per cent detritus decay displayed independent, significant effects of treatment and day (Table 1). *Drosophila* and cricket treatments had significantly greater decay than did either plant detritus type, although all treatments showed significant decay across days (Fig. 3). Oak (0.05, 0.25 g) showed the lowest decay of all treatments (Fig. 3).

Experiment 2

Prior to mosquito introduction on day 6, MR rates differed significantly among treatments for leaves ($F_{3,15} = 4.19$, $P = 0.033$) and fluid ($F_{7,31} = 4.96$, $P = 0.002$). We did not detect differences in MR between different amounts of leaf material regardless of leaf species (mean ± SE for pooled species, 0.05 g leaves = 1.76 ± 0.62 and 0.25 g leaves = 2.58 ± 0.90 μL of O_2 h^{-1}). Fluid MR rates differed significantly between 0.01 g (0.96 ± 0.40 μL of O_2 h^{-1}) and 0.05 g animal detritus (4.65 ± 1.00 μL of O_2 h^{-1}), and between 0.05 g (2.23 ± 0.72 μL of O_2 h^{-1}) and 0.25 g (6.07 ± 1.27 μL of O_2 h^{-1}) leaf detritus. Fluid MR for 0.25 g leaf detritus was significantly greater than that for 0.01 g animal detritus. Other contrasts, including 0.05 g animal versus 0.05 g leaf, 0.05 g animal versus 0.25 g leaf and 0.01 g animal versus 0.05 g leaf, were not significant.

Overall, there was no effect of mosquito presence on leaf-associated MR ($F_{15,26} = 0.75$, $P = 0.726$). Mosquito presence significantly affected fluid-associated

Table 1 ANOVA results for the effects of treatment (eight combinations of detritus type and amount) on each water chemistry measure over the five sampling dates

Factor (d.f., d.f.)	pH	Conductivity	TP	TN	Tannin	% Decay
Day (4, 96)	142.81*	5.29*	1.88[NS]	5.57*	11.29*	63.11*
Treatment (7, 96)	13.96*	329.70*	169.30*	339.92*	1540.09*	408.65*
Time × treatment (28, 96)	7.55*	9.73*	1.94[NS]	3.45*	2.52*	0.86[NS]

F-statistics are presented. An asterisk indicates significance after adjusting for multiple tests (i.e. $P \leq 0.008$).
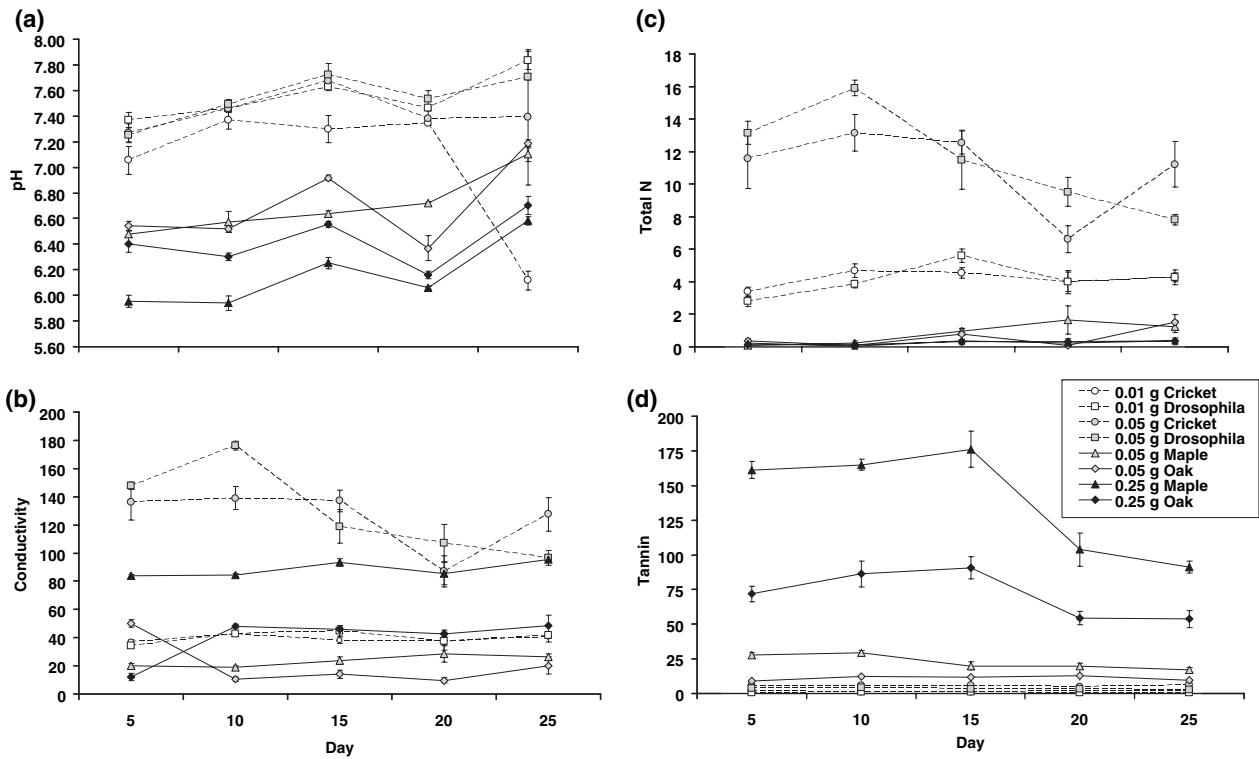NS, not significant; TP, total reactive phosphorus; TN, total N.

(a)

(c)

(b)

(d)

Legend:
- –○– 0.01 g Cricket
- –□– 0.01 g Drosophila
- –◇– 0.05 g Cricket
- –□– 0.05 g Drosophila
- –△– 0.05 g Maple
- –◇– 0.05 g Oak
- –▲– 0.25 g Maple
- –◆– 0.25 g Oak

Fig. 1 Mean (±1 SE) of the significant treatment (detritus type × amount (g)) by day effect on water chemistry measurements from laboratory microcosms. (a) pH, (b) conductivity (μMHOS cm^{-1}), (c) total nitrogen (mg L^{-1}) and (d) tannin (mg L^{-1}). Day corresponds to the day of measurement.
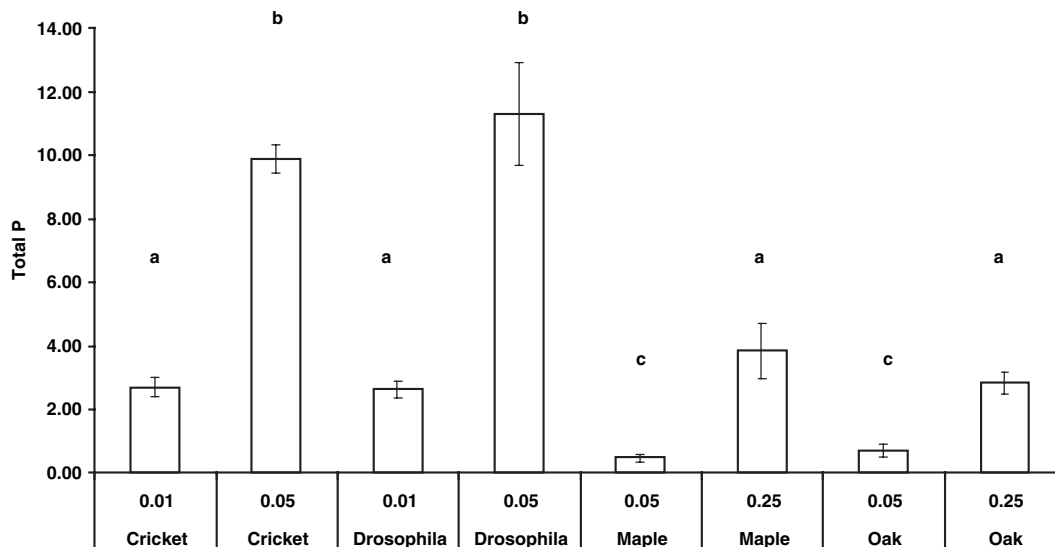
Fig. 2 Mean (±1 SE) total phosphorus (mg L^{-1}) values among different detritus type by amount (g) combinations. Different letters indicate significant differences among means.

MR ($F_{7,31} = 2.07$, $P = 0.004$), although contrasts for specific detrital types within each amount did not produce significant pairwise differences in MR with the presence of mosquitoes.

MANOVA yielded a significant effect of treatment ($F_{14,46} = 32.15$, $P < 0.001$) on larval mass and mean instar at day 12. Standardised canonical coefficients (SSC) for mean instar (SSC = 4.12) and mean mass
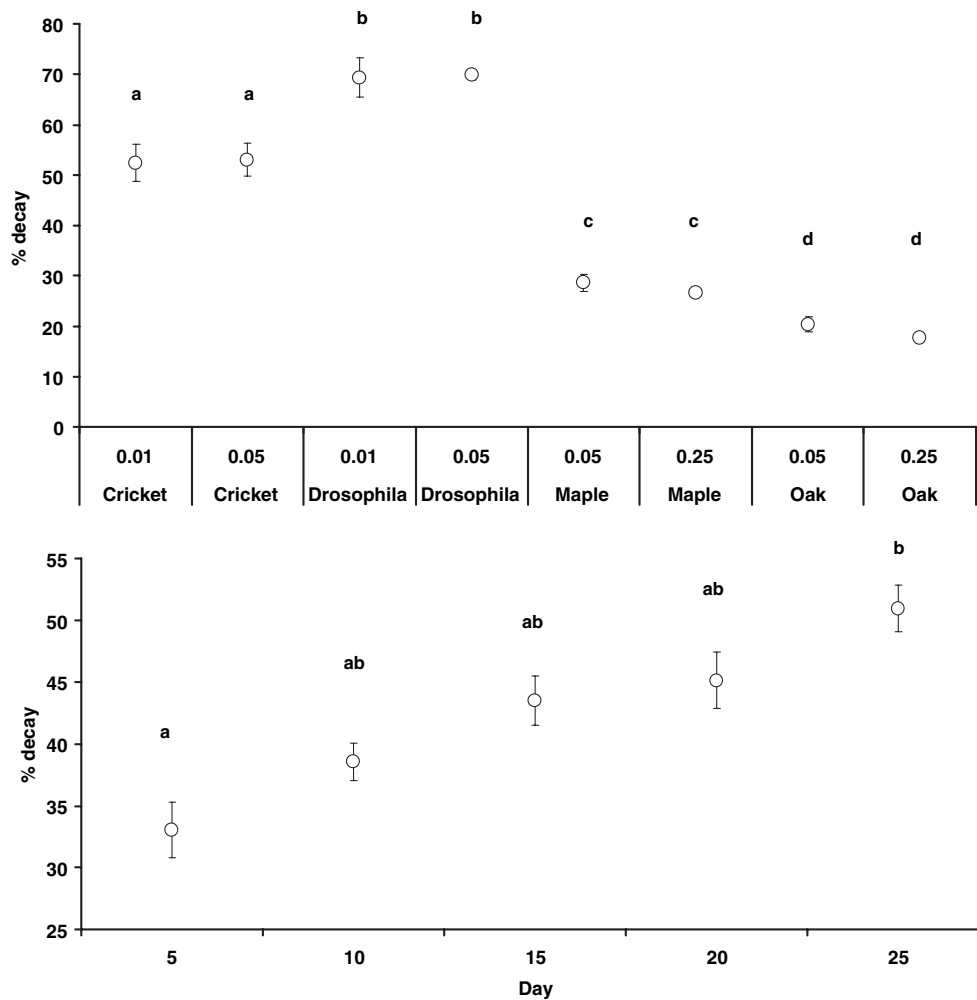
Fig. 3 Per cent decay (mean ± 1 SE) of initial detritus amounts (g). Significant treatment (upper figure) and time (lower figure) effects are illustrated. Means in the upper figure are calculated across all sample days, whereas means in the lower figure are calculated across all detritus types by amounts within each sample day. Day corresponds to the day of measurement. Different letters indicate significant differences among means.

(SSC = 1.06) were large, indicating that both contributed to the multivariate effect, but that the contribution of mean instar was considerably greater. Mosquitoes attained a later instar in animal containers versus leaf containers (Fig. 4). Larvae were larger in 0.05 g animal containers, somewhat smaller in 0.01 g animal containers and smallest in leaf containers (Fig. 4).

Experiment 3

Survival ($F_{7,68} = 16.49$, $P < 0.001$), mean female mass ($F_{7,46} = 82.49$, $P < 0.001$), and λ' ($F_{6,45} = 106.92$, $P < 0.001$) all displayed significant treatment effects

(Fig. 5). Survival was greatest in 0.05 g animal, 0.01 g animal, and 0.25 g leaf, and significantly lower in 0.05 g leaf (Fig. 5a). Females were largest in 0.05 g animal containers, but were approximately 50% smaller in containers with the same amount of leaf material (Fig. 5b). Values for λ' were above one for all treatments where it could be estimated (Fig. 5c), indicating that cohorts developing under all treatment conditions would be expected to increase. However, λ' values were significantly greater for 0.05 g animal detritus than for all other treatments (Fig. 5c). Equal λ' values for leaf and animal treatments were produced when there was 25 times more maple than cricket.
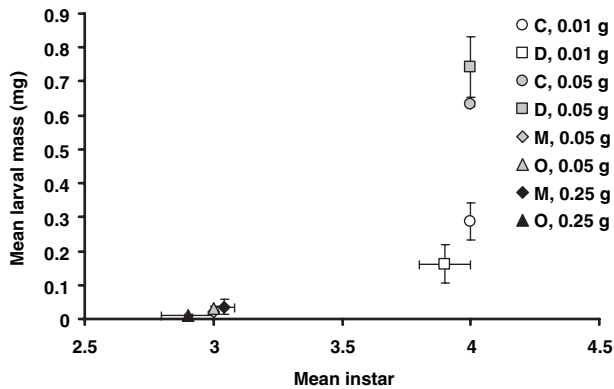
Fig. 4 Bivariate plot of the significant effect of detritus type and amount (g) on mean larval instar (±1 SE) and mean larval mass (±1 SE) on day 12 of experiment 2. Detritus types are: C, cricket; D, *Drosophila*; M, Maple; O, Oak.

Discussion

We tested three hypotheses concerning the importance of detritus type in laboratory microcosms simulating tree hole systems. The results of all our studies indicate that detritus type has multiple effects on different components of tree hole microcosms, and specifically highlight the importance of animal, as opposed to leaf, detritus in these small aquatic habitats. Animal detritus had significant positive effects on water nutrient content, on individual consumer performance and ultimately on consumer population growth estimates. Further, detritus type and amount had some effect on micro-organism metabolic rates. Allochthonous detritus affects communities and populations of animals across a wide array of habitat interfaces (Polis, Anderson & Holt, 1997). A growing body of evidence demonstrates the role of terrestrial donor habitats as strong influences on recipient aquatic systems, especially in affecting consumer biomass (Kawaguchi, Taniguchi & Nakano, 2003) and food web interactions (Cloe & Garman, 1996; Nakano *et al.*, 1999). The role of animal detritus in tree hole microsystems is largely unknown, at least in part because of the historical emphasis of research on the role of plant detritus in these communities (e.g. Lounibos *et al.*, 1993; Léonard & Juliano, 1995; Walker *et al.*, 1997; Srivastava & Lawton, 1998).

Our first hypothesis was that water chemistry of tree hole microcosms would differ depending on the detritus type. Animal detritus is a more nutrient-rich resource than leaf litter, as has been found in stream systems (Cloe & Garman, 1996), where large inputs of
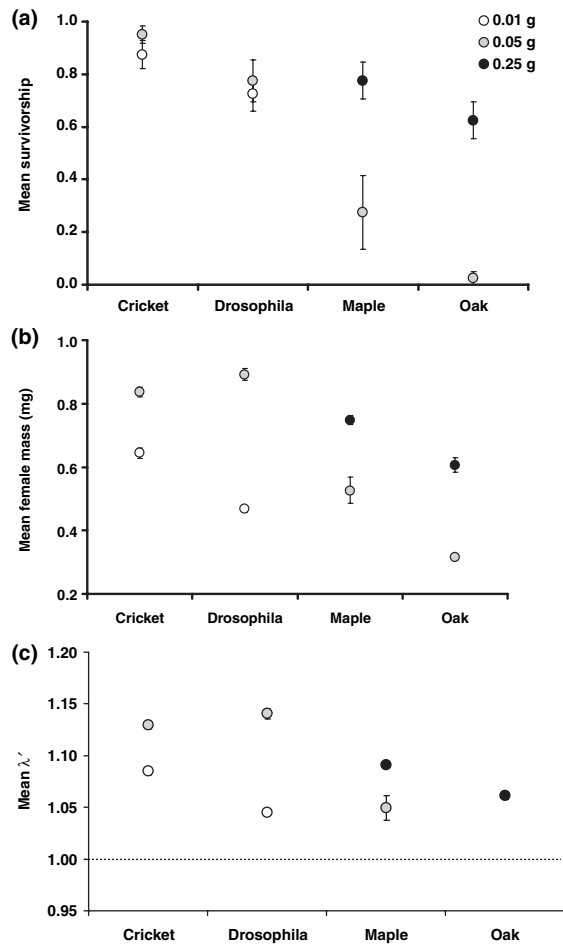

Fig. 5 Performance of adult *Ochlerotatus triseriatus* for experiment 3. (a) Mean proportion survival, (b) mean adult female mass (mg) and (c) mean estimated rate of increase (λ'). All means are ±1 SE. The dotted line at $\lambda' = 1$ corresponds to zero net growth of the experimental populations. Only one female was produced from 0.05 g Oak containers and thus a value for λ' could not be generated.

animal detritus can have direct (Kawaguchi *et al.*, 2003; Zhang *et al.*, 2003) and indirect (Nakano *et al.*, 1999) effects on consumer populations. Differential impacts have been hypothesised to be a consequence of the lower C : N ratio of animal carcasses (Cloe & Garman, 1996). Our animal-based microcosms had greater TN and TP, and greater detritus loss than plant-based containers. Animal-based microcosms contained approximately 16 times more TN than plant-based microcosms (mean N ± SE, Animal = 7.7 ± 0.5, Leaf = 0.5 ± 0.1 mg L^{-1}). The importance of leaf-derived nitrogen to mosquito growth has been shown repeatedly in microcosm studies (Carpenter, 1982; Walker *et al.*, 1991, 1997; Kaufman *et al.*, 2002).

Mosquitoes presumably benefit from greater nitrogen because of the positive relationship between micro-organism respiration rates (and presumably populations) and leaf nitrogen content (Kaufman *et al.*, 1995). Animal detritus generates even greater nitrogen than high-quality leaves, which is likely to provide even greater benefits for mosquito growth and survival. We did not, however, find that the greater TN in animal-based containers led to concomitant increases in micro-organism respiration rates. In fact, we found no differences in fluid-associated MR in leaf and animal containers with equal detritus mass, even though N clearly was higher in animal-based containers. The lack of a relationship between N and MR may be due to functional or taxonomic differences in the micro-organism community between animal and leaf microcosms, and may reflect the limited resolution that can be obtained from overall O_2 consumption. Fluid-associated MR also was not simply determined by detritus mass, as we found no difference in mean MR between Low Animal (0.01 g) and Medium Leaf (0.05 g) detritus containers. Based on these findings, our second hypothesis, that micro-organism productivity would be affected by detritus type, is supported at least in part, although there is a need for better ways to quantify the response of micro-organisms to detritus type.

The presence of mosquitoes did not affect leaf-associated MR values in our experiment, and although fluid-associated MR was affected by mosquitoes, we were unable to detect pairwise differences among detritus treatments. The fact that *O. triseriatus* affected micro-organisms in fluid is consistent with other studies (Kaufman *et al.*, 1999, 2001). Kaufman *et al.* (2001) found that while *O. triseriatus* larvae reduced surface micro-organism abundance, micro-organisms found within the leaf matrix were inaccessible to mosquito foraging. This suggests that in our study mosquitoes may have affected micro-organisms on leaf surfaces, but respiration by micro-organisms within the leaf itself may have obscured the effects of mosquito browsing.

Besides affecting MR in fluid, mosquitoes may have consumed directly portions of dead invertebrates. After 25 days animal-based microcosms had lost more than twice as much of the initial detritus mass as leaf containers (mean % detritus decay ± SE, Animal = 77.8 ± 2.4, Leaf = 32.4 ± 3.4). Animal detritus decomposes at a greater rate than plant material (Swift, Heal & Anderson, 1979; Begon, Harper & Townsend, 1990), but mosquito feeding may further accelerate the breakdown of animal detritus in two ways. First, mosquitoes may directly consume animal detritus, which we observed in this study and has been observed by other authors (Daugherty *et al.*, 2000). If mosquitoes can directly benefit from animal detritus via ingestion, then this would represent a more efficient path of energy flow than that for leaves, whose energy content first must be converted to micro-organism production before it is available to consumers (Merritt *et al.*, 1992). The fact that mosquitoes browse leaf surfaces (Merritt *et al.*, 1992) but do not shred leaves supports this hypothesis. Greater rate of transfer of animal-based versus plant-based energy to consumers has been hypothesised for stream systems (Garman, 1991; Cloe & Garman, 1996; Nakano *et al.*, 1999). In addition to direct ingestion, mosquito grazing of micro-organisms may stimulate micro-organism activity and increase mechanical breakdown of animal tissue, which itself may have enhanced ingestion of animal particles. Other aquatic detritivores have been shown to enhance micro-organism abundance or activity on biofilms through grazing (Lopez, Levinton & Slobodkin, 1977; Smith *et al.*, 1982).

Animal-based containers had three times greater TP than leaf-based microcosms (mean TP ± SE, Animal = 6.5 ± 0.5, Leaf = 2.0 ± 0.2 mg L^{-1}). Phosphorous limitation occurs in many different aquatic systems including coastal wetlands (Sundareshwar *et al.*, 2003) and lakes (Schindler, 1977). In addition, growth of many organisms (e.g. bacteria, Wetzel, 1995; Pace & Cole, 1996; Vadstein, 2000; *Daphnia*, Elser, Hayakawa & Urabe, 2001; Makino *et al.*, 2002), is thought to be limited by phosphorus. Phosphorus has also been shown to limit metabolic activity (i.e. respiration rates) in stream bacteria (Ramírez, Pringle & Molina, 2003). Perkins *et al.* (2004) suggested that phosphorus limitation may be common for insect larvae. Artificial additions of phosphorus (Na_2HPO_4) have small but significant effects on pupation rates of male and female *O. triseriatus* in laboratory microcosms (Carpenter, 1982). Tree holes may receive other inputs of phosphorus, such as bird guano (Walker *et al.*, 1991) or in stem flow, although the latter has been shown to contain only small amounts of phosphorus (<0.7 mg L^{-1}, Carpenter, 1982). Even though we are unable to conclude that phosphorus or

nitrogen alone was responsible for the large quantitative effects on mosquito performance, we can conclude that additions of both elements in animal carcasses are correlated with the profound effects on larval, adult and population performance of *O. triseriatus*.

When mosquitoes were reared with animal versus leaf detritus, larvae attained a later instar and were heavier after 12 days, and survivorship, adult mass and population performance were all greater. These results support our final hypothesis, that the performance of the dominant tree hole consumer is differentially affected by the inputs of animal versus plant detritus. Animal detritus inputs into pitcher plants are critical for the success of associated invertebrate populations and communities (Istock *et al.*, 1976; Cresswell, 1998, 2000; Sota *et al.*, 1998), and have been shown to have a positive effect on the abundance of the pitcher plant mosquito *W. smithii* (Bradshaw & Holzapfel, 1986). In addition, animal detritus has been shown to affect competitive interactions of mosquitoes in man-made containers (Barrera, 1996; Daugherty *et al.*, 2000). Our results support the conclusion that animal detritus can be an important factor contributing to invertebrate production in natural tree holes. The amounts of animal detritus entering real tree holes are largely unknown, but this detritus source is both potentially important and in need of quantification in the field.

We have found that animal detritus can be an important resource in tree hole microcosms, having specific and significant effects on water chemistry, micro-organism dynamics and larval and adult mosquito performance. What is striking is the disproportionate effect of animal detritus relative to leaf detritus, especially when equal amounts of each detritus type are compared. Daugherty *et al.* (2000) have suggested that nutrient pulses are common in aquatic microsystems. Such pulses have been thought to take the form of leaf or stem flow additions, although such additions alone may be insufficient to support tree hole invertebrates (Maćia & Bradshaw, 2000). Instead, we suggest that pulses of invertebrate carcasses may be an important driver of community and population dynamics in natural tree holes. We have shown that even small animal detritus additions can have strong effects on *O. triseriatus* populations through alterations in water chemistry and micro-organism communities. If the levels of animal detritus we utilised in this study are comparable to amounts encountered in natural tree holes (e.g. Daugherty *et al.*, 2000), then the importance of dead invertebrates for tree hole systems suggests that these containers have more in common with pitcher plants than previously thought.

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