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The roles of history: age and prior exploitation in aquatic container habitats have immediate and carry-over effects on mosquito life history

KATIE M. WESTBY* and STEVEN A. JULIANO

School of Biological Sciences, Illinois State University, Normal, Illinois, U.S.A

Abstract

1. Per-capita resource availability in aquatic habitats is influenced directly by consumer density via resource competition and indirectly via delayed resource competition when temporally non-overlapping cohorts of larvae exploit the same resources. In detritus-based systems, resources are likely to be influenced by the age of the aquatic habitat, as detritus changes in quality over time and may be replenished by new inputs.
2. For aquatic insects that exploit detritus-based habitats, feeding conditions experienced during immature stages can influence fitness directly via effects on development and survivorship, but also indirectly by influencing adult traits such as fecundity and longevity.
3. Larval habitat age and prior resource exploitation were manipulated in a field experiment using the container mosquito *Aedes triseriatus*.
4. It was found that *A. triseriatus* from older habitats had greater larval survival, faster development and greater adult longevity. Exploitation of larval habitats by a prior cohort of larvae had a significant negative effect on subsequent cohorts of larvae by delaying development.
5. It is suggested that extended conditioning of detritus probably resulted in conversion of recalcitrant resources to more available forms which improved the quality of the habitat.
6. In a parallel study, evidence was found of carry-over effects of habitat age and prior exploitation on adult longevity for *A. triseriatus* and *Aedes japonicus* collected from unmanipulated aquatic habitats.
7. These results indicate the importance of detritus dynamics and the discontinuous nature of resource competition in these mosquito-dominated aquatic systems.

Correspondence: Katie M. Westby, School of Biological Sciences, Illinois State University, Campus Box 4120, Normal, IL 61790, U.S.A. katiwestby206@gmail.com.

*Current address: Tyson Research Center, Washington University in St Louis, 6750 Tyson Valley Road, Eureka, MO 63025, U.S.A.

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Keywords

Aedes triseriatus, *Aedes japonicus*, *Culex restuans*; delayed density dependence; detritus; mosquito

Introduction

Animals with complex life histories, such as holometabolous insects, often occupy very different habitats during different phases of their life cycle. The fitness of these individuals is influenced not only by the environment they occupy during reproductive stages, but also by the environment they encountered during immature development. This phenomenon is sometimes referred to as the ‘carry-over’ effect and can be defined as non-lethal effects (or conditions) that have latent downstream consequences on later life-history characteristics such as fecundity and adult longevity. Such carry-over effects may occur between life cycle stages, seasons or years (Harrison *et al.*, 2011; O’Connor *et al.*, 2014). Conditions experienced during larval development can also exert strong immediate effects on immature survival and development rate, which will alter population dynamics (Radchuk *et al.*, 2013).

Immature mosquitoes of several genera occupy small, discrete, and ephemeral aquatic habitats that can be natural (e.g. tree holes and bromeliads) or human-made (e.g. discarded tyres, buckets, and other plastic containers). Detritus inputs primarily from terrestrial flora and fauna form the base of food webs in these habitats (Yee *et al.*, 2012). Leaves and animal carcasses, mainly arthropods, enter the system and provide substrates and dissolved nutrients that support microorganism growth. Mosquito larvae harvest the microorganisms by scraping detritus and the walls of the habitats or by filtering detritus-derived fine particulates in the water column, and some species may also gnaw on and directly consume plant or animal detritus (Merritt *et al.*, 1992). The presence of larvae can increase the rate of leaf decomposition, drastically reduce the number of bacteria on leaf surfaces, and change the community composition of microorganisms (Fish & Carpenter, 1982; Kaufman *et al.*, 2001, 2008; Kaufman & Walker, 2006; Pelz-Stelinski *et al.*, 2010). Although these habitats are regarded as ephemeral, the life cycles of mosquitoes and other insects that exploit them are often very short relative to the duration of the aquatic habitat. Because of this temporal difference, these habitats may vary greatly over time in the conditions (e.g. quantity and quality of detritus and microbial resources) encountered by larvae, and habitat history is likely to have multiple effects on its occupants. Some of this variation is a direct result of qualitative changes in detritus with microbial exploitation (Kaufman *et al.*, 1999, 2008), and some variation is an indirect result of the exploitation of the habitat, microorganisms and detritus resources by developing cohorts of larvae that export accumulated nutrients from the system by ecdysis (Fish & Carpenter, 1982; Aspbury & Juliano, 1998; Macia & Bradshaw, 2000; Paradise, 2004). Thus we expect immediate and carry-over effects of the history of an aquatic habitat on population dynamics and individual traits of mosquitoes and other insects that use those habitats. Mosquitoes that occupy these container habitats include important vectors of human diseases, such as dengue, chikungunya, West Nile and La Crosse viruses; thus, understanding how the history of aquatic habitats and the larvae that occupy them

carries over to adults of these species is an important part of understanding the ecology of mosquitoes that are of importance in public health (Hardy *et al.*, 1983).

Inter- and intraspecific competition for resources is often important in container habitats, with field and laboratory studies demonstrating frequent negative effects of density on mosquito larvae (Juliano, 2009; Walsh *et al.*, 2012, 2013; Camara *et al.*, 2016). The outcomes of these experiments are variable, with different detritus types (e.g. leaf species, plant versus animal) and ratios altering the outcome of density-dependent competition (Yee *et al.*, 2007; Murrell & Juliano, 2008; Juliano, 2009).

A handful of studies have investigated the potential effects of delayed density dependence, wherein larvae are negatively affected by the presence in a container of prior, temporally non-overlapping cohorts (Aspbury & Juliano, 1998; Walsh *et al.*, 2012, 2013). Prior exploitation of leaf detritus is an asynchronous and asymmetrical form of competition (Aspbury & Juliano, 1998), which can yield increased development time and reduced adult mass but may not affect larval survivorship for *Aedes triseriatus* (Say) (Aspbury & Juliano, 1998). Walsh *et al.* (2012, 2013) allowed container habitats to be naturally colonised by *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.), respectively. Each container was then restocked at larval densities that mimicked the naturally occurring density (2013) or were up to 10 times the natural density (2012). There was an effect of a prior cohort on development time and size, but not on larval survivorship, for *A. aegypti* (Walsh *et al.*, 2013; consistent with Aspbury & Juliano, 1998), and an effect on development time, size and survivorship for *A. albopictus* (Walsh *et al.*, 2012).

The ages of container habitats (i.e. how long they have held detritus and water) are rarely taken into consideration when assessing the effects of density (either direct or delayed) on mosquito populations and communities. It is very likely that aquatic containers are established at different times and have very different histories of detritus input, especially when the majority of input comes in seasonal pulses (Macia & Bradshaw, 2000; O'Neal & Juliano, 2013; Murrell *et al.*, 2014), or when colonised by species that have several generations over a single season repeatedly exploiting the same habitats. Habitat age has an effect on colonisation and community composition, independent of season itself in these systems (Murrell *et al.*, 2014), which suggests that age of an aquatic habitat is an important determinant of the properties of the associated invertebrate community. The effect of age, however, is probably complicated. Detritus quality may deteriorate over time as microorganisms exploit the detritus, which reduces bacterial abundance and productivity (Kaufman *et al.*, 2002; Kaufman & Walker, 2006). The ability of detritus to support microorganisms may, in some cases, increase over time if recalcitrant components of the detritus, such as cellulose and lignin, are broken down by early microbial colonists and converted to more readily available molecules like sugars (Moore *et al.*, 2004). There are potential confounding effects of toxic secondary compounds leached from leaves that can have negative effects on mosquito larvae, and those negative effects may either increase or decrease over time (David *et al.*, 2000). Finally, although the quality of detritus may deteriorate as microorganisms exploit it and are consumed by larvae, there may be new inputs of detritus that compensate for these losses over time (Paradise, 2004; O'Neal & Juliano, 2013).

In this paper, we tested the hypotheses that prior exploitation by a temporally non-overlapping cohort of larvae and habitat age both affect the survival, development time and size of developing female mosquitoes and also have carry-over effects on adult female longevity. We focus on females as they will typically suffer the consequences of reduced resource availability to a greater extent than do males, as they require a longer development time, more resources to attain a larger size, and pay higher fitness costs for being small (Hardy *et al.*, 1983; Wormington & Juliano, 2014). Female mosquitoes are also of practical concern as vectors of pathogens. We tested these hypotheses in a 2×2 factorial randomised block field experiment with prior exploitation and habitat age as the main effects. In addition to the experimental containers, we established a set of six unmanipulated, similar containers that were colonised naturally and received natural additions of detritus, to test for effects of natural variation in cumulative estimated larval densities and habitat age on female longevity.

Materials and methods

Aedes triseriatus used in this experiment were from a laboratory colony originating from collections at Tyson Research Center, 32 km west of St Louis, MO, and maintained in the laboratory for less than 1 year with regular additions of field-collected individuals. Black plastic 7.5 litres buckets were used as the experimental containers and while this size of artificial habitat is larger than most natural tree holes, it is within the range of sizes reported for natural tree holes and is experimentally tractable (Bradshaw & Holzapfel, 1983; Mercer & Anderson, 1994; Paradise, 2004). Human-made containers of this size are readily colonised by container-dwelling mosquitoes (Westby & Juliano, 2017). Each habitat received 3 litres of deionized water and 6 g of senescent white oak leaves (*Quercus alba*) collected from the forest floor the previous fall and stored dry at room temperature. White oak (2 g litre^{-1}) was chosen as the detritus source as it is a common species in the temperate oak-hickory hard-wood forest where this experiment was conducted; this amount is known to be sufficient for *A. triseriatus* to complete development and it facilitates comparison with other experimental studies on the same system (e.g. Yee & Juliano, 2006; Murrell & Juliano, 2013; Murrell *et al.*, 2014; Westby & Juliano, 2017). All experimental containers were covered with mosquito netting to prevent colonisation and additional detritus accumulation. Water levels were allowed to fluctuate naturally. Experimental containers were established as a 2×2 factorial randomised block design with six spatial blocks placed 1 m apart and containers within blocks only inches apart under the forest canopy at Parklands Merwin Nature Preserve in McLean County, IL. The treatments were prior exploitation by larvae (PE) or no prior exploitation (NPE). At the time of addition of the experimental cohort of larvae, containers were either 8 (old) or 4 (young) weeks old. Old habitats were established on April 25 and young habitats were established on May 23 so that further experimental manipulations happened in synchrony. This design enabled us to test explicitly for the effects of age and exploitation without the confounding effects of additional detritus inputs or mosquito colonisation. We also established one unmanipulated container in each block that received the same initial detritus and water as the experimental containers, and this was left uncovered to assess natural colonisation, mosquito community composition and larval

densities at this site. Unmanipulated containers were established at the same time as the old containers.

Containers assigned to the PE treatment had 200 first-instar *A. triseriatus* larvae added 1 week after the containers were filled. The NPE habitats were also unsealed briefly at the same time larvae were added to the PE treatments to account for any possible small addition of detritus that may have occurred when the habitats were unsealed to add larvae, although every attempt was made to minimise this. All habitats were unsealed regularly to remove living and dead adults from the water surface. Experimental larvae (150 first instars, or 50 larvae litre⁻¹) were added to all treatments at the same time (aged 4 weeks for young habitats and 8 weeks for old habitats). Previous field studies of similar buckets open to colonisation at forested sites in the Midwest often yield densities of mosquito larvae from 30 to 150 litre⁻¹ over the summer (Murrell & Juliano, 2013; Westby & Juliano, 2017) and occurred commonly in the open containers in this study (see Results; Fig. 3). Before experimental larvae were added, all habitats (both PE and NPE) were sieved through a 106 μ m mesh screen in the field to remove any remaining larvae from the prior cohorts. The water was returned to the habitats, leaves were carefully rinsed and inspected to remove any larvae and returned to the habitats. Sieve contents, including biofilm, fine particulate organic matter (FPOM) and larvae, were rinsed into a clean pan, and larvae were removed using a pipette. All biofilm and FPOM were then rinsed back into the habitat. Containers were checked regularly and when pupae were observed, the entire contents of each container were poured into a pan at the field site and pupae were removed and returned to the laboratory every 2 days. Pupae were held in 20 ml vials and adult emergence was recorded daily. Males were discarded and females were kept in groups by treatment, block and date of emergence in 1 litre plastic containers covered with mosquito netting and given *ad libitum* access to 5% sugar solution. Pupae and adult females were held at 25 °C with LD 14:10 h. Female mortality was recorded daily and dead females were dried at 50 °C. During the adult life, many of the wings were damaged and unmeasurable, so we measured the length of the hind femur to assess female size.

The unmanipulated containers were monitored weekly to assess the time to colonisation. All of the containers were colonised by week 7 and larval sampling began on week 8. A fine mesh aquarium net (42 cm² opening) with a vertical opening was placed in the bottom of the container and after waiting 2 min to allow the larvae to acclimate, the net was pulled vertically through the entire water column, yielding a sample of larvae from the entire height of the water column. We calculated the volume sampled by multiplying the depth of the water (measured at the time of the sample) by the opening area of the net. Larval densities in the habitats were then estimated by dividing the number of larvae in the sample by the sample volume. This enabled us to estimate density with minimal disturbance to the community as it was necessary to remove the larvae and return them to the laboratory for identification. Larvae were identified to species. Samples were taken weekly until the end of the experiment. Every 2–3 days, pupae were removed from the unmanipulated containers, returned to the laboratory, and allowed to eclose in the same manner as pupae from the experimental containers. We removed pupae, instead of larvae, to be followed for female longevity, as the pupal stage is a non-feeding stage and we assumed that by the pupal stage any impact of the larval habitat on resulting adults had already occurred. Adult females

emerging from the pupae were identified as *A. triseriatus* or *Aedes japonicus* (Theobald) and held in the same conditions as females from the experimental containers with daily mortality recorded. At the end of the study, detritus from all unmanipulated containers was collected, dried at 50 °C and weighed.

For the experimental containers, female larval survivorship (assuming a 50:50 sex ratio), median time to eclosion (females only), and mean female size were analysed as separate ANOVAS with individual buckets as the experimental unit and a random block effect (PROC GLM). Female longevity was analysed using a Cox proportional hazards model with effects of age, prior exploitation, interaction, and female size as a covariate, with a random block effect (PROC PHREG).

For the unmanipulated containers, cumulative densities of larvae over the course of the experiment were obtained for the dominant species and for all species combined, by block (see Fig. 3 for weekly densities). We expected that the effects of week and cumulative prior densities would be highly correlated and thus redundant. To determine whether models with week or with cumulative density better described our data on female longevity, we compared proportional hazards models (PROC PHREG) for Akaike's information criterion (AIC) values, which is an information theoretic approach allowing comparison of multiple models to determine which is most consistent with the data (Anderson, 2008). Obtaining AIC values required designation of block as a fixed effect in PHREG. The full model included week, cumulative *Culex restuans* (Theobald), *A. triseriatus* and *A. japonicus*. We also tested a model that included week and cumulative total larvae combining the three dominant species. We determined the best model based on the lowest AIC score, and the weight of evidence (ω_i) is interpreted as the probability that a model is actually the correct model (Anderson, 2008; Burnham *et al.*, 2010). The best model was then run again with block as a random effect to generate appropriate hypothesis tests for categorical effects and covariates. We did separate analyses for longevity of the two *Aedes* species (PROC PHREG). Because of damaged wings after determining longevity, we did not obtain sufficient size data for females from the unmanipulated habitats to include that variable in the models. All analyses were performed in SAS 9.3 (SAS User's Guide, SAS Institute 2011).

Results

There was a significant effect of habitat age on survivorship of female larvae to adulthood (Table 1) with greater survivorship in the older habitats (Fig. 1a). The effect of prior exploitation on female survivorship was non-significant (Table 1; Fig. 1a). There were significant effects of age, prior exploitation and their interaction on median female development time (Table 1; Fig. 1b). Females from young containers with the PE treatment took longer to develop than did females from the other three treatment combinations (Fig. 1b). There was no significant effect of container age, prior exploitation or the interaction on female size (Table 1).

Container age had a significant effect on adult female longevity ($\chi^2 = 3.719$; d.f. = 0.634; $P = 0.028$) but prior exploitation ($\chi^2 = 1.813$; d.f. = 0.629; $P = 0.102$), the interaction ($\chi^2 = 0.071$; d.f. = 0.623; $P = 0.609$) and female size ($\chi^2 = 1.876$; d.f. = 0.889; $P = 0.612$) did not.

Adult females from the younger habitats had a significantly greater hazard of death, although the difference was only observed after 40 days (Fig. 2).

The numerically dominant species in the unmanipulated containers were *Culex restuans* (total number collected = 465), *A. japonicus* (total number = 129) and *A. triseriatus* (total number = 173). Other rare mosquito species were *Orthopodomyia signifera* (total number = 61), *Anopheles barberi* (total number = 4) and *Culex territans* (total number = 1). *Culicoides* (Ceratopogonidae; total number = 21) and unidentified Chironomidae (total number = 5) were present but were not included in the analysis of the effect of the total number of individuals on adult longevity. *Culex restuans* colonised early (week 7) and were largely absent by week 13, after which the containers were dominated by *A. japonicus* and *A. triseriatus* (Fig. 3). *Aedes triseriatus* and *A. japonicus* pupae were only collected in sufficient numbers for the analysis of female longevity from weeks 13, 14, and 15. The best model for adult *A. triseriatus* longevity included only the main effect of week (Table 2). In contrast to *A. triseriatus* females from the controlled experiment, females from the unmanipulated habitats had decreased longevity as the containers aged ($n = 117$; $\chi^2 = 23.95$; d.f. = 2; $P = 0.001$). These females from week 15 had a more rapidly increasing hazard of death, particularly after about 30 days (Fig. 4), compared with females from weeks 14 and 13; weeks 13 and 14 did not differ (Fig. 4), suggesting that older habitats are for some reason producing poorer-quality adults. The best model for adult *A. japonicus* included week and cumulative density of *C. restuans* (Table 2). The effect of week was not significant ($n = 53$; $\chi^2 = 3.81$; d.f. = 2; $P = 0.149$), whereas the effect of *C. restuans* density was significant and detrimental ($\chi^2 = 6.59$; d.f. = 1; $P = 0.01$) with greater previous density of *C. restuans* in the container associated with greater hazard of death for this species (hazard ratio = 1.012). The mean ($\pm$ SE) dry mass of detritus in the unmanipulated habitats increased over the course of the experiment by 7.64 ± 1.4 g.

Discussion

Little attention has been paid to the effects of habitat age in aquatic container systems although it is likely that there is great natural variation in ages of container habitats. The quality of these habitats, as indicated by abundances and pupation success of larval mosquitoes, can be quite variable in containers the size of these buckets (Westby & Juliano, 2017). We observed greater female larval survival in our older habitats. For development time, container age interacted with prior exploitation such that females took significantly longer to develop when the habitat was young and had a prior cohort. We did not find an effect of either age or prior cohort on female size. There was a slight, though statistically significant, carry-over effect of larval habitat age on adult female longevity with an increase in the hazard of death for females developing in the younger versus the older containers (Fig. 2).

One hypothesis for the observed better performance (e.g. greater survival to adulthood, faster development) in older habitats is that as white oak leaves (used as the detritus source in this experiment) aged, they increased in ability to fuel growth of microorganisms. White oak leaves have been repeatedly shown to be a relatively poor resource for larval mosquitoes, mainly due to their slow rate of decay and high tannin-lignin content (Fish &

Carpenter, 1982; Murrell & Juliano, 2008). In detritus-based systems, leaves (along with animal detritus) enter the system and immediately begin leaching soluble organic and inorganic molecules and are exploited by microorganisms such as fungi and bacteria. These microorganisms also secrete enzymes that soften refractory leaf material (Webster & Benfield, 1986).

After extended periods of conditioning in the water, the oak leaves may have softened enough to allow for direct consumption of detritus material and associated fungi that would have been recalcitrant to larvae before lengthy conditioning. Golladay *et al.* (1983) found that Plecoptera had higher oak leaf ingestion and assimilation rates when the leaves had been conditioned for 8 weeks compared with 4 weeks, the same period as our experiment. Mosquito larvae are not typically thought to be shredders, although *A. triseriatus* has been classified as a shredder (Merritt *et al.*, 1992), has been observed consuming leaves (Carpenter, 1982) or having detritus particles in gut contents (Walker *et al.*, 1988). Kaufman & Walker (2006) speculated that fungi are the food source that larvae exploit to subsidise a diet consisting mainly of bacteria and that the greatest adult production in their experimental containers was probably due to fungal activity. We suggest that ageing may improve leaves as a source of mosquito nutrition, most likely via an increase in fungal colonisation. Those fungi may become more available to larvae, as leaves become more fragmented as they age (Kaufman & Walker, 2006), enabling larvae to ingest leaf particles containing digestible fungi within the leaf matrix.

We found some evidence of prior habitat exploitation (delayed density dependence) having an effect on a later non-overlapping cohort of mosquito larvae. Specifically, we observed a significant interaction of prior exploitation and habitat age with female development time, wherein females took significantly longer to develop in younger +PE containers than in the other three treatment groups (Fig. 1b). This highlights the likely complex interactions among habitat characteristics that influence mosquito dynamics. Slower development in this one treatment combination suggests that when resource quality is poor, delayed density dependence has an important effect on the adults produced, but that better resources (i.e. older litter which had a longer period of conditioning with being exploited by mosquitoes) can render the effect of delayed density dependence inconsequential.

Under natural conditions, many previous cohorts of mosquitoes will exploit container habitats, and there will be continuous additions (at variable rates) of new detritus. This pattern may go on for years, in both natural and human-made containers. To begin to address the question of how prior exploitation and habitat age affect mosquito performance in unmanipulated containers, we also established six habitats that we left open to colonisation by resident mosquitoes and to additions of detritus. We estimated the cumulative density of larvae over time and removed pupae regularly to assess the effects of natural larval community activity and habitat ageing on adult female longevity in the laboratory. Consistent with the results from the experimental containers, there was no effect of prior exploitation on adult *A. triseriatus* female longevity, suggesting that for this species under these circumstances, any negative effect of prior exploitation does not carry over to affect adult longevity. In the current study, we found a highly significant effect of week of collection on *A. triseriatus* longevity, although this result was not consistent with the effect

of manipulated container age in the experimental containers. Females collected from habitats that were older had reduced longevity compared with females collected when the habitats were younger (Fig. 4). Despite the seeming inconsistency, the data from the experiment and the unmanipulated containers are not easily comparable. In the experimental containers, ‘ages’ were either 4 weeks (young) or 8 weeks (old) before addition of experimental larvae, whereas for females collected from the unmanipulated habitats, we were comparing females collected as pupae from habitats that were 13, 14 or 15 weeks old with no way of determining, based on our design, how old the habitats were when eggs hatched and larvae commenced development. Finally, the unmanipulated containers were colonised by a mixture of species, whereas our experimental containers were single-species assemblages. Thus, although we refer to week of collection in this experiment as ‘age’, it is clear that differences between collection weeks incorporate much more than just differences in container age. These differences emphasise that the effects of habitat age and prior exploitation in nature are complicated and correlated and that without direct manipulation it is difficult to determine which effects are important.

In contrast with *A. triseriatus*, adult female *A. japonicus* from the unmanipulated containers were not affected by habitat age but exhibited greater hazard of death associated with cumulative density of prior *C. restuans* larvae in the habitat. This supports the hypothesis that, at least for some species and species combinations, prior exploitation by interspecific competitors has negative carry-over effects on adults. Laboratory experiments testing for effects of inter- and intraspecific competition between these species have not all reached the same conclusion, but when negative effects were detected, they were weak despite evidence from field surveys that *A. japonicus* is displacing *Culex* spp. and *A. triseriatus* in some habitats (reviewed by Kaufman & Fonseca, 2014). We did not detect any intraspecific or intrageneric effects on *Aedes* female longevity, which may result in part from the relatively low densities of *Aedes* in our unmanipulated containers. Combined *Aedes* density never exceeded 40 larvae litre⁻¹, which is lower than densities used in laboratory competition experiments for the ‘low density’ treatment (Hardstone & Andreadis, 2012). Our hypothesis for the stronger effect of *C. restuans* on *A. japonicus* than on *A. triseriatus* is differences among the species in habitat use. *A. japonicus* spends more time filtering and resting at the water surface than does *A. triseriatus* and this behaviour is similar to that of *C. pipiens* (Yee *et al.*, 2004; O’Donnell & Armbruster, 2007).

In conclusion, we report evidence that habitat history, particularly container age, affects larval performance and has a carry-over effect on adult female longevity in *A. triseriatus*. We also report a detrimental immediate effect of another aspect of habitat history, prior habitat exploitation, on female development time, and evidence of interspecific delayed density dependence on *A. japonicus* adult female longevity in containers that were allowed to be colonised naturally. Thus we provide evidence here of the multifaceted importance of habitat history in the complex life cycle of these insects.

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Fig. 1. (a) Female larval survivorship (significant effect of age but not prior exploitation) and (b) female development time (significant main effects and interaction) from the manipulated experiment.

Fig. 2. Survival probabilities for adult female *Aedes triseriatus* from the manipulated experiment for young (4 weeks) and old (8 weeks) container habitats.

Fig. 3. Density (no. of larvae litre⁻¹; mean $\pm$ standard error) of the dominant species collected from the unmanipulated habitats by date of collection. Unmanipulated habitats were established on April 25 and colonised by June 1. The sample dates presented represent weeks 8–15 after habitat establishment.

Fig. 4. Survival probabilities for adult female *Aedes triseriatus* stratified by the week they were collected as pupae from the unmanipulated containers. Week refers to the number of weeks since the container was established.

Table 1

ANOVA table for the effects of habitat age and prior exploitation for the manipulated experiment on female larval survivorship, female development time (median day to eclosion) and female size (hind femur length).

Source	d.f.	Female survivorship		Female development time		Female size	
		F-value	P-value	F-value	P-value	F-value	P-value
Age	1	9.94	0.0067	24.11	0.0002	1.05	0.322
Prior	1	4.25	0.057	24.11	0.0002	1.56	0.231
Age × prior	1	0.53	0.478	12.19	0.0033	0.69	0.418
Block	5	1.44	0.267	0.25	0.932	2.14	0.116

P values in bold are statistically significant.

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Table 2

Model parameters used to determine the best model describing adult female longevity for *Aedes triseriatus* and *Aedes japonicus* originating from the unmanipulated containers.

Model	<i>A. triseriatus</i>			<i>A. japonicus</i>		
	AIC	AIC	ω_i	AIC	AIC	ω_i
Week, <i>A. triseriatus</i> , <i>A. japonicus</i> , <i>C. restuans</i>	887.0	5.43	0.034	309.3	3.8	0.097
Week, <i>A. japonicus</i> , <i>C. restuans</i>	885.6	4.03	0.068	307.5	2	0.237
Week, <i>C. restuans</i>	883.6	2	0.188	305.5	0	0.645
Week	881.6	0	0.511	314.6	9.12	0.007
Week, total larvae	883.5	1.9	0.198	313.617	8.09	0.011
Total larvae	892.8	11.2	0.002	316.01	10.5	0.003

The effect of block was included as a fixed effect in each run in PROC PHREG (SAS 9.3) which is the only way to generate Akaike's information criterion (AIC) values for determining model fit. The model with the lowest AIC value is considered the best model and is indicated in bold. We also report AIC_c , which is a numerical value demonstrating how distant a particular model is from the best model, and ω_i , which is the weight of evidence that supports the model and can be interpreted as the probability that a model is the correct one.