

Stage-dependent predation on competitors: consequences for the outcome of a mosquito invasion

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Summary

1. Predator-mediated coexistence occurs when predation allows competitors to coexist, due to preferential consumption of a superior competitor relative to an inferior competitor. Differences between the native treehole mosquito (*Aedes triseriatus*) and the co-occurring Asian tiger mosquito (*Aedes albopictus*) in anti-predatory larval behaviours account, in part, for the greater vulnerability of this invasive species to native predatory midge (*Corethrella appendiculata*). We test the hypothesis that stage-dependent differences in the sizes of *A. albopictus* and *A. triseriatus* larvae, relative to the size-limited *C. appendiculata*, contribute to differential consumption and the likelihood of predator-mediated coexistence of these competitors.

2. In all instars, larvae of *A. triseriatus* were larger than *A. albopictus* of the same stage. Third and fourth instar *C. appendiculata* selectively consumed late-stage *A. albopictus* in preference to same-stage *A. triseriatus*. Small, early-stage prey larvae did not differ in vulnerability to predation, but large, late-stage larvae differed significantly in vulnerability to predation, probably owing to size-limited predation by fourth instar *C. appendiculata*. This effect was less pronounced for third instar *C. appendiculata*.

3. Prey size, in conjunction with anti-predatory behavioural responses, alters the probability of predator-mediated coexistence. A stage-structured predation model showed that equally vulnerable early stages reduce the range of environmental conditions (productivities) in which predator-mediated coexistence is possible, increasing the likelihood of both competitive exclusion of the resident species or failure of the invasive to establish. These results underscore the importance of stage-dependent interspecific differences in predator–prey interactions for determining how predators may affect community composition.

Key-words: container mosquitoes, predatory midge, prey selection, stage-dependent predation

Introduction

Predator-mediated coexistence of competitors occurs when a superior competitor is preferentially consumed relative to an inferior competitor. In many instances, trade-offs may exist between a species' life-history traits that determine competitive ability and vulnerability to predation. Body size is an important trait that changes during ontogeny and may alter predator–prey interactions (Werner & Gilliam 1984; Olson, Mittelbach & Osenberg 1995; Chase 1999; Crumrine 2005), potentially influencing predator-mediated coexistence among

competing prey. Predator-foraging components, such as encounter rates, attack probabilities and capture probabilities, may be influenced by relative sizes of predators and prey (Osenberg & Mittelbach 1989; Aljetlawi, Sparrevik & Leonardsson 2004). For size-limited predators, the relative size of prey during ontogeny may be more important than anti-predator behavioural responses in determining predator–prey interactions, because prey of certain sizes may be invulnerable (Paine 1976; Chase 1999; Wellborn 2002; Aljetlawi *et al.* 2004; Mills, Rader & Belk 2004). Moreover, prey size-refuges may have significant community-level consequences beyond the direct effects of predator–prey interactions (Persson *et al.* 1996), and size-limited predators may alter interactions (e.g. competition) among differently sized prey species (e.g. Morin 1983; Griswold & Lounibos 2005a). Thus, differences in the relative sizes of predators and prey during ontogeny is common in many systems, but its role altering

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predator-mediated coexistence among competing prey species is unclear.

Communities of aquatic insects in water-filled treeholes and artificial containers (e.g. tyres, cans, vases) provide an attractive model system to investigate topics of broad interest including the role of ontogenetic body-size changes in predator–prey interactions. Treehole mosquito *Aedes triseriatus* (Say) is a mosquito native to eastern North America whose immature stages are confined to water-filled containers. Asian tiger mosquito *Aedes albopictus* (Skuse) is an invasive species from Asia that invaded container habitats in the USA, Europe, Africa and South America during the last 20–30 years (Lounibos 2002). In south-eastern North America, mosquito *Toxorhynchites rutilus* (Coquillett) and predatory midge *Corethrella appendiculata* (Grabham) are the most common predators of insect larvae in container habitats (Bradshaw & Holzapfel 1983). Although *A. albopictus* larvae are usually competitively superior to *A. triseriatus* (Livdahl & Willey 1991; Teng & Apperson 2000; Aliabadi & Juliano 2002), there has been little evidence of reductions in *A. triseriatus* in container habitats in Florida since the invasion of *A. albopictus* (Lounibos *et al.* 2001). Coexistence of these competitors seems to result, in part, from macrohabitat segregation (Lounibos *et al.* 2001), and from differential effects of predation (Griswold & Lounibos 2005a, b; Juliano & Lounibos 2005). *Aedes albopictus* and *A. triseriatus* differ in vulnerability to *T. rutilus* and *C. appendiculata*, in part because of anti-predatory behaviours of *A. triseriatus* that are absent or less evident in *A. albopictus* (Kesavaraju & Juliano 2004; Kesavaraju *et al.* 2007). *Corethrella appendiculata* is a small, size-limited predator. Size of fourth instar *C. appendiculata* is similar to the sizes of second–third instar mosquito prey and, thus, late-instar mosquito prey may achieve a size refuge by exceeding the size limit of *C. appendiculata* (Griswold & Lounibos 2006). Because of the important consequences of larval growth in absolute and relative sizes of predators and prey (see Werner & Gilliam 1984; Olson *et al.* 1995; Chase 1999; Crumrine 2005), understanding how vulnerabilities to *C. appendiculata* change during development could be important for understanding the contribution of this predator to coexistence of these competitors.

The overall goal of the study was to determine whether predators exhibit stage-dependent predation and how it influences coexistence among competing prey species. In particular, we use insects that reside in water-filled containers as a model system to experimentally test the hypothesis that stage-dependent differences in the sizes of *A. albopictus* and *A. triseriatus* larvae, relative to the size-limited predatory midge *C. appendiculata*, contribute to differential consumption, and the likelihood of predator-mediated coexistence, of the competing prey species. In order to address this hypothesis, we: (i) determined the relative sizes of predator and prey developmental stages; (ii) tested whether stage-dependent differences in the sizes of *A. albopictus* and *A. triseriatus* larvae, relative to the size-limited predatory midge *C. appendiculata*, contribute to differential consumption of the competing prey species; and (iii) developed a model to determine the role of

stage-structured predation on the outcome of coexistence between competing prey species.

Materials and methods

DETERMINING RELATIVE SIZES OF PREDATOR AND PREY DEVELOPMENTAL STAGES

Laboratory microcosm experiments, conducted in August 2005, were used to evaluate prey species- and stage-selective predation by *C. appendiculata*. *Aedes albopictus* used in the experiments were F₁–F₂ progeny of larvae field collected in Vero Beach, FL, and *A. triseriatus* and *C. appendiculata* were obtained from laboratory colonies that originated from the same field location in FL. Field-collected larvae of both *A. triseriatus* and *C. appendiculata* were added at irregular intervals to colonies of these species.

Measurements of larvae were made to compare stage-specific differences in the relative sizes of *A. triseriatus*, *A. albopictus* and *C. appendiculata*. We measured head capsule widths and body lengths of first, second and third instars of both prey species and third and fourth instars of *C. appendiculata* thawed after freezing at –20 °C. Samples were the same age as larvae used for subsequent predator–prey experiments. Thawed specimens were photographed with a digital camera attached to a dissecting microscope, and head capsule widths and body lengths (anterior thorax to distal eighth abdominal segment) were measured using iSolution Lite 6.1 (Advanced Imaging Concepts Inc., Princeton, NJ, USA) software. MANOVA was used to determine effects of species, instar and their interaction on the response variables (head width and body length). Significant effects were subsequently analysed by multivariate contrasts with sequential Bonferroni adjustment and standardized canonical coefficients (SCCs; Scheiner 2001). The latter analysis allowed us to compare the relative sizes of each larval stage within and between prey species.

EMPIRICAL TESTS OF STAGE-STRUCTURED PREDATION

We tested whether stage-dependent differences in the sizes of *A. albopictus* and *A. triseriatus* larvae, relative to the size-limited predatory midge *C. appendiculata*, contribute to differential consumption of the competing prey species. Prey species were reared separately in enamel pans with 1.0-L tap water and 0.3 g of a 1 : 1 albumin : yeast mixture. To initiate all parts of the experiment on the same day, eggs of both prey species were hatched for 5 consecutive days to obtain sufficient numbers of first, second and third instar prey. We added either first, second or third instars of 40 prey larvae at ratios of 40 : 0, 30 : 10, 20 : 20, 10 : 30 and 0 : 40 (*A. triseriatus* : *A. albopictus*) to containers with 200-mL distilled water. Number of prey offered to the predator was greater than the maximum number of first instar mosquitoes consumed daily (maximum feeding rate) by *C. appendiculata* (Griswold & Lounibos 2005b; Lounibos *et al.* 2008). Shortly thereafter, one 4th instar *C. appendiculata* (< 2 days since moulting) was added to each container. Each unique treatment was replicated 10 times (3 prey instars × 5 prey ratios × 10 replicates = 150 total). Experiments lasted for 24 hours at 25 °C ± 1 SE and 14 : 10 (L : D) after which time predators were removed and all contents of the containers were frozen at –20 °C. The duration of the experiment used here follows established methods for determining functional responses of both *C. appendiculata* and *T. rutilus* predators to these prey species (Griswold & Lounibos 2005b). Experimental samples were later thawed and remaining *A. triseriatus* and *A. albopictus* were identified and counted.

This experiment was maintained at defined environmental conditions, including food, density and temperature, which are known to modify growth and development rates of both mosquito prey and *Corethrella* predators. For *A. albopictus*, under optimal nutrition, larval development takes *c.* 5–10 days at 25 °C (Hawley 1988). Development time of *A. triseriatus* is longer than *A. albopictus* ranging from *c.* 9–30 days for 50% pupation by a cohort (Lounibos, Nishimura & Escher 1993). The predatory third and fourth instar stages of *C. appendiculata* can be completed in 11.1 ± 0.2 days (mean \pm SE) with *c.* 3 days of this period spent in the third instar (Lounibos *et al.* 2008). *Corethrella appendiculata* consume *c.* 200 first instar *A. albopictus* during this period of development (Lounibos *et al.* 2008).

A second experiment was carried out identically to the first experiment except that one 3rd instar *C. appendiculata* (< 2 days since moulting) was added to each container, instead of a fourth instar. Similarly, each treatment was replicated 10 times ($3 \times 5 \times 10 = 150$ total). Ideally, experiments involving both third and fourth instar predators should have been performed in the same temporal block. However, logistic constraints, including the ability to coordinate the developmental stages of all prey and predator species simultaneously precluded the temporal block design. We did not include treatments without predators because background mortality for these prey species is expected to be negligible over the length of the experiment (24 hours).

Product moment correlation analyses showed that prey size and stage were significant and positively related to one another (*A. albopictus* body length vs. instar 0.92, $P < 0.01$; *A. albopictus* head width vs. instar 0.98, $P < 0.01$; *A. triseriatus* body length vs. instar 0.84, $P < 0.01$; *A. triseriatus* head width vs. instar 0.97, $P < 0.01$). Thus, we are confident that the use of stage in our analyses accurately captures size-related effects of predation for these prey species. More importantly, size measurements allow us to quantify the relative sizes of prey and predators of different stages. A three-way ANOVA was used to test for effects of predator instar, prey species, prey instar and their interactions on the number of prey consumed by *C. appendiculata* for treatments with only one prey species present (i.e. 40 : 0 and 0 : 40). Significant effects were further analysed by pairwise contrasts of treatment means using a Tukey–Kramer adjustment for multiple comparisons (SAS Institute 1989). Prey choice for *C. appendiculata* was determined by calculating an index of electivity (α) for treatments with varying ratios of prey species (i.e. 30 : 10, 20 : 20, 10 : 30) (Manly 1974; Osenberg & Mittelbach 1989):

$$\alpha_i = \frac{d_i/n_i}{\sum d_j/n_j}, \quad \text{eqn 1}$$

where d_i is the number of species *i* consumed (*A. albopictus* or *A. triseriatus*) and n_i is the number of species *i* originally present at the start of the experiment (*A. albopictus* or *A. triseriatus*). The summation is from $j = 1$ to k (in this case, $k = 2$). α_i ranges between 0 (no prey of that species consumed) to 1 (all prey of that species consumed), and $\alpha_i = 0.5$ indicates prey are consumed in the proportion that they occur in the environment. Individual three-way ANOVAs, performed separately for *A. albopictus* and *A. triseriatus*, were used to test for treatment effects of predator instar, prey instar, prey ratio and their interactions on *A. triseriatus* and *A. albopictus* α values calculated after *C. appendiculata* predation. Significant treatment effects were subsequently analysed by contrasts of means using a Tukey–Kramer adjustment for multiple comparisons (SAS Institute 1989) or the sequential Bonferroni method (Rice 1989).

MODELLING EFFECTS OF STAGE-STRUCTURED PREDATION

We further investigated the role of stage-structured predation as a determinant of the outcome of invasion and competition between our invader and the native species. We investigated the consequences of stage-structured predation by incorporating stage structure of prey into the model described by Juliano & Lounibos (2005) of invasion by a superior competitor (*I*, *A. albopictus*) into a community consisting of a resident prey (*R*, *A. triseriatus*), a predator (*P*, *C. appendiculata*) and micro-organisms (*M*) fed upon by the prey. This model is an extension of Leibold's (1996) model, adding a lower trophic level (*M*) that is a resource for the competitors, and type-2 functional responses for competing prey (*R*, *I*), *P* and *M*. Feeding rates of micro-organisms on a resource [$f_M(C)$], invader and resident on micro-organisms [$f_I(M)$ and $f_R(M)$ respectively], and predator on invader [$f_P(I)$] and resident [$f_P(R)$] all take on the general form: $f(X) = FX/(K + X)$, where *X* is the food abundance (non-living resource, micro-organisms or prey mosquitoes, depending on which consumer is being considered), *F* is the maximum feeding rate and *K* is the half-saturation constant, or the food abundance yielding one half maximum feeding rate. The two-species functional responses for the predator feeding on resident and invader are $f(R) = F_{PR}R/(R + K_{PR} + K_{PR}I/K_{PI})$ and $f(I) = F_{PI}I/(I + K_{PI} + K_{PI}R/K_{PR})$ respectively (Juliano & Lounibos 2005). Lower *K*s result in greater competitive ability for the consumer (i.e. ability to feed at low food availability) and greater vulnerability for the prey. Conversion efficiencies indicate the rate at which consumed food is converted into growth for micro-organisms (c_M), invader (c_I), resident (c_R), and predator feeding on invader (c_{PI}) and resident (c_{PR}). Death rates (d_P , d_R , d_I , d_M) for each species are density independent. Resources (*C*) for micro-organisms become available at a rate logistically dependent on their abundance, with a maximum availability of *S*. Mathematical details on the model are given in Appendix S1.

The stage-structured model (Fig. 1, Appendix S1) consists of two aquatic stages (young, old) for the two prey, corresponding to first + second instar larvae, third + fourth larvae, plus adults, which mature from older larvae and produce young larvae. For purposes of modelling, the predator is regarded as having only one stage. The structure of two-species, two-stage functional responses of the predator is similar to the two-species functional response (Juliano & Lounibos 2005), but more complex because of stage structure (see Appendix S1 for details). For the mosquito prey, maturation to the next stage is positively related to feeding. The predator consumes the two prey species, but has different half saturation constants (*K*), so that the invader is more vulnerable to predation (lower *K*). The *K* of the competing mosquito prey for their feeding on micro-organisms determines competitive ability. The only interactions of prey are through the micro-organism population (resource competition) and through the predator (apparent competition).

Based on empirical results (Fig. 5), older invasive larvae (*A. albopictus*) are more vulnerable than older resident larvae (*A. triseriatus*) ($K_{PIO} < K_{PRO}$ for predator). Based on earlier results (Livdahl & Willey 1991; Teng & Apperson 2000; Aliabadi & Juliano 2002), *A. albopictus* (invasive) is a better competitor than *A. triseriatus* (resident) in all stages ($K_I < K_R$) for micro-organism prey. All conversion efficiencies, death, maturation, pupation and reproductive rates, and maximum feeding rates are set equal for the two competitors (Appendix S1). We then vary parameters for vulnerability of young larvae to predation (K_{PIY} , K_{PRY}) to determine effects on predator-mediated coexistence. Young invader and resident larvae may be equally vulnerable to predation ($K_{PIY} = K_{PRY}$), or may have

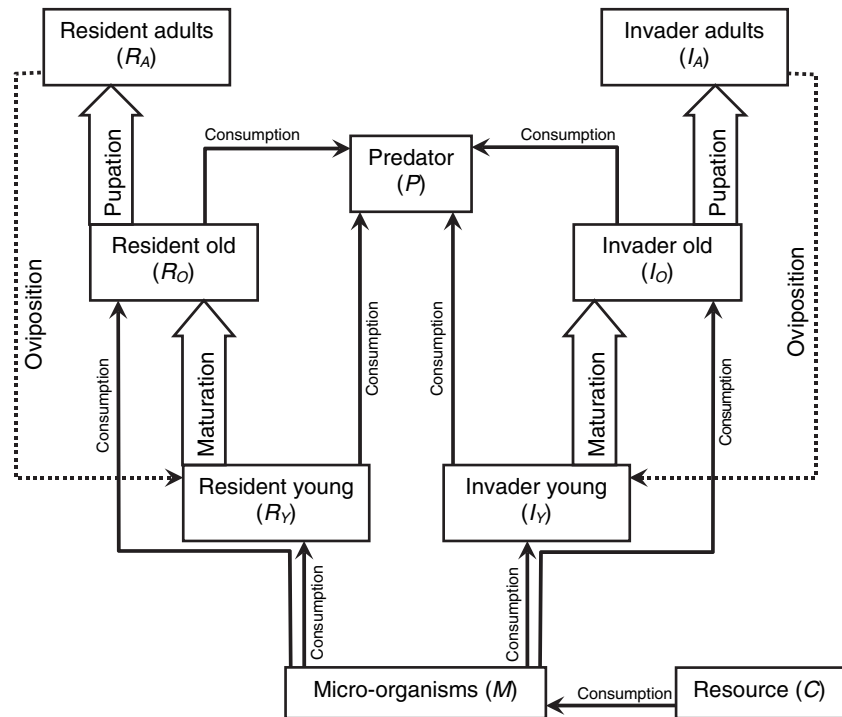


Fig. 1. Stage-structured model based on the *Aedes albopictus*–*Aedes triseriatus*–*Corethrella appendiculata* system. The model consists of a stable system of a predator (*P*) preying on a resident prey (*R*), which feeds on a micro-organism population (*M*) growing on a non-living resource (*C*), that is invaded by a prey species (*I*) that is a superior competitor but more vulnerable to predation.

vulnerabilities that are the same as those of intermediate-sized larvae ($K_{PIY} < K_{PRY}$). Parameter values and mathematical details of the model are given in Appendix S1.

Whether the predator can have a keystone effect depends on environmental productivity (*S*, maximum availability of resources to micro-organisms) (Leibold 1996; Juliano & Lounibos 2005). We are specifically interested in how size-dependent predation by *C. appendiculata* may alter the range of productivities associated with the different possible outcomes of invasion by *A. albopictus*. These outcomes include: stable coexistence of *A. triseriatus* and *A. albopictus*, competitive exclusion of *A. triseriatus* and failure of the invasion by *A. albopictus* (Leibold 1996; Juliano & Lounibos 2005). At low *S*, competition dominates and (if the predator can persist) the equilibrium community consists of the invader and predator (i.e. resident excluded via competition). If *S* is high, predation dominates and the equilibrium community consists of the resident and predator (i.e. the invasion fails due to the presence of the predator). At intermediate *S*, predator-mediated coexistence is possible and the equilibrium community consists of the resident, invader and predator. Based on our empirical results we ask: what is the effect of equal vulnerability to predation of the two competitors as young larvae on the range of productivities associated with each of these three outcomes? To evaluate this question, we compare the range of *S* necessary for coexistence of competitors as vulnerability of young larvae ranges from a difference which is equivalent to that of older larvae to equality.

Results

DETERMINING RELATIVE SIZES OF PREDATOR AND PREY DEVELOPMENTAL STAGES

MANOVA on mosquito prey measurements showed significant effects of prey species (Pillai’s trace = 0.66, d.f. = 2, 1497, $P < 0.0001$), prey instar (Pillai’s trace = 1.05, d.f. = 4, 2996, $P < 0.0001$) and species × instar interaction (Pillai’s

trace = 0.16, d.f. = 4, 2996, $P < 0.0001$). Body length (SCC = 2.75) was the major contributor to the interaction effect relative to head width (SCC = 0.76). Although body lengths were similar for *A. triseriatus* and *A. albopictus* as first and third instars, body length of second instar *A. triseriatus* was greater than that of *A. albopictus* (Fig. 2). In contrast head width was the major contributor to main effects (SCC > 6.0 in both cases), and body length a relatively minor contributor (SCC < 1.0 in both cases). We performed 11 bivariate contrasts to address the significant species × instar interaction, adjusting for multiple comparisons using the sequential Bonferroni method (Table 1). All con-

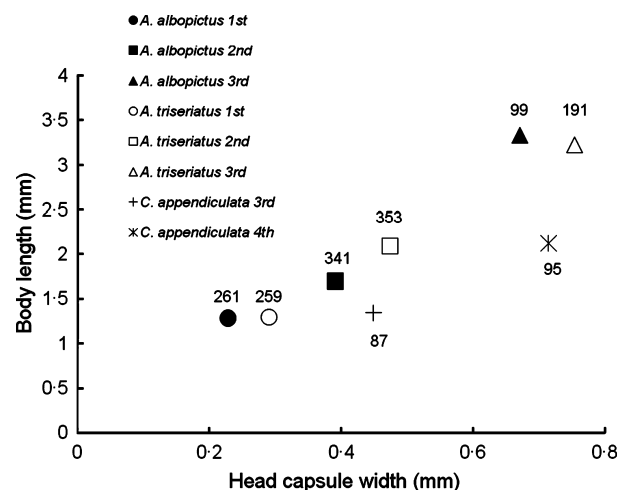


Fig. 2. LS mean head capsule widths and body lengths of *Aedes triseriatus*, *Aedes albopictus* and *Corethrella appendiculata*. SE bars are too small to be shown. Numbers adjacent to symbols show the number of larvae measured.

Table 1. Multivariate contrasts of bivariate (head width, body length) means to address the significant interaction of species \times instar for *Aedes triseriatus* and *Aedes albopictus*

Contrast	Pillai's trace	P	Standardized canonical coefficients	
			Head width	Body length
Conspecific				
1st <i>A. albopictus</i> vs. 2nd <i>A. albopictus</i>	0.80	< 0.0001	6.68	0.14
2nd <i>A. albopictus</i> vs. 3rd <i>A. albopictus</i>	0.88	< 0.0001	5.90	0.98
1st <i>A. triseriatus</i> vs. 2nd <i>A. triseriatus</i>	0.85	< 0.0001	6.31	0.62
2nd <i>A. triseriatus</i> vs. 3rd <i>A. triseriatus</i>	0.91	< 0.0001	6.38	0.55
Heterospecific				
1st <i>A. albopictus</i> vs. 1st <i>A. triseriatus</i>	0.35	< 0.0001	6.88	-0.49
1st <i>A. albopictus</i> vs. 2nd <i>A. triseriatus</i>	0.91	< 0.0001	6.55	0.34
2nd <i>A. albopictus</i> vs. 1st <i>A. triseriatus</i>	0.62	< 0.0001	6.40	0.53
2nd <i>A. albopictus</i> vs. 2nd <i>A. triseriatus</i>	0.58	< 0.0001	6.21	0.72
2nd <i>A. albopictus</i> vs. 3rd <i>A. triseriatus</i>	0.95	< 0.0001	6.35	0.59
3rd <i>A. albopictus</i> vs. 2nd <i>A. triseriatus</i>	0.79	< 0.0001	5.76	1.09
3rd <i>A. albopictus</i> vs. 3rd <i>A. triseriatus</i>	0.95	< 0.0001	6.35	0.59

Degrees of freedom for numerator and denominator were 2 and 1497 respectively. The larger species or instar, as indicated by head width, is shown in boldface. An adjustment for multiple comparisons was made using the sequential Bonferroni method.

trasts of bivariate means of head width and body length were highly significant indicating size differences between larval stages and that *A. triseriatus* was larger than *A. albopictus* (Table 1). Bivariate pairwise contrasts and associated SCCs indicated that head width was the principal source of differences between pairs (SCCs; Table 1; Fig. 2). For both species, each subsequent developmental stage was significantly larger than the previous instar, as shown by head width (Fig. 2). Within a given developmental stage, *A. triseriatus* was larger than *A. albopictus* in one or both dimensions (Fig. 2). In all cases of significant interspecific differences between developmental stages, the earlier instar of one species was significantly smaller than a later instar of the other species (e.g. second *A. triseriatus* vs. third *A. albopictus*; Table 1).

MANOVA on *C. appendiculata* body-size measurements showed a significant instar effect (Pillai's trace = 0.96, d.f. = 2, 179, $P < 0.0001$). Larval head width (SCC = 4.96) was the major contributor to the instar effect, whereas body length (SCC = -0.08) contributed little. Fourth instar *C. appendiculata* were significantly larger than third instars (Fig. 2).

EMPIRICAL TESTS OF STAGE-STRUCTURED PREDATION

The ANOVA for treatments with a single-species present (i.e. 40 : 0 and 0 : 40) showed significant two-way interaction effects of predator instar \times prey instar ($F_{2,107} = 11.55$, $P < 0.0001$), predator instar \times prey species ($F_{1,107} = 11.65$, $P = 0.0009$) and prey instar \times prey species ($F_{2,107} = 3.11$, $P = 0.04$), but no significant three-way interaction. Significantly more first instar larvae were consumed than second instars, and significantly more second instar larvae were consumed than third instars (Fig. 3), but the difference in prey consumption between prey instars (e.g. first vs. second) was differentially larger for fourth instar *C. appendiculata* than third instar *C. appendiculata* (Fig. 3). The predator instar \times prey species interaction resulted from similar con-

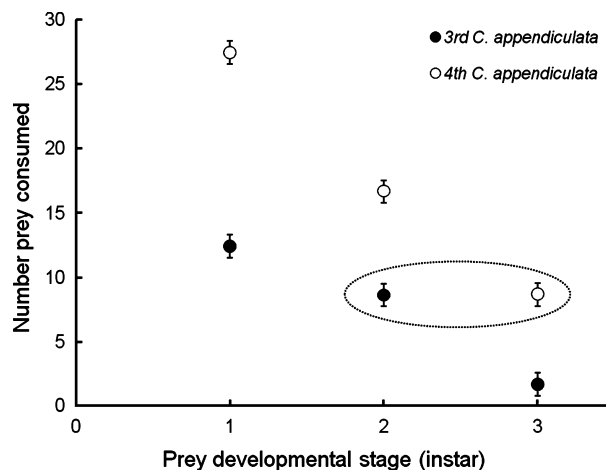


Fig. 3. LS mean \pm SE number of larval prey, pooled across mosquito prey species, in each developmental stage (instar) consumed by third and fourth instar *Corethrella appendiculata* in single-species treatments (i.e. number of *Aedes triseriatus* : *Aedes albopictus*, 40 : 0 and 0 : 40). All possible contrasts of means showed all means were significantly different from each other, except those means found within the dashed ellipse.

sumption of both species of mosquito prey by third instar *C. appendiculata*, but preferential consumption of greater numbers of *A. albopictus* than *A. triseriatus* larvae by fourth instar *C. appendiculata* (Fig. 4). The prey instar \times prey species interaction resulted from similar *C. appendiculata* consumption of *A. albopictus* and *A. triseriatus* as first and second instars, but preferential consumption of *A. albopictus* over *A. triseriatus* as third instars (Fig. 5).

For both *A. triseriatus* and *A. albopictus* with varying ratios of prey species (i.e. *A. triseriatus* : *A. albopictus*, 30 : 10, 20 : 20, 10 : 30), there were significant predator instar, prey instar, prey ratio and predator instar \times prey instar interaction effects on α values from *C. appendiculata* predation (Table 2). The prey ratio effect showed greater

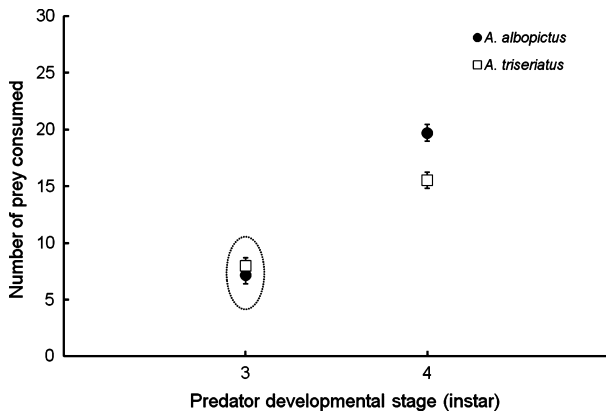


Fig. 4. LS mean \pm SE number of *Aedes triseriatus* and *Aedes albopictus* prey, pooled across developmental stages, consumed by third and fourth instar *Corethrella appendiculata* in single-species treatments (i.e. number of *A. triseriatus* : *A. albopictus*, 40 : 0 and 0 : 40). All possible contrasts of means showed all means were significantly different from each other, except those means found within the dashed ellipse.

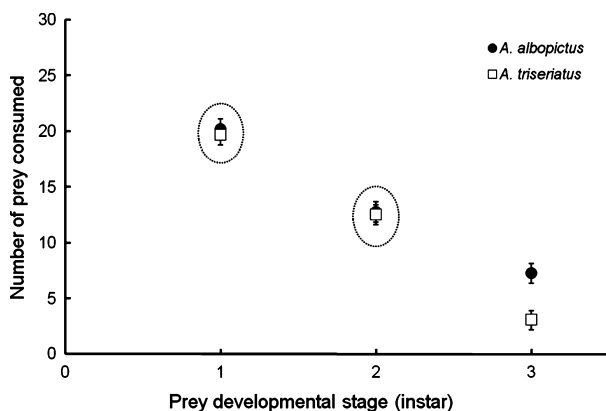


Fig. 5. LS mean \pm SE number of *Aedes triseriatus* and *Aedes albopictus* prey in each developmental stage (instar) consumed by *Corethrella appendiculata*, pooled across developmental stages (i.e. third and fourth instar) in single-species treatments (i.e. number of *A. triseriatus* : *A. albopictus*, 40 : 0 and 0 : 40). All possible contrasts of means showed all means were significantly different from each other, except those means found within the dashed ellipses.

electivity of *A. albopictus* at 20 : 20 than 30 : 10 treatments. For *A. triseriatus*, there was lower electivity at 20 : 20 than 30 : 10 treatments (Fig. 6). All other comparisons were not significant (Fig. 6). Because the interactions were significant, we used six pairwise contrasts for each prey species, *A. triseriatus* and *A. albopictus* (12 total), of prey instar within predator instar treatment (three prey instars by two predator instars). We adjusted for multiple comparisons using the sequential Bonferroni method (Rice 1989). Pairwise contrasts showed that as prey size (instar) increased, large predators consumed proportionately more *A. albopictus* and less consumption of *A. triseriatus* (Fig. 7). However, for small predators, pairwise contrasts showed a different pattern. Although the general pattern of prey consumption of first and third

Table 2. Analysis of variances for effects of predator instar, prey instar, prey species and interactions on *Corethrella appendiculata* electivity (Manly's index α) of *Aedes triseriatus* and *Aedes albopictus*

Effect	d.f.	F	P
<i>A. triseriatus</i>			
Predator instar	1	6.19	0.01
Prey instar	2	11.77	< 0.0001
Prey ratio	2	3.06	0.04
Predator instar \times prey instar	2	4.24	0.01
Predator instar \times prey ratio	2	0.88	0.41
Prey instar \times prey ratio	4	0.56	0.69
Predator instar \times prey instar \times prey ratio	4	0.79	0.53
Error d.f.	149		
<i>A. albopictus</i>			
Predator instar	1	4.67	0.03
Prey instar	2	15.72	< 0.0001
Prey ratio	2	2.96	0.05
Predator instar \times prey instar	2	4.87	< 0.0089
Predator instar \times prey ratio	2	0.41	0.66
Prey instar \times prey ratio	4	0.59	0.67
Predator instar \times prey instar \times prey ratio	4	1.31	0.26
Error d.f.	148		

Electivity values are for treatments with varying prey ratios: *A. triseriatus* : *A. albopictus*, 30 : 10, 20 : 20 and 10 : 30.

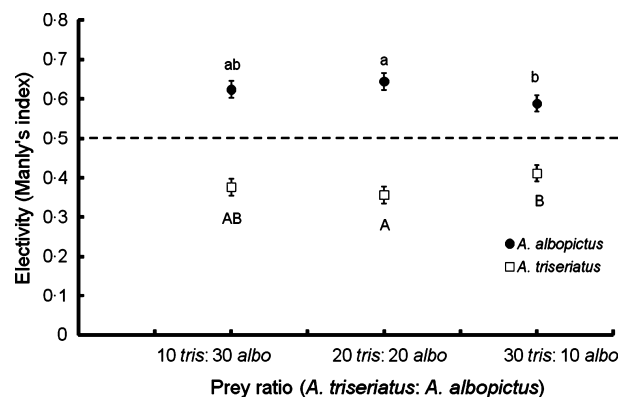


Fig. 6. LS mean \pm SE electivity values of *Aedes triseriatus* and *Aedes albopictus* (treatments *A. triseriatus* : *A. albopictus*, 30 : 10, 20 : 20, 10 : 30) by *Corethrella appendiculata* for significant prey ratio effects. $\alpha = 0.5$ (dashed line) indicates prey are consumed in the proportion that they occur in the environment. Lower- and upper-case letters indicate significant differences for *A. albopictus* and *A. triseriatus*, respectively. Means sharing similar letters are not significantly different from each other.

instar prey by small predators was similar to those observed by large predators, electivity values for second instar prey differed dramatically (Fig. 7). Consumption of second instar prey by small *C. appendiculata* was lower than consumption of first and third *A. albopictus* (Fig. 7). The situation was reversed for *A. triseriatus* so that the consumption of second instar prey by small *C. appendiculata* was greater than first and third prey (Fig. 7).

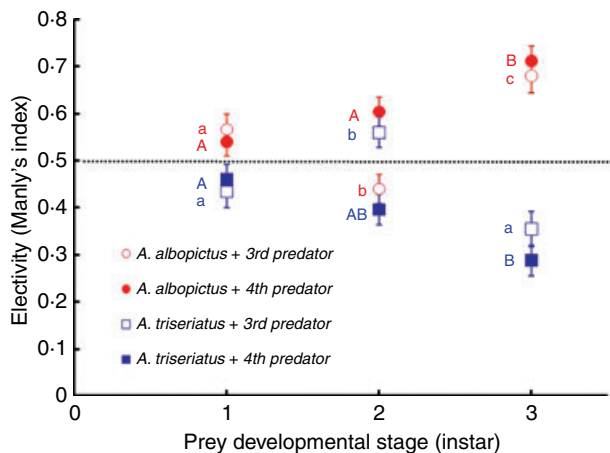


Fig. 7. LS mean \pm SE electivity values of *Aedes triseriatus* and *Aedes albopictus* (treatments *A. triseriatus* : *A. albopictus*, 30 : 10, 20 : 20, 10 : 30) by third and fourth instar *Corethrella appendiculata* for significant interactions of predator instar \times prey instar. $\alpha = 0.5$ (dashed line) indicates prey are consumed in the proportion that they occur in the environment. Red symbols and letters are for *A. albopictus* and blue symbols and letters are for *A. triseriatus*. Lower- and upper-case letters indicate significant differences for third and fourth instar *C. appendiculata* respectively. Means sharing similar letters are not significantly different from each other.

MODELLING EFFECTS OF STAGE-STRUCTURED PREDATION

For all simulations, coexistence of invader, *A. albopictus*, and resident, *A. triseriatus*, is impossible in the absence of predators, because the invasive species replaces the resident by interspecific competition. In the presence of the predator, coexistence of competing resident and invader is possible at intermediate productivities (*S*), with exclusion of the resident at low *S*, and failure of the invader to become established at high *S*. Simulations show that as vulnerabilities to predation of young larvae of the two competing species become more similar, invasion and coexistence become possible over a smaller range of productivities, but competitive exclusion or failure of invasion are the result over a wider range of productivities (Fig. 8). These effects of equally vulnerable young larvae are independent of whether vulnerability of young larvae is greater or less than that of older larvae (results not shown). Thus, equal vulnerability of young larvae increases the likelihood of competitive exclusion of the resident by the invader and also increases the likelihood that the resident and predator will act as a barrier to the invader.

Discussion

Predator-mediated coexistence may be an important mechanism allowing for competing species to coexist. Here we show stage-dependent differences in predators and prey result in differential consumption and the likelihood of predator-mediated coexistence of competing prey species. Our results confirm selective consumption by large predators of the superior competitor, the invasive *A. albopictus*, compared to the

Young larvae vulnerability varies from equal to Invader \gg Resident; $K_{PIO}=50, K_{PRO}=300$

	Equal			Diff.
K_{PIY}	175	150	100	50
K_{PRY}	175	200	250	300
Productivity (<i>S</i>)				
300	I	I	I	I
400	IP	IP	RIP	RIP
500	RIP	RIP	RIP	RIP
600	RIP	RIP	RIP	RIP
700	RIP	RIP	RIP	RIP
800	RP	RP	RIP	RIP
900	RP	RP	RP	RIP
1000	RP	RP	RP	RP
1100	RP	RP	RP	RP
1200	RP	RP	RP	RP
1300	RP	RP	RP	RP
1400	RP	RP	RP	RP

Fig. 8. Results of simulations testing the effect on coexistence of stage-structured predation, with vulnerabilities of young larvae to the predator ranging from equality to vulnerabilities equal to those of older larvae. In this system, the older larvae of the Resident (*R*) are less vulnerable to predation by the predator (*P*) (determined by the parameters K_{PIO} and K_{PRO} , with lower values leading to greater vulnerability). Vulnerabilities to predation for the young larvae are determined by the parameters K_{PIY} and K_{PRY} . Competitive ability of the larvae is determined by the ability to feed on a micro-organism population (*M*). The invader is a better competitor ($K_I = 500$) than the resident ($K_R = 600$). Abbreviations in the figure show which members of the invaded system coexist stably (with micro-organism population *M* always present). I, invader only; IP, invader + predator; RIP, resident + invader + predator; RP, resident + predator. The different shaded areas highlight these different categories. Solid lines indicate the thresholds for the persistence of the predator and the failure of the invasion. Further details are found in the text and Appendix S1.

native *A. triseriatus* and show that this pattern becomes more evident with prey development. For all instars, *A. triseriatus* were larger than *A. albopictus* and so differences in stage-dependent predation were most likely attributable to the relative sizes of predators and prey, as observed in other systems (Wilbur 1989; Aljetlawi *et al.* 2004). Additionally, prey behavioural responses to perceived risk, such as reduced activity, may result in a reduction in predation (Dixon & Baker 1988; Lind & Cresswell 2005). Species-specific differences between *A. triseriatus* and *A. albopictus* in anti-predator behaviours may also act in conjunction with size effects and facilitate coexistence between these prey species (Kesavaraju & Juliano 2004; Kesavaraju *et al.* 2007). The present study was limited to an investigation of stage-dependent predation among *A. albopictus* and *A. triseriatus*, which are largely attributable to size effects.

In single-species prey treatments, more *A. albopictus* than *A. triseriatus* were consumed, but this relationship was dependent on development of both the predator and prey. Species-specific differences in consumption rate by *C. appendiculata* could represent differences in prey handling time, such that larger prey, especially *A. triseriatus*, require longer handling time (e.g. digestion) compared to smaller prey, such as *A. albopictus* (Jeschke, Kopp & Tollrian 2002; Van Gils

et al. 2005; Van Rijn *et al.* 2005; Whelan & Brown 2005). Consistent with the present studies' results, another container-dwelling predator, *T. rutilus*, required significantly greater handling time for *A. triseriatus* than for *A. albopictus* (Griswold & Lounibos 2005b). For treatments with both prey species present at variable ratios, *A. albopictus* were usually preferred over *A. triseriatus*, indicating that prey 'switching' probably does not occur (Griswold & Lounibos 2005b). Species-specific differences in electivities became more pronounced in later prey instars, which indicated a greater disparity of predator preference for invasive over native larvae during the course of development, placing greater predation pressure on *A. albopictus*.

The simulation results show that equal vulnerability of young larvae reduces the range of productivity environments in which invader and resident can have predator-mediated stable coexistence. This outcome can be understood by viewing equal vulnerability of early stages as a reduction in the niche difference between resident and invader (Chase & Leibold 2003). The likelihood of predator-mediated coexistence of competitors is reduced because equal vulnerability of young larvae of the two competitors renders the species ecologically more similar. With equally vulnerable young larvae (Fig. 5), only a limited range of productivity (*S*) environments is capable of producing the balance between interspecific competition and predation necessary for stable coexistence of all three populations. Paradoxically, equal vulnerabilities of early-stage larvae simultaneously increases the community's ability to resist invasion (i.e. range of productivity environments in which the invader succeeds decreases, Fig. 8) and increases the community's vulnerability to extinction of a resident if invasion occurs (i.e. range of productivity environments in which the resident is excluded increases). Our model did not incorporate differences between third and fourth instar *C. appendiculata* in their ability to consume prey of different sizes. In general, interspecific differences in vulnerability to third instar predators are less than those for fourth instar predators. Low interspecific differences in vulnerability to third instar predators should have an effect similar to that of equal vulnerability of young prey larvae: it should render the two prey species more ecologically similar and reduce the range of conditions in which predator-mediated coexistence is possible. However, Lounibos *et al.* (2008) showed that the development time of third instar of *C. appendiculata* is relatively short compared to the fourth instar, and far fewer prey are consumed, which may potentially mitigate these effects.

In conclusion, we have shown that interspecific differences in prey size structure may contribute to predator-mediated coexistence between *A. albopictus* and *A. triseriatus*. Together with studies on anti-predatory behavioural responses (Kesavaraju *et al.* 2007), population-level impacts of predation (Griswold & Lounibos 2005a) and patterns of distribution and abundance of the three species (Kesavaraju, Damal & Juliano 2008), there is now strong circumstantial evidence that *C. appendiculata* can promote coexistence between the invasive superior competitor, *A. albopictus*, and the

native inferior competitor, *A. triseriatus* (Juliano & Lounibos 2005; Kesavaraju *et al.* 2007, 2008). *Toxorhynchites rutilus* is a large predator with its first instar size being similar to fourth instar *C. appendiculata*. Thus, size differences between *A. triseriatus* and *A. albopictus* may play much lesser role in the outcome of predation when encounters occur with *T. rutilus*. Regardless, *A. triseriatus* is still less vulnerable to predation by *T. rutilus* than *A. albopictus*, attributable to species-specific differences in anti-predatory behavioural responses (Kesavaraju & Juliano 2004). However, abundance of *T. rutilus* may be less than *C. appendiculata*. In North America, there is considerable overlap in the distribution of these two species and so predators may play an important role in their coexistence. However, the Northern range of *A. triseriatus* exceeds *A. albopictus*, perhaps in part attributable to the physiological adaptation to cold temperatures allowing greater range extension (Dudley *et al.* 2007). Our study shows that the predator-mediated coexistence in this system is highly dependent on stage-dependent differences in prey-predator interactions. Further, the relative importance of prey size and behaviour may interact with environmental factors, such as resource levels (*S*) (Griswold & Lounibos 2005a; Juliano & Lounibos 2005). Differential responses of the two species to resource levels would likely shift growth (size) and development time, thus changing both stage-dependent prey vulnerability and the time over which prey are at risk. Further refinements of the model may include development rates of both prey species and predator. Environmental factors known to affect the population dynamics, especially larval growth and development, include temperature, food quality and quantity, and pH. It seems clear that an assessment of the potential for predator-mediated coexistence in this system will require more detailed investigations of the ontogeny of these interactions among predators and competing prey.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Model of stage-structured predation in this system with invasive and resident competitors, micro-organisms that are their food, and which feed on a nonliving resource, and a predator.

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