Linking phenological shifts to species interactions through size-mediated priority effects

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Summary

1. Interannual variation in seasonal weather patterns causes shifts in the relative timing of phenological events of species within communities, but we currently lack a mechanistic understanding of how these phenological shifts affect species interactions. Identifying these mechanisms is critical to predicting how interannual variation affects populations and communities.

2. Species' phenologies, particularly the timing of offspring arrival, play an important role in the annual cycles of community assembly. We hypothesize that shifts in relative arrival of offspring can alter interspecific interactions through a mechanism called size-mediated priority effects (SMPE), in which individuals that arrive earlier can grow to achieve a body size advantage over those that arrive later.

3. In this study, we used an experimental approach to isolate and quantify the importance of SMPE for species interactions. Specifically, we simulated shifts in relative arrival of the nymphs of two dragonfly species to determine the consequences for their interactions as intraguild predators.

4. We found that shifts in relative arrival altered not only predation strength but also the nature of predator–prey interactions. When arrival differences were great, SMPE allowed the early arriver to prey intensely upon the late arriver, causing exclusion of the late arriver from nearly all habitats. As arrival differences decreased, the early arriver's size advantage also decreased. When arrival differences were smallest, there was mutual predation, and the two species coexisted in similar abundances across habitats. Importantly, we also found a nonlinear scaling relationship between shifts in relative arrival and predation strength. Specifically, small shifts in relative arrival caused large changes in predation strength while subsequent changes had relatively minor effects.

5. These results demonstrate that SMPE can alter not only the outcome of interactions but also the demographic rates of species and the structure of communities. Elucidating the mechanisms that link phenological shifts to species interactions is crucial for understanding the dynamics of seasonal communities as well as for predicting the effects of climate change on these communities.

Key-words: climate change, community assembly, dragonfly, intraguild predation, *Pantala flavescens*, phenological mismatch, seasonal dynamics, size-structured interactions, trait-mediated priority effects, *Tramea carolina*

Introduction

Seasonal weather patterns play a key role in the timing of life-history events, or phenologies, of many species (Inouye 2008; Tottrup *et al.* 2008). Within a community, these phenological events determine when species 'arrive' in the habitat each year relative to one another (e.g. via

migration, emergence from dormancy and birth). The relative timing of arrival determines when and at what ontogenetic stages species interact (Both *et al.* 2009; Yang & Rudolf 2010). Species within communities, however, often differ in their responses to weather cues (Saenz *et al.* 2006; Miller-Rushing & Primack 2008). Consequently, interannual variation in seasonal weather patterns can differentially affect phenologies of species, thereby altering the amount of time between arrival events of species and

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even arrival order. Despite the increasing evidence indicating that relative shifts in phenologies of interacting species are ubiquitous in nature (Parmesan 2007; Tylianakis *et al.* 2008), we do not have a good understanding of how these relative shifts affect species interactions (Yang & Rudolf 2010). Determining consequences for species interactions is critical to understanding and predicting how environmental variation affects demographic rates of populations and structure of communities (Forrest & Miller-Rushing 2010; Yang & Rudolf 2010).

To address this problem, it is useful to consider the consequences of phenological timing in the framework of community assembly. Many aspects of the annual cycles of community development that occur in seasonal habitats represent bouts of (re)colonization (e.g. seeds germinating, birds returning to nesting habitat, insects emerging from diapause and frogs laying eggs). When organisms colonize a habitat, they encounter and interact with organisms that arrive both before and after them. This can result in priority effects, whereby early arrivers affect the survival, growth and fitness of late arrivers (Connell & Slatyer 1977), and the strength of these priority effects can vary among years as relative arrival times shift. As differences in arrival become larger, early arrivers have more time to (i) increase in population density (Robinson & Dickerson 1987; Olito & Fukami 2009), (ii) alter environmental conditions (e.g. via resource monopolization, Hernandez & Chalcraft 2012), and/or (iii) change their individual traits (e.g. increase in body size and/or aggressiveness, Geange & Stier 2009; Rudolf & Singh 2013) prior to the appearance of late arrivers. This often results in negative consequences for late arrivers, even when arrival differences are quite small (Alford & Wilbur 1985; Munday 2004). Priority effects are widespread and have been observed in a diversity of communities including, but not limited to, insects (Shorrocks & Bingley 1994; Padeffke & Suhling 2003), plants (Kardol, Souza & Classen 2013), fungi (Dickie *et al.* 2012), amphibians (Alford & Wilbur 1985; Hernandez & Chalcraft 2012), fishes (Munday 2004; Geange & Stier 2009) and sessile marine invertebrates (Dean & Hurd 1980). While priority effects are increasingly recognized as a major factor structuring natural communities (Chase 2003; Fukami 2010), few studies have attempted to isolate the different types of priority effects to determine their importance (Hernandez & Chalcraft 2012).

Here, we focus on the importance of 'size-mediated priority effects' (SMPE) as a mechanism to explain how phenological shifts affect species interactions. SMPE occur when earlier-arriving organisms have time to grow and achieve a body size that affords them an advantage in interactions with later-arriving organisms. In the annual cycles of offspring colonization, SMPE are likely important because variation among years in relative arrival of species causes variation in the relative body size at which they interact (Sutherland & Karlson 1977; Geange & Stier 2009). Relative size, in turn, can alter per-capita interaction strength, or the effect of one individual on an individual of another species (reviewed by Werner & Gilliam 1984; Schwinning & Weiner 1998; Yang & Rudolf 2010). Relative size also can change over ontogeny due to the interplay between relative arrival, relative growth rates and interaction feedbacks (Yang & Rudolf 2010; Miller & Rudolf 2011), which causes interactions to be dynamic and their outcomes to be difficult to predict *a priori*.

We hypothesize that phenological shifts could alter interactions in diverse ways via SMPE, including changes to the strength, symmetry and even the nature of interactions (e.g. competition vs. predation). If two generalists that compete for a shared resource arrive at approximately the same time (and size), interactions could be symmetric. However, as disparity in arrival increases, the early arriver has more time to grow before colonization by the late arriver, and the body size disparity increases. As a result, interactions could become more asymmetric, oftentimes favouring the larger, older organism. In the case of predatory organisms, increasing arrival disparity could even change the interaction type from competition to predation, with the larger, older organism eating the smaller, younger one. If there are also differences in relative growth rates, interactions could become more complex. For example, if the late arriver has relatively higher growth rates, it can exceed the early arriver in size over time, especially if arrival differences are small. This could switch which species is competitively dominant or reverse the direction of predator–prey interactions (i.e. prey becomes predator and vice versa).

In this study, we took an experimental approach to determine the importance of SMPE as a mechanism driving the effects of phenological shifts on species interactions. We used simple pond communities dominated by the nymphs of two dragonfly species, which interact as intraguild predators. First, we used a mesocosm experiment that manipulated shifts in relative arrival of the dragonflies in a way that isolated SMPE from other types of priority effects. This experiment also spanned the entire period in which these dragonflies interacted as nymphs, so we could determine the full effect of SMPE on demographic rates and community structure at this life stage. Then, we used a combination of growth models and results from a laboratory experiment to link the observed effects of phenological shifts in the mesocosm experiment to changes in size-dependent interactions over ontogeny. We found that SMPE altered predation strength, which scaled up to affect dragonfly demographic rates and community structure.

Materials and methods

STUDY ORGANISMS

The aquatic nymphs of dragonflies serve as an ideal system for understanding how interactions between species change with shifts in relative arrival. Dragonflies exhibit intra- and interspecific variation in reproductive phenology (Morin 1984; Wissinger

1988; Corbet 1999), and their aquatic nymphs increase in body size by several orders of magnitude during ontogeny (Benke *et al.* 1999; NLR, unpublished data). Consequently, nymph communities are highly size-structured, and interactions consist of a combination of competition and predation (i.e. intraguild predation; Polis, Myers & Holt 1989; Johnson 1991; Wissinger 1992). Also, previous work has indicated that priority effects can alter interactions between the nymphs of dragonfly species (Fincke 1999; Padeffke & Suhling 2003). In this study, we used the nymphs of two libellulid dragonflies, *Tramea carolina* (Carolina Saddlebags) and *Pantala flavescens* (Wandering Glider), hereafter referred to simply as *Tramea* and *Pantala,* respectively. The annual timing of reproduction for these two species in eastern Texas overlaps significantly, but *Tramea* adults typically begin ovipositing earlier in the season (late spring to late summer) compared with those of *Pantala* (early summer to early fall) (NLR, personal observation). Therefore, nymphs of *Tramea* generally establish before those of *Pantala* in the semipermanent and permanent lentic habitats in which they commonly co-occur. Nymphs of these two species are very similar in size at hatching and in the final instar (NLR, unpublished data). However, previous work suggests that nymphs of *Pantala* exhibit higher growth rates than those of *Tramea* (Fig. S1, Supporting information, Suhling *et al.* 2004), and thus, these species exhibit different development times. This means that if the disparity in arrival between *Tramea* and *Pantala* nymphs is small, it is possible for *Pantala* nymphs to 'catch up to' or even exceed the earlier-arriving *Tramea* nymphs in body size.

MESOCOSM EXPERIMENT: IMPORTANCE OF SIZE-MEDIATED PRIORITY EFFECTS

The goal of this experiment was to determine how shifts in relative arrival affect communities. Specifically, we wanted to determine whether SMPE are an important mechanism underlying how phenological shifts affect dragonfly demographic rates and community structure. Manipulating relative arrival directly would not result in a clear answer to our question because the effects of SMPE would be confounded by the other types of priority effects that can operate concomitantly (Hernandez & Chalcraft 2012). For example, as arrival differences become larger, the early arriver has more time not only to grow larger but also to modify the habitat (e.g. reduce food quantity and/or quality). To isolate SMPE, we used four experimental treatments that manipulated relative differences in initial size as a surrogate for differences in arrival. Experimental units received *Tramea* nymphs belonging to one of four sequential instars, which we labelled Very Small, Small, Medium and Large (described in Table 1), or received no *Tramea* nymphs (control). Newly hatched *Pantala* nymphs were exposed to these five types of initial conditions, which represented increasingly earlier arrival of *Tramea* relative to *Pantala*. Treatments were replicated six times in a randomized complete block design.

The experiment was conducted in an open field at the Rice University South Campus (RUSC) research site in Houston, Texas. We arranged 30 cylindrical plastic wading pools (diameter = 100 cm, depth = 19 cm) in six spatial blocks with the five pools within each block arranged into a circle. This configuration increased the likelihood that the number of eggs laid by female *Pantala* would be similar across pools within a block (see introduction of *Pantala* below). On 20 August 2010, we filled pools with water (depth = 11 cm, volume = 86 L) and immediately fitted them with lids made of 60% shade cloth to prevent unwanted

Table 1. Size classes of *Tramea carolina* nymphs used to create treatments in the mesocosm experiment. Relative size = initial difference in head width between the two species (*Tramea*/*Pantala*)

	Size class			
	Very Small	Small	Medium	Large
Estimated age (days)	7	9	11	14
Head width (mm)	1·85	2·34	2·90	3·47
Relative size	3·2 ×	4·0 ×	5·0 ×	6·0 ×
Body length (mm)	3·8	4·6	6·1	7·1
Dry mass (mg)	0·75	1·56	3·05	5·39

colonization. To establish natural prey communities, we added primary consumers [zooplankton and water boatmen insects (*Trichocorixa calva*)] from local ponds. Each pool received three concentrated aliquots of zooplankton (August 20: 780 mL, September 4: 800 mL and September 8: 700 mL), and one introduction of water boatmen (September 10: 20 adults). We made multiple subsequent additions of both zooplankton (September 18 and 29, October 9: 800 mL per pool per date) and water boatmen (September 19 and 29: 40 per pool per date, October 9: 100 per pool and November 6: 50 per pool per date) during the course of the experiment because the lids excluded organisms that would otherwise contribute to the prey community through natural colonization. These measures prevented prey depletion that would artificially increase intraguild predation. We also added ~30 g dry mass of oak leaf litter for habitat structure and nutrients. This experimental setup has been successfully used for decades to answer a diversity of ecological questions and has been particularly useful for understanding community assembly processes (e.g. Wilbur 1997; Resetarits & Binckley 2009; Rudolf & Rasmussen 2013).

Introduction of *Pantala* nymphs to our experimental pools was accomplished by allowing natural oviposition by *Pantala* females. We controlled timing of oviposition by only removing lids from pools for 3 days (August 29–September 1). Pools remained covered for the remainder of the experiment. There was no evidence that other predatory insects colonized during this 3-day period or that *Pantala* successfully oviposited outside of this period. On September 9, we surveyed hatchlings using standardized scoops with fine mesh aquarium nets. We found that six of 30 pools had somewhat low densities relative to other pools within their respective blocks, so we added additional nymphs to these pools from several extra pools that had been set up specifically for this purpose. Later on this same day, we repeated our standardized surveys in those six pools to confirm that we had increased densities to levels comparable to other pools within their respective blocks. Although differences in hatchling density among spatial blocks existed, ultimately there were no differences among treatments [mean ± standard deviation (SD): 164 ± 76 nymphs per pool, $\chi^2_{4,20} = 2·01$, $P < 0·7340$, Fig. S2, Supporting information].

We collected all *Tramea* nymphs from a pond at the Sam Houston State University Center for Biological Field Studies (SHSU CBFS; 5 km northeast of Huntsville, TX) on September 4. We used only recently moulted individuals in the experiment to assure that all nymphs within a given instar were at the same point in development. The experiment was initiated on September 11 with the introduction of *Tramea* nymphs to the appropriate treatments. For all treatments that received *Tramea*, we added

seven nymphs to each pool (81·40 nymphs m^{-3}), which is representative of natural densities during this time of year ($N = 4$ ponds, mean $\pm$ SD: 195·87 nymphs m^{-3} $\pm$ 218·03, range: 12–560 nymphs m^{-3}). We chose to control for initial density (instead of biomass) because (i) we were interested in the per-capita effect (not per unit biomass effect) and (ii) we anticipated that survival of *Tramea* during the experiment would generally be high (which it was, see Results) and that *Tramea* nymphs in the Very Small treatment would catch up in size to those in the Large treatment over the course of the experiment (early instar nymphs grow faster than later instar nymphs). Consequently, over the duration of the experiment, density and biomass would be relatively similar across treatments. Had we instead controlled for initial *Tramea* biomass, both biomass and density would have strongly diverged among treatments over the course of the experiment (e.g. much higher values for both in the Very Small treatment relative to the Large treatment).

We checked pools daily to collect adult dragonflies as they emerged. By November 17, nearly all surviving *Pantala* nymphs in pools containing *Tramea* had either emerged as adults or were in the final instar, so we emptied the pools and preserved all remaining nymphs in 70% ethanol. To determine the treatment effects on dragonfly survival, growth and biomass, we counted survivors (nymphs and adults), measured nymph size (i.e. head width) and then dried and weighed all survivors (nymphs and adults). We also collected and counted all remaining water boatmen to determine whether treatment effects on dragonflies were strong enough to alter prey abundance.

RESOLVING SHIFTS IN TEMPORAL DYNAMICS OF SIZE-DEPENDENT SPECIES INTERACTIONS

To better understand the mechanisms underlying the patterns in survival that we observed in the mesocosm experiment, we used a combination of growth modelling and results from a laboratory experiment. Specifically, we wanted to know how interactions changed over ontogeny and how those changes differed among relative arrival scenarios. The growth models estimated changes in relative size over ontogeny in the mesocosm experiment, and the laboratory experiment determined how interactions changed with relative size.

Laboratory experiment

In October 2012, we collected nymphs of these two species from the same localities used in the mesocosm experiment (*Pantala*: RUSC – October 23, 24 and 26; *Tramea*: SHSU CBS – October 25) and in all instars available at this time [*Pantala*: 8 instars, head width (HW) range = 1·21–5·74 mm; *Tramea*: 6 instars, HW range = 2·57–6·37 mm]. Head width was used as a measure of size because the head capsule is a rigid structure unaffected by growth within instars and because it correlates strongly with labium size, which determines the size of prey a nymph can overpower (Wissinger 1989, 1992). The experiment was conducted in an environmentally controlled room (21 °C, 14 h light:10 h dark) using an array of square polypropylene containers (12 × 12 × 6 cm) filled with pond water. To provide traction for dragonflies, we added just enough pre-rinsed sand to cover the container bottoms. We used white sheets of paper to create visual barriers between containers. We added to each container one nymph of each species in all possible instar combinations (8 *Pantala* instars × 6 *Tramea* instars = 48

combinations), which resulted in a wide range of head width ratios (*Tramea*/*Pantala*: 0·45–5·28). We replicated each size combination as much as possible, but ultimately replication depended upon availability of nymphs for particular sizes (average: 3 replicates, range: 1–9 replicates, 137 experimental units total). The experiment was started at 13:00 on October 26 and ran for 72 h. We recorded whether predation had occurred in each container at six times during the experiment.

Growth models

Because these dragonfly species differ in growth rates, relative size ratios can shift during ontogeny. This, combined with differences in relative arrival, can alter species interactions in complex ways. Therefore, we developed growth curves for both species in each of the four treatments of the mesocosm experiment in which they co-occurred to compare changes in relative size over time among treatments. Data from a comparable experiment (NLR, unpublished data) indicated that logarithmic functions perform well to describe growth of these species, so we used functions of this form for this experiment (see Appendix S1, Fig. S1, Supporting information). In the logarithmic equations where size = m * ln(time) + b, we used m (hereafter called the growth rate coefficient) to make comparisons between species and across treatments. For *Tramea*, all survivors were still nymphs, so we developed minimum, average and maximum growth curves based on changes over time in head width. For *Pantala*, survivors were a mix of nymphs and adults, but all pools with survivors had at least one adult emerge. Therefore, we developed maximum growth curves based on minimum time to the adult stage. For *Pantala*, the maximum growth curve is the most biologically interesting because it indicates when/if during ontogeny the direction of predation begins to switch from *Tramea* eating *Pantala* to vice versa. We combined the size-based mortality results from the laboratory experiment with the estimates of changes in relative size derived from these growth models to determine the probability of mortality for each species at key time points during the mesocosm experiment and to determine when/whether the direction of predation began to switch. For more details on growth models, see Appendix S1 (Supporting information).

STATISTICAL ANALYSIS

Mesocosm experiment

We used logistic regression to determine how initial *Tramea* size affected the proportion of pools that exhibited recruitment of at least one *Pantala* nymph into the adult population. To evaluate the model, we conducted a likelihood ratio test comparing the full model to the intercept-only model, and we conducted a le Cessie – van Houwelingen – Copas – Hosmer unweighted sum of squares test for global goodness-of-fit (Hosmer *et al.* 1997). For the goodness-of-fit test, $P > 0·05$ indicates a failure to reject the null hypothesis that the model provides a good fit to the data.

We used regression to test for effects of initial *Tramea* size on *Pantala* survival, *Pantala* total biomass, *Tramea* survival, and water boatmen survival. For each regression analysis, we initially included a quadratic predictor term to determine whether the relationship between the predictor and response was curvilinear. If the quadratic term was not significant in a given analysis, it was excluded from the final analysis. If it was significant, we

conducted the Mitchell-Olds & Shaw (MOS) test to determine whether there was evidence of a hump-shaped or U-shaped relationship (Mitchell-Olds & Shaw 1987). For all of these models, we also initially included as a predictor the estimated density of *Pantala* hatchlings at the start of the experiment. However, this predictor was not significant in any cases and was therefore excluded from the final models. To create the *Pantala* total biomass response variable, we added together for each pool the dry mass of nymphs and the estimated final instar nymph dry mass of adults. We developed these estimates for adults using empirically derived relationships between final instar nymph and adult dry masses. This formulation of the response variable is less likely to exhibit treatment bias compared with simply adding together the dry mass of nymphs and adults. This is because dragonflies lose a significant amount of mass during the transformation from nymph to adult, and the proportion of *Pantala* survivors that emerged as adults varied among the treatments. We did not analyse *Tramea* total biomass because this response was confounded with the experimental treatments.

Laboratory experiment

We used logistic regression to determine how relative size affected survival for each dragonfly species after 72 h of interaction. In these analyses, the predictor variable was the ratio of head widths for interacting pairs. Initially, we also included as a covariate the absolute head width of one species when looking at survival of the other, in case, the size ratio at which the predation switch occurred was dependent upon absolute size. However, this covariate was not significant in either model and consequently was not included in the final models. Model evaluation methods were the same as those used for the mesocosm experiment. In some experimental units, one or both nymphs moulted before a predation event, so we used the effective size ratios instead of the original ratios. We excluded three experimental units from analyses because *Tramea* nymphs died of causes unrelated to predation (i.e. no signs of injury).

General

All analyses were performed using the R statistical computing environment (R Development Core Team 2013). We used the 'rms' package for logistic regression (Harrell 2013), and the 'vegan' package for the MOS test (Oksanen *et al.* 2013). When block effects were not significant, block degrees of freedom were pooled with the error term degrees of freedom for the final analysis (Zuur *et al.* 2009). Assumptions of normality of residuals and homogeneity of variances were satisfied for all analyses.

Results

MESOCOSM EXPERIMENT: IMPORTANCE OF SIZE-MEDIATED PRIORITY EFFECTS

Late arriver recruitment success

Across the 24 pools in which *Tramea* co-occurred with *Pantala*, only 15 (63%) had *Pantala* survivors. Logistic regression revealed that an increase in initial *Tramea* size caused the proportion of pools producing at least one adult *Pantala* to decline from 97% to 14% [Fig. 1a;
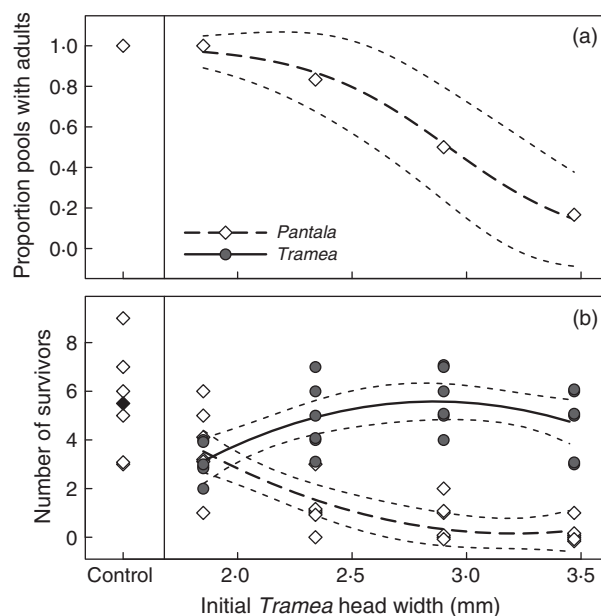

Fig. 1. Response to differences in initial size of *Tramea carolina* nymphs for (a) the proportion of pools with at least one survivor of *Pantala flavescens*, and (b) the number of survivors per pool for *Pantala* and *Tramea*. The number of survivors is out of an estimated average of 164 *Pantala* nymphs that colonized pools and seven *Tramea* nymphs initially added (see 'Materials and methods' for details). Thick lines are model predictions, and thin dashed lines are the corresponding 95% confidence intervals. The control treatment did not contain any *Tramea* nymphs and is only included in plots for comparison. The mean for *Pantala* survival in the control treatment is represented by the black diamond. When necessary, points were jittered to increase visibility of the six replicates per treatment.

intercept: $9\cdot46 \pm 3\cdot60$ standard error (SE), $P < 0\cdot0086$, slope: $-3\cdot24 \pm 1\cdot25$ SE, $P < 0\cdot0094$; overall model evaluation: $\chi^2 = 12\cdot17$, d.f. $= 1$, $P < 0\cdot0005$; goodness-of-fit test: $\hat{S} = 0\cdot5271$, d.f. $= 1$, $P = 0\cdot5981$].

Late arriver survival

Regression indicated that *Pantala* survival declined nonlinearly with increasing initial *Tramea* size [Fig. 1b; survivors $= -10\cdot23 *$ size (mm) $+ 1\cdot65 *$ size (mm)$^2 + 15\cdot65$; adjusted $R^2 = 0\cdot48$, $F_{2,21} = 16\cdot88$, $P < 0\cdot0001$]. Survival in the Very Small treatment was $3\cdot1 \times$, $5\cdot5 \times$, and $22\cdot0 \times$ greater than that in Small, Medium and Large treatments, respectively. Though the relationship was curvilinear, the MOS test indicated that it was not U-shaped.

Late arriver total biomass

Pantala total dry biomass declined linearly with increasing initial *Tramea* size [biomass (mg) $= -118\cdot74 *$ size (mm) $+ 407\cdot04$; adjusted $R^2 = 0\cdot57$, $F_{1,21} = 31\cdot52$, $P < 0\cdot0001$]. For the Control, Very Small, Small, Medium and Large treatments, total dry biomass was $249\cdot0$ mg $\pm 83\cdot5$ SD, $215\cdot6$ mg $\pm 66\cdot9$ SD, $94\cdot7$ mg $\pm 73\cdot0$ SD, $50\cdot8$ mg $\pm 59\cdot8$ SD, and $13\cdot2$ mg $\pm 32\cdot3$ SD, respectively.

Early arriver survival

Regression indicated that *Tramea* survival increased non-linearly with increasing initial *Tramea* size and was greatest in the Medium treatment [Fig. 1b; survivors = 13·70 * size (mm) − 2·39 * size (mm)^2 − 14·02; adjusted R^2 = 0·33, $F_{2,21}$ = 6·68, P < 0·0057]. Survival was 45%, 69%, 81% and 67% in Very Small, Small, Medium and Large treatments, respectively. Though the relationship was curvilinear, the MOS test indicated that it was not hump-shaped.

Prey survival

Regression indicated no effect of initial *Tramea* size on water boatmen survival (adjusted R^2 = 0·05, $F_{1,28}$ = 2·66, P < 0·1139; 9·47 per pool ± 4·96 SD). This is possibly due to the fact that, although the final composition of the dragonfly community differed among treatments, the final total number of dragonflies did not (5·90 per pool ± 1·65 SD; $\chi^2_{4,25}$ = 2·44, P < 0·6555). Water boatmen survival simply declined linearly with increasing final dragonfly abundance [water boatmen = −1·99 (dragonflies) + 21·21; adjusted R^2 = 0·42, $F_{1,28}$ = 21·71, P < 0·0001].

LABORATORY EXPERIMENT: CHANGES IN INTERACTIONS WITH RELATIVE SIZE

Predation occurred in 107 of 134 experimental units (80%, 45 *Tramea* and 62 *Pantala* eaten). Generally, the larger nymph became a predator of the smaller one, regardless of species, even when size differences were quite small (Fig. 2). Based on head width ratios (*Tramea*/*Pantala*), interactions between species fit into five distinct categories: (i) ratio = 0·45–0·86: *Tramea* always eaten; (ii) ratio = 0·90–1·17: if either eaten, then it was *Tramea*; (iii) ratio = 1·18: no predation; (iv) ratio = 1·20–2·02: if either eaten, then it was *Pantala*; and (v) ratio = 2·13–5·28: *Pantala* always eaten. Logistic regression models indicated that, for both species, the predicted probability of mortality increased with head width ratio values (i.e. as size of the focal species decreases relative to the other species; Table 2). This increase in probability of mortality occurred rapidly around a head width ratio of 1·0 (Fig. S3, Supporting information). However, *Tramea* must be larger in head width to eat *Pantala* than vice versa. For example, probability of mortality for *Pantala* becomes 0·5 when the size ratio is 1·60, but mortality probability for *Tramea* become 0·5 when the ratio is 1·05.

GROWTH MODELS: TEMPORAL DYNAMICS OF SIZE-DEPENDENT SPECIES INTERACTIONS

At the beginning of the mesocosm experiment, *Pantala* nymphs were highly vulnerable to predation from *Tramea* across all treatments (*Tramea* HW/*Pantala* HW ≥ 4·0, Pr [mortality] for *Pantala* = 0·9999, based on laboratory experiment results). However, the maximum growth rate
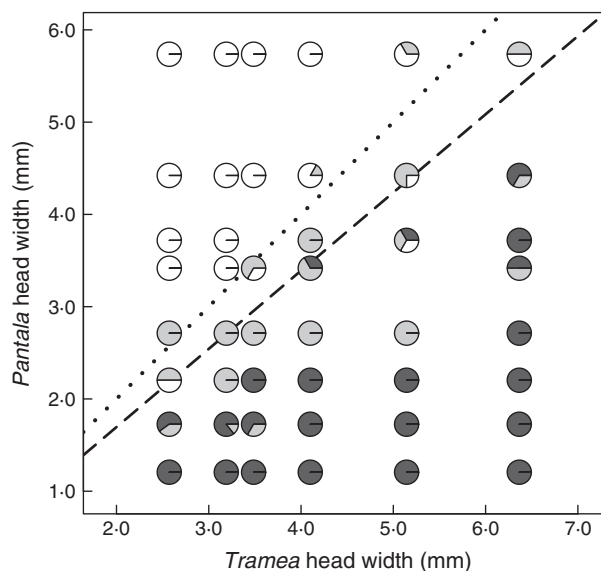

Fig. 2. Predation outcomes for pairings of nymphs of *Tramea carolina* and *Pantala flavescens* in the laboratory experiment. Dark grey indicates that *Tramea* ate *Pantala*, white indicates that *Pantala* ate *Tramea*, and light grey indicates that neither species was eaten. Each point represents one to eight replicates. The dotted line denotes a head width ratio of 1·00 and the dashed line denotes the head width ratio at which the direction of predation switched (1·18).

Table 2. Logistic regression analysis of survival of nymphs of *Pantala flavescens* and *Tramea carolina* after 72 h of interaction in the laboratory experiment when the size ratio of the two species was manipulated

	β	SE β	Wald Z	d.f.	*P*-value
Pantala survival					
Constant	−8·36	1·51	−5·540	1	< 0·0001
Size ratio (T/P)	5·23	0·95	5·480	1	< 0·0001

Overall model evaluation: χ^2 = 130·017, d.f. = 1, P < 0·0001

Goodness-of-fit test: Ŝ = 1·4376, d.f. = 1, P = 0·1505

Tramea survival					
Constant	−13·89	3·27	−4·247	1	< 0·0001
Size ratio (P/T)	14·28	3·36	4·248	1	< 0·0001

Overall model evaluation: χ^2 = 126·8024, d.f. = 1, P < 0·0001

Goodness-of-fit test: Ŝ = 0·5164, d.f. = 1, P = 0·6056

d.f., degrees of freedom; P, *Pantala*; SE, standard error; T, *Tramea*.

of *Pantala* exceeded that of *Tramea* in the Very Small, Small, Medium and Large treatments by 1·6 × [*Pantala* growth rate coefficient (m_p) = 3·54, *Tramea* growth rate coefficient (m_t) = 2·24], 1·4 × (m_p = 3·20, m_t = 2·23), 1·7 × (m_p = 3·28, m_t = 1·97), and 2·1 × (m_p = 3·01, m_t = 1·46), respectively. Consequently, the fastest-growing *Pantala* nymphs achieved sizes that rivaled or exceeded those of *Tramea* nymphs over ontogeny, and the time required for *Pantala* to catch up to *Tramea* decreased
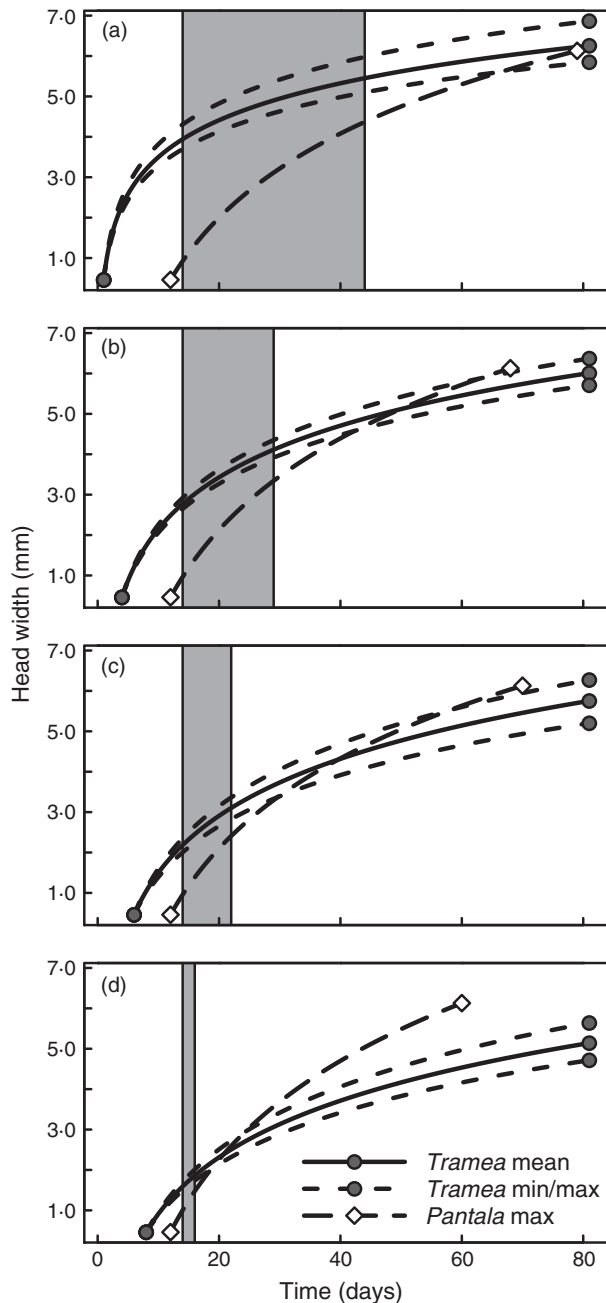
Fig. 3. Logarithmic growth curves for nymphs of *Pantala flavescens* and *Tramea carolina* in four treatments that manipulated initial *Tramea* nymph size: (a) Large, (b) Medium, (c) Small and (d) Very Small. In all plots, days 1, 12 and 14 represent the estimated hatching date for Large *Tramea*, hatching date for *Pantala*, and start of the experiment, respectively. The grey shaded regions indicate the estimated temporal windows during which *Tramea* could prey upon *Pantala* and was invulnerable to predation from *Pantala*. End points of *Pantala* growth curves are the dates on which the fastest-growing nymphs became adults, while end points for *Tramea* curves represent the end of the experiment (day 81, none emerged as adults in any treatment).

with initial size differences (Fig. 3). Growth models estimated that the fastest-growing *Pantala* nymphs became large enough to consume the slowest-growing *Tramea* nymphs by 4, 10, 17 and 32 days after hatching in the

Very Small, Small, Medium and Large treatments, respectively (i.e. smallest *Tramea* HW/largest *Pantala* HW ≤ 1·17, based on laboratory experiment results), indicating initiation of a switch in predation direction. Therefore, the duration of ontogeny over which predation was restricted to *Tramea* consuming *Pantala* diminished from 30 days in the Large treatment to 15, 8 and 2 days in the Medium, Small and Very Small treatments, respectively (Fig. 3). By the time they emerged as adults, the fastest-growing *Pantala* nymphs in all treatments were at a size immune to predation from *Tramea* and were capable of eating even the largest of *Tramea* nymphs (Pr[mortality] for *Tramea*: Very Small = 0·92, Small = 0·68, Medium = 0·66 and Large = 0·34, based on laboratory experiment results).

Discussion

Relative shifts in phenological timing of interacting species are ubiquitous in seasonal habitats, but we are just beginning to understand how these shifts influence species interactions. In this study of intraguild predators, we show that shifts in arrival by one species relative to another can have strong effects on species interactions via size-mediated priority effects (SMPE). These effects on interaction strength determined not only the relative abundances of dragonfly species within habitats but also whether the dragonflies could coexist. Importantly, we also found a nonlinear scaling relationship between shifts in relative arrival and interaction strength because small shifts in relative arrival caused large changes in interaction strength while subsequent changes had relatively minor effects. Thus, even small phenological shifts can determine the success or failure of species to colonize and persist in a community. These results highlight the importance of SMPE as a mechanism linking phenological shifts to species interactions and the dynamics of natural communities.

SIZE-MEDIATED PRIORITY EFFECTS AND COMMUNITY DYNAMICS

Size-mediated priority effects have been implicated as a driving force in determining the outcome of species interactions, but surprisingly little experimental work has rigorously evaluated their role. Presently, our knowledge of these priority effects is largely derived from two types of studies: (i) those that manipulate relative arrival in experiments that span the development of the study organisms (e.g. Alford & Wilbur 1985; Hodge, Arthur & Mitchell 1996; Boone, Scott & Niewiarowski 2002) and (ii) those that manipulate relative size of study organisms in experiments that span a small fraction of development time (e.g. Hurd 1988; Fincke 1999; Von May, Reider & Summers 2009). The former cannot readily disentangle SMPE from other types of priority effects, and the latter does not provide information about the dynamics of relative body size, and thus species interactions, that occur over ontogeny. To isolate and quantify SMPE under different

scenarios of phenological shifts, we took an integrative approach that involved a mesocosm experiment, laboratory experiment and growth modelling. We found that, when differences in arrival between species were relatively large (i.e. Large treatment), the early arriver maintained a size advantage long enough to eliminate the late arriver from nearly all habitats through predation. However, when arrival differences were small (i.e. Very Small treatment), the faster growth rates of the late arriver allowed at least some individuals to achieve a size refuge from predation and even gain a size advantage. Consequently, in this scenario, there was mutual predation (i.e. large *Tramea* ate small *Pantala* and large *Pantala* ate small *Tramea*), which ultimately allowed the two species to coexist in similar abundances. Taken together, this integrative approach demonstrated that SMPE can strongly affect interactions between growing predators, even when all other types of priority effects are excluded and when the range of phenological shifts is quite small (i.e. 5-day vs. 12-day arrival difference). Importantly, these results also indicate that species interactions are not fixed, as is typically assumed, but instead are dynamic and can vary among years with changes in relative arrival phenologies.

FUNCTIONAL RELATIONSHIP OF PHENOLOGICAL SHIFTS AND INTERACTION STRENGTH

While there is increasing evidence that phenological shifts have the potential to alter species interactions, the way in which per-capita interaction strength scales with phenological shifts is largely unknown. If this relationship is linear, we can simply use the magnitude of these shifts to determine the magnitude of change in interactions. However, if it is nonlinear, we will need to develop a mechanistic framework to understand this scaling relationship. Based on studies of competition that have examined interaction strength over a range of relative arrival times, it appears that this relationship can be nonlinear (Shorrocks & Bingley 1994; Hodge, Arthur & Mitchell 1996). Nonlinearity can arise from a variety of nonexclusive (and interacting) mechanisms including positive feedback loops, nonlinear scaling of per-capita interaction strength with species' size ratios, and changes in interaction type (e.g. from competition to predation). In competition, larger early arrivers can dominate resources, which promotes their own growth while reducing growth of late arrivers, and this can lead to a positive feedback between size and growth rates. Because most studies have focused upon competition, it is difficult to know whether this nonlinearity prevails for other types of interactions.

In our study of intraguild predators, we had treatments representing four levels of phenological shifts, and we observed a nonlinear relationship between these shifts and per-capita interaction strength, as measured by survival. Small changes in relative arrival (i.e. Very Small vs. Small treatments, 2-day relative shift) led to a dramatic decline

(68%) in survival of the late arriver, while further increasing differences in relative arrival had relatively minor consequences (i.e. Small vs. Medium treatments, additional 2-day shift, 14% further decline, Fig. 1b). While this nonlinearity could be due, in part, to mean survival of the late arriver levelling off as it approached zero, we think that the change to interactions that occurred between the Very Small and Small treatments was more important in generating this nonlinear relationship. This conclusion is further supported by the nonlinear scaling relationship also observed for mean survival of the early arriver, which never approached zero (Fig. 1b). The small arrival differences represented by the Very Small treatment likely allowed many individuals of the faster-growing late arriver to 'outgrow' the early arriver to become the dominant predator, while all greater arrival differences resulted in the late arriver suffering intense predation from the early arriver. Whether the interactions involve competition, predation or both, it is unlikely that the phenological shift-species interaction relationship will be linear given that the majority of interactions occur among growing individuals.

Conclusions

The timing of phenologies naturally varies among years with seasonal weather patterns, which could affect the outcome of species interactions. Theory suggests that such context-dependent outcomes of species interactions can alter long-term persistence of species and thus community structure because different years favour different species (Chesson & Warner 1981; Polis, Myers & Holt 1989). In support of this, our results indicate that the relative timing of phenological events can change the outcome of interactions between species strongly enough to alter demographic rates of species and community structure. When differences in relative arrival were great, SMPE allowed the early arriver to gain the advantage and exclude the late arriver, but when arrival differences were small (and SMPE were weak), the two species coexisted in similar abundances. If the difference in relative phenological timing, and thus the outcome of interactions, varies more or less randomly among years, species could exhibit long-term coexistence. However, anthropogenic climate change is causing directional shifts in species' phenologies (Parmesan 2007; Tylianakis *et al.* 2008), which could consistently place certain species at a disadvantage across years. This could lead to the exclusion of these species from the community over the long term. While this study provides important novel insight into how phenological shifts affect species interactions, there is still much work to be carried out to develop a predictive framework for understanding the long-term consequences of phenological shifts for natural communities. In the development of this framework, it is clear that empirical studies that carefully isolate and quantify mechanisms are crucial (Forrest & Miller-Rushing 2010; Miller-Rushing *et al.* 2010).

Acknowledgements

Data accessibility

All data associated with the study are deposited in the Dryad repository: http://doi.org/10.5061/dryad.rb590 (Rasmussen *et al.* 2014).

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Supporting Information

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

Appendix S1. Detailed description of methods for developing growth models.

Fig. S1. Plot showing minimum and maximum logarithmic growth curves fitted to data for *Tramea carolina* nymphs in an outdoor mesocosm experiment similar to the one presented in this study.

Fig. S2. Plot showing the average number of *Pantala flavescens* hatchlings in the five treatments at the outset of the mesocosm experiment.

Fig. S3. Plots showing the probability of mortality for nymphs of *Pantala flavescens* and *Tramea carolina* with different relative head width ratios of the two species when paired in the laboratory experiment.