Toxicity of Sulfide and Ammonium to *Aedes triseriatus* Larvae (Diptera: Culicidae) in Water-Filled Tree Holes and Tires

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Abstract

Ammonium and sulfide in water of tree hole and tire habitats of *Aedes triseriatus* Say larvae could accumulate to toxic levels, limiting growth and production of larvae and adults. Both ions were detected in water samples taken in longitudinal series over 11 dates from 10 habitats of each type during the larval growth season, at concentrations suggestive of reducing conditions in these habitats. Ammonium was more concentrated overall in water of both habitat types, while sulfide was more concentrated in tires than in tree holes. Water of tree holes was more acidic, whereas water in tires tended to be more basic, an important difference relative to the tendency of ammonium to form the more toxic ammonia moiety under basic conditions. Oxygen saturation was low in both habitat types, indicative of hypoxic conditions such that aerobic respiration would be limited. First- and fourth-instar larvae were sensitive to ammonium and sulfide in acute dose-response assays, but LC₅₀ values were above maximum concentrations observed under field conditions, suggesting that toxic effects of ammonium and sulfide on larvae are not acute, but could be chronic.

Key words: sulfide, ammonium, toxicity, *Aedes triseriatus*

Survival, growth, and production of larval mosquitoes, such as the eastern tree hole mosquito *Aedes triseriatus* Say, in their aquatic habitats vary greatly and depends upon a variety of interacting biotic and abiotic factors (e.g., food availability, competition, predation, parasitism, temperature, hydrologic regime, water chemistry, and insolation; Sinsko and Craig 1981, Haramis 1984, Lounibos 1985, Laird 1988, Walker and Merritt 1988, Ellis et al. 2006). Chemical composition of water is known to affect distribution, abundance, and growth of mosquito larvae and may be particularly important for container mosquitoes (e.g., Petersen and Chapman 1969, Laird 1988, Clements 1992, Mercer 1993, Mercer and Anderson 1994).

Mosquito larvae are extraordinarily tolerant to chemicals in their aquatic environments. For example, they tolerate a wide range of hydrogen ion (pH) concentrations and have been described as “what must surely be one of the most pH-insensitive groups of animals in existence,” even when salinity simultaneously imposes narrower, species-specific tolerances due to variation in osmoregulatory capacity (Clark et al. 2004). Paradoxically, increased sodium ion concentration in tree hole water actually enhanced production of *Ae. triseriatus*, an inhabitant of typically low saline waters, possibly by reducing energetic costs of regulating sodium balance (Paradise and Dunson 1998). Hypoxic, sulfide-rich waters often form habitat

for mosquito larvae such as salt marshes and brackish impoundments (Greene and Van Handel 1992, Rey et al. 1992). When waters are hypoxic, certain toxins such as sulfide anions may accumulate from decomposing organic matter and sulfate reduction to concentrations sufficient to be toxic to fish as well as invertebrates (Bagarinao 1992). Although sulfide is a strong metabolic poison through its interference with oxidative respiration (Bagarinao 1992), the toxicity of sulfide to mosquito larvae in aquatic environments is poorly known and apparently has not been assessed in relation to its natural concentrations.

Several studies suggest that larval mosquitoes of some species excrete into the surrounding water substances inhibitory to larval growth, termed “overcrowding” or “growth retarding” factors (Moore and Fisher 1969, Moore and Whitacre 1972, Kuno and Moore 1975, Broadie and Bradshaw 1991). These substances have not been identified. Because mosquito larvae excrete ammonium ions in their urine (Cochran 1985, Clements 1992), ammonium toxicity could be a population regulatory factor at high larval densities, if ammonium concentrations increase in water to chronic or acutely toxic levels. For example, Mitchell and Wood (1984) found that the acute LC₅₀ of ammonium for *Aedes aegypti* L. larvae was 3.6 mM (i.e., 64.9 mg per liter), and that larval susceptibility could be increased with genetic selection. Chemical competitive interference

has been examined in previous studies but was not a factor in density-dependent larval development of *Ae. aegypti* (Dye 1982) nor *Wyeomyia smithii* (Coquillett) larvae (Broberg and Bradshaw 1995). Dye (1982) was unable to reproduce the growth-retardant factor results (e.g., Kuno and Moore 1975). Later, however, Dye (1984) found interference to vary with the strain of *Ae. aegypti* used in experiments, suggesting a chemical factor was involved in reducing larval growth rate. Carpenter (1982) observed that concentrations of various chemicals in tree hole water might be stimulatory or inhibitory to growth of larvae of the eastern tree hole mosquito, *Aedes triseriatus* Say. In particular, he documented poorer larval performance when ammonium concentrations exceeded 9.1 mg per liter. Kurihara (1983) observed mortality of larval mosquitoes (of several different species) in bamboo stumps at ammonium concentrations >200 mg per liter. Thus, ammonium could occur in container habitats of larval mosquitoes in concentrations sufficient to cause acute or chronic toxicity (Schubauer-Berigan et al. 1995). By contrast, ammonium sulfate fertilizer stimulated *Anopheles arabiensis* Liston and *Culex* spp. larval growth in rice fields (Mutero et al. 2004), although the direct effect was unclear and was attributed to enhanced oviposition in fertilized versus unfertilized plots.

The objectives of this study were to quantify the concentrations of ammonium and sulfide ions in water of tree holes and tires forming habitat for larvae of the mosquito *Ae. triseriatus*, compare it to oxygen saturation, and to assess the acute toxicity of sulfide and ammonium to first- and fourth-instar larvae of this species.

Materials and Methods

Study Site

The study site was Hudson Woods, a woodlot on the south part of the main campus of Michigan State University, East Lansing, MI (described in Walker et al. 1991). The site is dominated by mature American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marsh), and basswood (*Tilia americana* L.) trees. Many of the beech trees have basal, water-filled tree holes that contain *Ae. triseriatus* larvae from March through September. For experimental purposes, used automobile tires were stacked against trees in the woods beginning in 1988, and allowed to fill with rainwater naturally. The tires and tree holes sampled here were located within 200 m of each other. Owing to the presence of a natural population of *Ae. triseriatus* there (Walker and Merritt 1988), mosquitoes of this species naturally colonized the tires in the years prior to the work reported here. A colony of mosquitoes was established from this local population and used in the experiments described here, at the time in its fifth generation.

Water Sampling and Chemical Analyses

Ten basal, pan type tree holes (i.e., those with a complete bark lining) and 10 tires, all chosen for their propensity to harbor *Ae. triseriatus* larvae and to retain water throughout the summer, were sampled for physicochemical characteristics of their water on 11 separate occasions from 25 June to 31 August 1990. Presence of *Ae. triseriatus* larvae in the tree holes and tires selected for this study was confirmed with preliminary sampling, and sampling was conducted to coincide with the seasonality of larval development at this latitude (Walker and Merritt 1988, Walker et al. 1991). Intervals between sampling times ranged from 3 to 10 d. Determination of sulfide ion concentration in the water was done with the methylene blue colorimetric assay (Cline 1969, Van Handel 1987). Water samples (6 ml) were drawn from a depth of ca. 4 cm with a sterile pipette

and immediately transferred to a tube containing reagent, thus samples were fixed in the field to prevent oxidation of sulfide prior to analysis (Van Handel 1987). Determination of ammonium ion concentration was done using the phenolhypochlorite colorimetric assay (Solorzano 1969, Degobbi 1973). Water samples (5 ml) were similarly drawn, held on ice, and returned to the laboratory, and then filtered (0.22 μ m polycarbonate membranes, Nuclepore, Pleasanton, CA), and diluted 1:10 or 1:20 with purified (Milli-Q, Merck Millipore, Billerica, MA) water prior to processing. Standard curves were established for all sets of reagents at each sampling date by creating 10-fold serial dilutions of sodium sulfide or ammonium chloride, and were conducted in triplicate with appropriate blank controls. Tubes containing sulfide standards were provided with a nitrogen-gas headspace to prevent oxidation. Samples or standards were transferred to cuvettes and optical densities read at 650 nm (sulfide) or 630 nm (ammonium) in a spectrophotometer.

The concentration of dissolved oxygen (DO) was determined in the field with a YSI Model 51B dissolved oxygen meter (Yellow Springs Instrument Co., Yellow Springs, OH) fitted with a polarographic, membrane probe consisting of a gold cathode and silver anode. This instrument automatically adjusted the dissolved oxygen readings with water temperature, also recorded. It was calibrated with oxygen-saturated water prior to each field trip. Also, for some dates, the pH of water in two tree holes and two tires was determined with a calibrated, portable pH meter (Orion Research Inc., Cambridge, MA).

Data from water samples were analyzed with univariate repeated-measures analysis of variance (ANOVA; Steel and Torrie 1980, Von Ende 2001) where the type of container (tree hole or tire) was the main treatment effect. To complete the data matrix, a missing datum value for sulfide from a spilled vial was estimated using an analysis of covariance method described by Steel and Torrie (1980, p. 427 to 428), treating individual containers as treatments and sampling days as blocks. For a posteriori comparisons of mean values, least significant difference values (at $P < 0.05$) were calculated using the appropriate standard errors from mean squares (Steel and Torrie 1980).

Acute Toxicity Tests

Acute toxicity of sulfide and ammonium for first- and fourth-instar larval *Ae. triseriatus* was quantified in laboratory experiments. In the first set of experiments, solutions of sodium sulfide and ammonium chloride were prepared across a broad range of concentrations bracketing those observed in tree hole and tire water, in order to estimate the range of concentrations which are acutely toxic. Accordingly, one molar solutions of sodium sulfide and ammonium chloride were prepared in volumetric flasks using distilled water, and stored at 4°C with a nitrogen head space provided to the sulfide flask to prevent oxidation. Serial, 10-fold dilutions were prepared from these stock solutions. First- and fourth-stage larvae from a laboratory colony of *Ae. triseriatus*, established from the field site in 1988 and continuously maintained with free mating, were transferred into clean, 10-ml glass culture tubes (10 larvae per tube), and excess water carefully removed with a Pasteur pipette, stranding larvae at the bottom of the tube. Tubes containing larvae were filled with 10 ml of one of the serially diluted solutions, or with water as a control. Tests were conducted in quadruplicate. After 24 h, the number of dead and live larvae was recorded per tube, and percent mortality was calculated and compared among dosages using the Kruskal-Wallis (K-W) nonparametric rank test (Steel and Torrie 1980).

To estimate median lethal concentrations (LC_{50}) to first- and fourth-instar *Ae. triseriatus*, a second set of experiments was conducted with quadruplicate tubes of varied, increasing concentrations of sulfide or ammonium, with 10 larvae per tube; the experiment included distilled water controls. Data of percent mortality were subjected to probit analysis (Finney 1971) using a computer program (SAS 2006) to estimate LC_{50} values and 95% confidence limits around these estimates.

Results

Concentrations of DO, sulfide, and ammonium for tree holes and tires among the sampling dates are shown in Fig. 1. Individual values for DO in tree holes ranged from 0.2 to 3.2 mg per liter, and in tires ranged from 1.4 to 6.2 mg per liter, and were higher in tires, overall. In general, DO was present in all samples and generally low, and varied over time in tree holes and tires, but in no consistent pattern as the summer season progressed (Fig. 1). Overall, sulfide was present but typically in low concentrations in tree hole and tire water, sometimes ranging below the limit of detection for the assay (0.05 mg per liter, Van Handel 1987). Values of sulfide concentration in tree holes ranged from 0.0 (i.e., undetectable) to 0.82 mg per liter, and in tires ranged from 0.0 (i.e., undetectable) to 0.54 mg per liter. Sulfide was initially higher in concentration and then decreased in tires, but in contrast was lower in tree holes initially, then increased by the date of the last sample (Fig. 1). Values of ammonium concentration in tree holes ranged from 0.0 to 41.4 mg per liter, and in tires ranged from 0.0 to 41.4 mg per liter. Ammonium peaked in concentration at the fourth sampling day in tires and then decreased, whereas ammonium in tree holes peaked at the third sampling day, decreased, then increased again for the last three sampling days (Fig. 1).

Repeated-measures ANOVA showed that tires had significantly higher concentrations of DO ($F=22.2$; $df=1, 18$; $P<0.001$) and sulfide ($F=4.5$; $df=1, 18$; $P<0.05$) than did tree holes, but there was no significant difference in ammonium concentrations between these types of containers ($F=0.5$; $df=1, 18$; $P>0.05$). There was highly significant variation in DO, sulfide, and ammonium concentrations among sampling dates (F -statistics, respectively, were 8.1, 4.4, and 3.2; $df=10, 180$; $P<0.001$). There were significant interactions between the type of container and date of sampling for concentrations of DO ($F=4.0$; $df=10, 180$; $P<0.001$), sulfide ($F=4.0$; $df=10, 180$; $P<0.001$), and ammonium ($F=3.5$; $df=10, 180$; $P<0.05$).

Temperature ranged from 11 to 19.5°C in tires and 11.5 to 22°C in tree holes. Tree holes were on average 0.6°C warmer than were tires, a statistically significant difference (repeated-measures ANOVA, $F=12.1$; $df=1, 18$; $P=0.003$; Fig. 2A). Temperature in tree hole and tire water also varied significantly over time ($F=292.1$; $df=10, 180$; $P<0.0001$), and there was a significant interaction of the habitat type (tire or tree hole) and time in the variation in temperature as well ($F=16.2$; $df=10, 180$; $P<0.0001$). The pH of tree hole water tended to be more acidic (range of values, 5.2 to 7.5) and that of tires more basic (range of values, 5.3 to 8.7; Fig. 2B). Because only two tree holes and two tires were examined for pH, no statistical analysis was done on these data.

Acute toxicity tests of sulfide and ammonium (Fig. 3) from the first experiment showed that survival declined significantly for first instars exposed to increasing concentrations of ammonium (K-W test, $H=25.2$, $df=6$, $P<0.001$) and sulfide (K-W test, $H=25.0$,

Fig. 1. Mean concentration of (A) oxygen, (B) ammonium, and (C) sulfide in water of tree hole ($n=10$) and tire ($n=10$) habitats among 11 sampling dates in Hudson woods, Michigan. Vertical bars are least significant differences from pooled standard errors (Steel and Torrie 1980).

$df=6$, $P<0.001$). A similar trend was observed for fourth instars exposed to ammonium (K-W test, $H=21.9$, $df=6$, $P<0.001$) and sulfide (K-W test, $H=20.8$, $df=6$, $P<0.001$). First instars were more sensitive than fourth instars to both sulfide and ammonium, as survival decreased more rapidly with increasing concentration than did the curves for fourth instars (Fig. 3). Estimates of LC_{50} values from the second experiment (Table 1) confirmed differences in acute toxicity of first- and fourth-instar larvae to sulfide. There was no overlap in the 95% confidence intervals surrounding the estimated LC_{50} values for the two different instars, and the LC_{50} for first instars was an order of magnitude lower than that of fourth instars. The LC_{50} estimate for ammonium for first instars was lower than that for fourth instars; however, the 95% confidence interval around the estimate for first instars overlapped with the LC_{50} estimate for fourth instars.

Fig. 2. (A) Mean water temperature of tree hole ($n=10$) and tire ($n=10$) habitats among 11 sampling dates in Hudson woods, Michigan. Vertical bars are least significant differences from pooled standard errors (Steel and Torrie 1980). (B) pH of water of two tree hole and two tire habitats in Hudson woods, Michigan.

Discussion

Ammonium and sulfide, both well-known toxins of aquatic vertebrates and invertebrates (Bagarinao 1992, Schubauer-Berigan et al. 1995), occurred in natural (tree hole) and artificial (tire) habitats of *Ae. triseriatus* larvae. The observations confirm those of others (Walker and Merritt 1988, Walker et al. 1991) that tree hole water is rich in nitrogenous and sulfurous compounds and suggest that these habitats are unlikely to be limited by either, when nitrogen and sulfur serve as assimilable nutrients or as energy sources. These ions varied in concentration between tire and tree hole water types, and over time, probably as a function of hydrodynamics, larval density and excretion, sources and fluxes of sulfur- and nitrogen-containing compounds, and transformations of these compounds by microbial activity. Of the two ions, ammonium occurred at higher concentrations than did sulfide (Fig. 1). Ammonium appears in aquatic environments typically as a metabolite from microbially mediated decomposition of nitrogen-containing organic matter (Ward 2007) or as an excretory product of nitrogenous wastes from invertebrates, including mosquito larvae (Cochran 1985, Clements 1992). Ammonium–nitrogen content was much higher in water with *Ae. triseriatus* larvae than without them, greatly exceeded concentrations of oxidized forms of nitrogen (nitrate, nitrite), and declined as larval cohorts proceeded to pupation and adult emergence (Walker et al. 1991). Similarly, Bradshaw and Creelman (1984) found that insect inhabitants of pitcher plants increased nitrogen loading in pitcher plant water. Water of field-deployed microcosms with or without decomposing leaves did not differ in ammonium content. This finding indicated that ammonification directly from leaf detritus, without having first been cycled through the microbial loop and consumed and processed by mosquito larvae or other insects, was not a major source of ammonium–nitrogen directly to the water column (Walker et al. 1991). Tires (not having stems or trunks) do not receive stemflow; however, throughfall water could

Fig. 3. (A, B) Mean percent mortality of first- and fourth-instar larvae of *Ae. triseriatus* after exposure for 24 h to different concentrations of ammonium and sulfide. Vertical bars are least significant differences from pooled standard errors (Steel and Torrie 1980). Horizontal bars along lower left axis of each graph show the range of concentration of compounds detected in tree hole and tire water, to allow comparison of toxicity results.

Table 1. Acute toxicity, as median lethal concentration (LC₅₀) values and upper and lower 95% confidence limits in mg per liter, of first- and fourth-instar *Ae. triseriatus* larvae for sulfide (S²⁻) and ammonium (NH₄⁺)

Compound (Instar)	LC ₅₀	Lower confidence limit _{95%}	Upper confidence limit _{95%}
S ²⁻ (First)	77.5	41.9	147.6
S ²⁻ (Fourth)	699.8	473.8	936.4
NH ₄ ⁺ (First)	1,341.0	653.1	2,414.9
NH ₄ ⁺ (Fourth)	2,377.3	2,271.4	5,481.3

introduce ammonium after passing through the forest canopy, and indeed in a previous study throughfall water contained ammonium, albeit at lower quantities than stemflow water (Walker et al. 1991). Interestingly, water of tires and tree holes had similar ammonium content here (Fig. 1), suggesting that the developing and excreting larvae, and not allochthonous water input, loads ammonium into tire and tree hole water. Thus, the primary source of ammonium in tree hole or tire water is the larvae themselves, and its effect should operate in a density-dependent fashion.

Tires could be an important source of sulfur in tire water, because sulfur compounds are used in the vulcanization (heat polymerization) of rubber (Ghosh et al. 2003), likely explaining why tire water had higher sulfide content than did tree hole water (Fig. 1). Stemflow and throughfall water both contain sulfate ions, more abundant in the former than in the latter, but both providing a source for tree hole and tire water. We previously observed a rapid (within 24 h) flux of sulfate in field-deployed microcosms receiving stemflow or throughfall water, suggesting in situ sulfate reduction to sulfide by microbial metabolism (Walker et al. 1991). This anaerobic process is likely the most important source ultimately of sulfide ions in tree holes and tires, and is driven by guilds of sulfate-reducing bacteria in hypoxic sediments for which organic matter serves as electron donor and sulfate as electron acceptor (Luther et al. 2011). Because oxygen tends to show vertical zonation in mesocosms meant to simulate nature tree holes (Harlan and Paradise 2006), likely the metabolism of these guilds and the resultant sulfide are primarily confined to the bottoms of tree holes and tires, although sulfide zonation was not studied here. Our samples were taken at ca. 4 cm depth regardless of volume and conformation of the tree holes or tires. It could be, therefore, that sulfide concentrations were underestimates of the highest values in the sulfate-to-sulfide flux, had we sampled sediments directly. We have observed that grab samples of the bottoms of tree holes often smell strongly of rotten eggs, indicative of sulfide, thus much of the sulfide in tree holes may be confined to anoxic, packed sediment layer whose characteristics deserve further study. Certainly, *Ae. triseriatus* larvae visit the bottom of tree holes during foraging bouts and may as a result encounter sulfide there (Walker and Merritt 1988, 1991). Interestingly, the secondary production of fish in a sulfidic cave stream, lacking phototrophy entirely, depended on metabolism and biomass of sulfate-reducing bacteria, emphasizing the importance of sulfide as an energy source as much as a potential toxin to the fish living there (Roach et al. 2011). Similarly, sulfate reduction to sulfide could indirectly be responsible for some mosquito production in tree holes through production of bacterial biomass serving as larval food.

Both first- and fourth-instar larvae of *Ae. triseriatus* tolerated sulfide and ammonium, surviving exposures at concentrations far exceeding those they might experience in tree hole or tire water based upon our field measurements of 10 containers of each type through a 3-mo period. The toxicity responses for *Ae. triseriatus* larvae observed here revealed substantially higher LC₅₀ values than those observed for *Ae. aegypti* by Mitchell and Wood (1984), suggesting also variation across species of mosquitoes whose larval habitats include tree holes and artificial containers. Beringao (1992) noted that many aquatic organisms have the ability to tolerate sulfide despite its toxicity, including avoidance of sulfide-loaded zones, and metabolic mechanisms. Although dose-mortality response for first and fourth instars was measurable in both the first (Fig. 3) and second (Table 1) experiments with resultant LC₅₀ estimates, yet acute mortality occurred in ranges higher than all natural values (compare with measured ranges in Fig. 1). First instars were more sensitive to both ions and amongst all comparisons most sensitive to sulfide, but the field concentrations of sulfide were below the lowest concentrations used in tests here that even resulted in 20% mortality (see Fig. 3A). Kurihara (1983) observed mortality of larval mosquitoes in bamboo stumps when ammonium exceeded 200 mg per liter, but this value is about five times above the highest values measured here as well as in previous studies (Walker and Merritt 1988, Walker et al. 1991).

Carpenter (1982) observed growth-inhibiting effects of ammonium at 9.1 mg per liter concentration, which is within the range of field values here. There could be at least two explanations for this effect. First is the possibility that *Ae. triseriatus* larvae experience sublethal effects of chronic exposure to subacute concentrations of ammonium, which was not measured here for either ammonium nor sulfide and should be the subject of future research. For example, subacute concentrations of sulfide reduced feeding rates of larvae of the mayfly, *Hexagenia limbata* (Serville) (Oseid and Smith 1974). Chronic effects of tannins on growth of larvae of the western tree hole mosquito, *Aedes sierrensis* Ludlow, suggests that such subacute effects may be common for mosquito inhabitants of container habitats (Mercer 1993, Mercer and Anderson 1994). A limitation in toxicity testing here is the possible requirement for flow-through systems to maintain constant exposure, which require that the test organisms can respire while entirely submerged, such as midge larvae or amphipods (e.g., Smith et al. 1976). Mosquito larvae must be able to surface to breathe, thus test conditions require an air-water interface, creating an atmosphere into which the test substances (sulfide, ammonia) could volatilize or oxidize, thereby reducing exposure strength during acute toxicity tests. Secondly, the accumulation of ammonium, a reduced form of nitrogen, in tree holes and tires indicates the development of a reducing environment, which could stall larval growth through indirect effects, discussed further below. Importantly, toxicity of ammonium is pH dependent owing to the dissociation constant of ammonium to ammonia; basic pH tends to liberate the fourth proton from NH₄⁺ and generate the more highly toxic NH₃ compound from the relatively less toxic ammonium ion (Erickson 1985, Sheehan and Lewis 1986, Schubauer-Berigan et al. 1995). Although our pH data were limited here, observed values ranged from slightly basic to neutral to slight acidic, thus dissociation of ammonium to ammonia would be less likely (Erickson 1985). Kaufman and Walker (2006) predicted that pH of tree hole water should tend to acidify, due to release of organic acids into solution during decomposition of leaf detritus. Indeed, Alto et al. (2005) found that water of container habitats of larval mosquitoes became increasingly acidic with increasing amounts of leaf litter.

All average values and most individual measurements of dissolved oxygen in tree hole water in this study were below the lower limit of hypoxia of 2 mg per liter set by international standards (Rabalais et al. 2010). Values in tire water tended to be slightly above this limit (although still relatively low), suggesting that oxygen is less limiting, and indeed tires produced numerically more *Ae. triseriatus* adults than did tree holes when both were shaded (Haramis 1984). Tire water may present larger surface area than tree hole water, thus allowing more oxygen exchange into tire water. Although not anoxic, the hypoxic status of tree holes and tires indicates high oxygen demand to meet respiratory needs of the aerobic microbial community in the water column, and associated with the decomposing organic matter in these containers. Hypoxia in mesocosms simulating tree holes resulted in lower mosquito productivity and reduced species richness compared with more oxygenated mesocosms (Harlan and Paradise 2006); the range of dissolved oxygen values in that study and this one were similar. Oxygen functions as an electron acceptor in microbial chemotrophic respiration of ammonium and sulfide, and also as an oxidizing agent for abiotic oxidation of sulfide (Ward 2007, Luther et al. 2011), thus its presence in water could alleviate the toxic or growth-inhibiting pressure presented by sulfide and ammonium on mosquito larvae. Experimental increases in oxygen saturation progressively diminished toxic effects of sulfide to freshwater aquatic invertebrates

(Oseid and Smith 1975). However, the relatively low amount of oxygen in the water in tree holes and tires, observed in this study, must severely limit this process and allow ammonium and sulfide to accumulate and persist. Further, the low saturation of oxygen and high concentration of ammonium in tree hole water reported here and elsewhere (Walker and Merritt 1988, Walker et al. 1991), as well as presence of sulfide, and the rapid disappearance of nitrate, nitrite, and sulfate (all electron acceptors in microbial respirations) suggests that tree holes are reducing environments, that is, lacking an adequate and steady supply of electron acceptors relative to electron donors sufficient to allow heterotrophic decomposition of organic matter to proceed. It could be one of the limiting factors in container mosquito habitats in general, which have been described as “heterotrophic, small ponds” (Lounibos 1985) and depend upon decomposition of organic material as the primary energy and carbon source for mosquito production (Kaufman et al. 2010).

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