

Priority effects on the life-history traits of two carrion blow fly (Diptera, Calliphoridae) species

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Abstract. 1. Third instars of the invasive blow fly *Chrysomya rufifacies* are facultative predators on larvae of the native blow fly *Cochliomyia macellaria*.

2. The effects of priority arrival time on the survivorship and fitness of *C. rufifacies* and *C. macellaria* were investigated in laboratory experiments.

3. *Cochliomyia macellaria* colonising a resource within 1–2 days after *C. rufifacies* resulted in a 20–70% reduction in survivorship, pupal weight and fecundity compared with those colonising a resource more than 2 days before or after *C. rufifacies*. Inversely, *C. rufifacies* exhibited a 50% increase in survivorship and fecundity when closely (~2 days) associated temporally on the resource with *C. macellaria* and was negatively affected by disparate arrival.

4. These results demonstrate that arrival sequence significantly affects the fitness of both *C. rufifacies* and *C. macellaria*. Early colonisation may allow *C. macellaria* to persist in a community, while there are fitness benefits for *C. rufifacies* colonising after *C. macellaria*.

5. The 60% reduction in *C. macellaria* survivorship when in close temporal association with *C. rufifacies* may act as an agent of selection for *C. macellaria* to colonise a resource early and develop quickly to avoid predation on resources colonised by *C. rufifacies*.

6. Selection for such traits may explain how *C. macellaria* is able to persist despite intraguild predation by this invasive species. In contrast, the 50% increase in survivorship and fecundity exhibited by *C. rufifacies* when arriving after *C. macellaria* may select for *C. rufifacies* to delay colonisation.

Key words. *Chrysomya rufifacies*, *Cochliomyia macellaria*, forensic entomology, interspecific competition, intraguild predation.

Introduction

Patchy distribution of exploitable resources is widespread throughout nature, and interspecific competition for such patches is often intense. Species' colonisation patterns have been considered random or governed by individual physiology (Hodge, 1996). A temporal disparity between ecologically similar species may confer a competitive advantage to primary colonisers under exploitative competition circumstances. An early coloniser is relatively unaffected by the presence of later species, but resource depletion may be a detriment to later-arriving conspecific or interspecific individuals (Bryant,

1971; Beaver, 1984; Shorrocks & Bingley, 1994; Hodge, 1996). Competitively inferior species exploiting resources before patch invasion by more competitively dominant species may survive and consequently persist in an environment (Schoener, 1974; Hodge, 1996). For example, offspring of *Drosophila* sp. (Diptera, Drosophilidae) arriving to Instant *Drosophila* Medium (IDM) was significantly earlier than ecologically similar species, had a 35% increase in survivorship, a 22% decrease in developmental time, and a 17% increase in adult size when compared with those resulting from adults arriving later (Hodge, 1996). This 'priority effect' (Alford & Wilbur, 1985) has been documented in other systems, such as fish (Geange & Stier, 2009), crustaceans (Irving *et al.*, 2007) and amphibians (Eitam *et al.*, 2005). In each case, early-arriving species gained a fitness advantage by exploiting a patch before a competitor arrived. However, priority effects have not been

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examined in detail for blow flies (Diptera, Calliphoridae) that often colonise discrete resource patches – carrion.

Priority effect may be responsible for the coexistence of competing Calliphoridae on a carrion resource (Denno, 1975; O'Flynn, 1983; Schoenly, 1992). Primary colonisers of carrion tend to exhibit efficient detection, location and colonisation (Tomberlin *et al.*, 2011a,b), along with rapid feeding and growth while on the resource (Beaver, 1984). These traits lead to carrion colonisers arriving at a patch early in the decomposition process and consuming the maximum amount of resource before competitors arrive (Schoenly, 1992). Secondary colonisers must contend with reduced nutrient value and high competitor diversity (Beaver, 1984). Natural selection would seemingly therefore favour the earliest and most efficient colonisers of a carcass (Kneidel, 1984a,b). Secondary colonisers of carrion require an advantage over those already in residence (Lane, 1975). Two common hypotheses about such an advantage are that: (i) the species must be an inferior competitor when in direct competition with other species on a resource (Atkinson & Shorrocks, 1981); or (ii) the species requires the resource to be modified by early colonisers in a way that enhances the fitness of the secondary colonising species (Lang *et al.*, 2006).

Central Texas has 10 commonly occurring species of Calliphoridae that naturally colonise and feed on decomposing animal tissue of carrion (Tenorio *et al.*, 2003) and are considered a major part of the necrobiome (Benbow *et al.*, 2013). Of these, *Cochliomyia macellaria* (Fabricius) and *Chrysomya ruffifacies* (Macquart) are the most abundant species during the warm months of the year (Goddard, 1988; Tenorio *et al.*, 2003; Bucheli *et al.*, 2009). *Cochliomyia macellaria* is native to the New World (Baumgartner, 1993) with a distribution from southern Canada, throughout the U.S.A., Mexico, Central America, and as far south as central Argentina. *Cochliomyia macellaria* is a primary coloniser of vertebrate carrion arriving early (within minutes in some cases) in the decomposition process (Tomberlin & Adler, 1998). Large collective egg masses may result in > 1000 larvae cm² in large, aggregate masses, which can quickly consume a carcass or cadaver (Laake *et al.*, 1936; dos Reis *et al.*, 1999; Slone & Gruner, 2007; Oliveira & Vasconcelos, 2010).

Chrysomya ruffifacies is native to Southeast Asia. It was introduced to Central America in the mid- to late 1970s (Baumgartner, 1993), arrived in the U.S.A. by 1980 (Gagne, 1981), and has become well established across North America, including southern Canada (Rosati, 2007). *Chrysomya ruffifacies* acts as a secondary coloniser of vertebrate carrion (Bohart, 1951; Norris, 1959), with adults arriving within minutes after death and suspected to colonise 1–2 days later (Baumgartner, 1993). As second and third instars, they are intraguild predators (Fuller, 1934) on *C. macellaria* larvae (Baumgartner, 1993). Researchers hypothesize that *C. ruffifacies* exhibits predatory behaviors when food resources are limited due to competition with other necrophagous blow flies (Norris, 1965; Goodbrod, 1990). This tendency has led researchers to hypothesize that *C. ruffifacies* will dominate carrion patches and eventually eradicate *C. macellaria* from North America (Wells & Greenberg, 1992a; Baumgartner, 1993).

As *C. macellaria* and *C. ruffifacies* larvae exploit carrion as their primary resource, they are considered direct competitors (Kneidel, 1984a; Baumgartner, 1993; De Jong, 1997). Studies documented competition among carrion flies in the field (Denno, 1975; Denno *et al.*, 1995; Archer & Elgar, 2003), and in experimental caged populations illustrated that some species regularly outcompete others to the point of extinction (Hanski & Kuusela, 1977). However, these results do not completely apply to *C. macellaria* and *C. ruffifacies*, as both species continue to persist in the southern U.S.A. The objective of this study was to determine the priority effects between *C. macellaria* and *C. ruffifacies* larvae on liver. These effects on each species were determined by measuring the impact of temporal variation in colonisation on the fitness-related life-history traits of survivorship, pupal weight, adult longevity and egg production of resulting adults.

Materials and methods

Laboratory colonies of *C. ruffifacies* and *C. macellaria* larvae used in this study were initiated from flies collected in Brazos County, Texas, U.S.A., during spring and summer of 2009 and 2010. Larvae were reared on fresh bovine liver provided *ad libitum* in 3-litre plastic containers (Sterilite Corporation, Townsend, Massachusetts) housed in a walk-in growth chamber at 27.0 ± 1.0 °C, 60.0% RH, and with a LD 12:12 h photoperiod. Dispersing third-instar larvae were transferred to 3-litre containers with autoclaved sand (Town & Country Landscape Supply Co., Chicago, Illinois) for pupation. Resulting adults were maintained in 30 × 30 × 30 cm³ cages (Bioquip Products, Rancho Dominguez, California) held in the growth chamber previously described. Granulated sugar (Imperial Sugar Co., Sugar Land, Texas), buttermilk powder (Saco foods Inc., Middleton, Wisconsin), and water were provided *ad libitum*, and 20 g of bovine liver was placed in the cage between 4 and 15 days post-emergence for 4 h to induce oviposition as needed.

Collected eggs were homogenized prior to placement in treatments and controls. For mixed species treatments, 100 eggs (determined gravimetrically) of the pioneer species were introduced to 100 g of fresh bovine liver in a 20.5 × 34.5 × 20.5 cm plastic tub with 1.5 litres of sand. Eggs were transferred using a camel hair paintbrush. The competing species (100 eggs per species) was introduced 0, 1, 2, 3 or 4 days after introduction of the pioneering species (Fig. 1). To ensure that results were not due to resource age, a pure culture of 200 eggs of each species independently (controls) was placed on liver aged 0, 1, 2, 3 or 4 days in the growth chamber under conditions described in the previous paragraph and retained in the same environment (Fig. 1). These controls tested for the effect of resource age on the life-history traits of each species (see later) without testing priority effects. All treatments and controls were established on the same day. All replicates were placed in the rearing room under the conditions previously described. Nine treatments and 10 controls were established for this study. Each was replicated a minimum of three times over three generations.

Life-history traits to evaluate fitness effects for each species included larval survivorship, pupal weight, adult longevity and

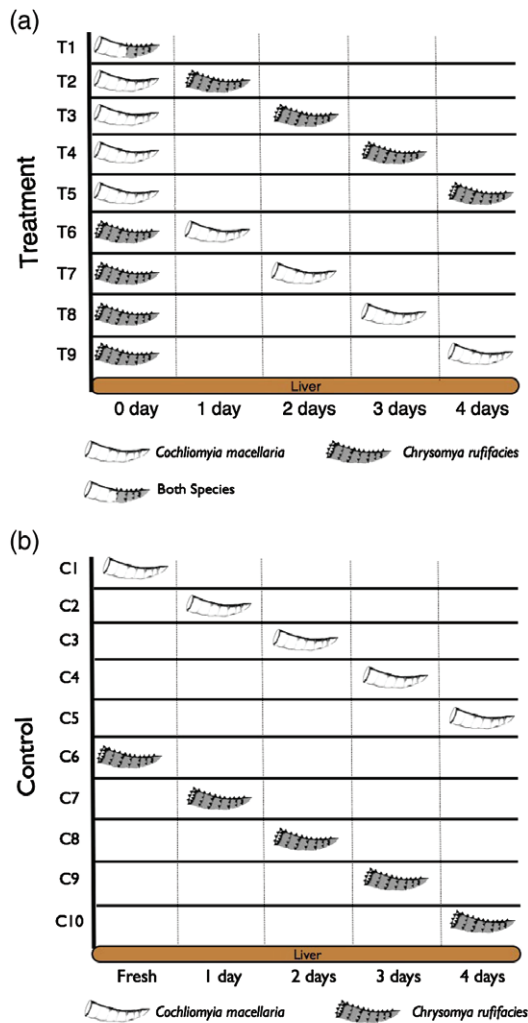


Fig. 1. (a) Treatments for mixed species experiments for *Cochliomyia macellaria* and *Chrysomya rufifacies*. The pioneer species was placed on fresh (0-day-old) liver, while the competing species was placed simultaneously as a mixed treatment (T1) or 1 day (T2, T6), 2 days (T3, T7), 3 days (T4, T8) or 4 days (T5, T9) after the pioneering species. (b) controls For the effect of liver age on fitness. Pure (single species) cultures of larvae from each species (*C. macellaria* and *C. rufifacies*) were placed on fresh (C1, C6), 1-day-old (C2, C7), 2-day-old (C3, C8), 3-day-old (C4, C9) or 4-day-old (C5, C10) liver.

egg production (Allen, 2004). Containers were checked every 24 h for pupae, which were then collected from each container and weighed. In order to measure adult longevity, the first 20 pupae were placed individually in 30-ml clear plastic cups (Bio-Serv, Frenchtown, New Jersey) with 5 g autoclaved sand, covered with breathable lids and returned to the growth chamber. Pupae were observed daily for eclosion. Resulting adults were fed 100 μ l of 21% concentration sucrose syrup daily via pipette until death.

Remaining pupae from each treatment and control were placed in respective cages and held in the growth chamber. Resulting adults were provided granulated sugar, water and commercial

buttermilk powder *ad libitum*. Colonies were presented with fresh bovine liver for 12 h day⁻¹, and the resulting eggs were counted. Dead adults were removed from colonies and sexed. The total number of eggs produced by each colony was divided by the total number of females present in that colony, for a mean number of eggs produced per female.

A MANOVA was employed to test for the effect of resource age or priority effects on blow fly life-history traits. If there was not a significant main effect within a species, paired *t*-tests were used to test for differences between species for each life-history trait. Where there was a significant effect, ANOVAs with Dunnett's multiple comparison tests were used to test for priority effects on each life-history trait within a species compared with liver age controls. Survival percentages were arcsine square-root-transformed prior to all analyses and the other life-history traits were log + 1-transformed. These statistics were performed in JMP 9.0.0 (SAS Institute Inc., Cary, North Carolina), IBM SPSS STATISTICS 18 (SPSS Inc. 2009, Chicago, Illinois) and GRAPHPAD PRISM 5.0 (GraphPad Software, Inc., La Jolla, California).

Results

Single species resource age effects

An intraspecific comparison indicated that there was not a significant ($P < 0.05$) effect of resource (i.e. liver) age on the life-history traits of *C. macellaria* (Wilks' $\lambda = 0.562$, d.f. = 12, $P = 0.737$) or *C. rufifacies* (Wilks' $\lambda = 0.691$, d.f. = 12, $P = 0.939$) when reared in pure culture (Table 1). An interspecific comparison indicated that survival to pupation was not significantly different between species ($t_{(29)} = 0.618$, $P = 0.544$) (Table 1). However, *C. rufifacies* pupae were significantly (24%) larger than *C. macellaria* pupae ($t_{(17)} = 9.032$, $P < 0.0001$) (Table 1). *Cochliomyia macellaria* adults lived significantly longer (~34.0 days) than *C. rufifacies* adults (~23.5 days) ($t_{(19)} = 3.257$, $P = 0.004$; Table 1). Further, there was not a significant difference in fecundity between the two species ($t_{(19)} = 0.637$, $P = 0.532$; Table 1).

Priority effects

There was a significant priority effect on the life-history traits of *C. macellaria* and *C. rufifacies* (Wilks' $\lambda = 0.031$, d.f. = 56, $P < 0.027$). There was a nearly significant overall ($F_{(9)} = 1.88$, $P = 0.094$) priority effect on *C. macellaria* survival to pupation, and in post-tests there was a significantly ($P < 0.05$) lower survival when *C. rufifacies* was introduced 1 and 2 days earlier (Fig. 2). There was not a significant ($F_{(9)} = 1.58$, $P = 0.168$) priority effect on *C. rufifacies* survival (Fig. 2). Similar to survival to pupation there was a significant ($F_{(9)} = 3.08$, $P = 0.009$) priority effect on *C. macellaria* pupal weight when *C. rufifacies* eggs were introduced 1 or 2 days prior (both $P < 0.05$ in post-tests) to *C. macellaria* (Fig. 3). The mean pupal weight of *C. rufifacies* was significantly ($F_{(9)} = 3.64$, $P = 0.004$) affected by priority introduction; however, there were no significant post-test differences even though, on average, *C. rufifacies* pupal weight was

Table 1. Life parameters for control treatments ($n = 9$) for *Cochliomyia macellaria* and *Chrysomya rufifacies* larvae reared in pure culture.

Species	Age of liver at inoculation (days)	0	1	2	3	4
<i>C. macellaria</i>	Survival rate (%)	62.25 ± 0.09	71.25 ± 0.05	70.88 ± 0.05	62.63 ± 0.05	51.00 ± 0.11
	Pupal weight (g)	0.055 ± 0.015	0.054 ± 0.001	0.051 ± 0.00	0.046 ± 0.002	0.050 ± 0.001
	Longevity (days)	32.16 ± 2.06	34.50 ± 2.30	34.43 ± 1.95	34.18 ± 2.28	34.94 ± 2.10
	Fecundity (eggs/adult)	429.74 ± 359.49	678.53 ± 358.41	626.67 ± 487.43	651.09 ± 190.98	335.56 ± 185.69
<i>C. rufifacies</i>	Survival rate (%)	65.50 ± 0.06	70.17 ± 0.02	68.13 ± 0.13	77.13 ± 0.07	69.88 ± 0.05
	Pupal weight (g)	0.073 ± 0.001	0.070 ± 0.002	0.064 ± 0.001	0.068 ± 0.001	0.065 ± 0.001
	Longevity (days)	24.58 ± 2.08	19.89 ± 1.54	25.22 ± 1.46	21.22 ± 1.33	25.38 ± 1.47
	Fecundity (eggs/adult)	582.33 ± 582.33	532.74 ± 318.97	374.06 ± 255.52	514.11 ± 391.66	421.10 ± 181.08

$n =$ replicates.

about half that of other treatments when introduced 3–4 days after *C. macellaria* (Fig. 3). There was also a significant priority effect ($F_{(9)} = 2.73$, $P = 0.020$) on the life span of *C. macellaria*, but not for *C. rufifacies* ($F_{(9)} = 0.200$, $P = 0.993$) (Fig. 4); however, even though the life span of *C. macellaria* was, on average, an order of magnitude lower than in the other treatments when *C. rufifacies* was introduced 1 and 2 days earlier, there were no significant post-test comparisons (Fig. 4). Mean egg production was highly variable for both species, leading to no statistical priority effect for either species (*C. macellaria*, $F_{(9)} = 0.955$, $P = 0.495$; *C. rufifacies*, $F_{(9)} = 0.394$, $P = 0.928$). This variability masks potential biological effects of priority on *C. macellaria* where egg production was nearly 0 whenever *C. rufifacies* was introduced earlier in sequence (Fig. 5). In *post hoc* power analyses, the statistical power of these tests was quite low, ranging from 0.1 to 0.3, and possibly leading to type II errors. Thus, we suggest that there may be biologically important effects of the earlier arrival of *C. rufifacies* on the life span and egg production of *C. macellaria* in addition to the significant effect on survival to pupation and pupal weight.

Discussion

The results of this study demonstrate colonisation priority effects between *C. rufifacies* and *C. macellaria*. Our results imply that the predator–prey relationship between the two species may be more important than competition for food. Priority effects are documented in Calliphoridae, as Hanski (1987) determined that carrion flies arriving first at a carcass dominated the resource, effectively preventing further colonisation by later species (Hanski, 1987). Kneidel (1983) also demonstrated priority effects in carrion-breeding Diptera, finding that *Megaselia scalaris* (Loew) (Diptera, Phoridae) reduced secondary coloniser diversity when it acted as a primary coloniser (Kneidel, 1983). Schoenly and Reid (1987) postulated facilitation of arthropod community change depended heavily upon primary colonisers, which altered the resource, thus allowing location (Spivak *et al.*, 1991) and colonisation by secondary species (Schoenly & Reid, 1987). This suggests that secondary species rely on the presence of primary colonisers to prepare the resource for their colonisation, resulting in higher fitness and increased offspring survivorship.

Chrysomya rufifacies has historically been considered a primary necrophage and a facultative predator (Goodbrod, 1990; Baumgartner, 1993; Rosa *et al.*, 2006). It was assumed that predation of other blow fly larvae occurred only when the resource was scarce (Rosa *et al.*, 2006), allowing *C. rufifacies* to survive even as a secondary coloniser on a depleted resource (Faria, 2004). Results from this study, however, indicate otherwise. *Chrysomya rufifacies* larvae that were present on the resource concurrently with *C. macellaria* larvae had significantly higher fitness than those that were on the resource before or after. This is unexpected if it is assumed that *C. rufifacies* maintains secondary colonisation status to avoid competition with primary colonisers. An inferior coloniser would show substantially lower fitness when forced to compete directly with equivalent species (Hanski, 1987). As this was not the case, it appears that *C. rufifacies* is not delaying colonisation to avoid competition.

A second possibility for remaining as the secondary coloniser is the modification of resource by primary colonisers. The community of arthropods inhabiting a carrion resource early in decomposition changes the quality and characteristics of the resource (Schoenly & Reid, 1987; Spivak *et al.*, 1991), which may allow secondary colonisers to use the resource more efficiently. However, this experiment showed that the fitness characteristics of *C. rufifacies* on fresh and aged resources were similar. If *C. rufifacies* required modification of the resource to enable efficient nutrient intake, fresh resource should have led to a decline in fitness. As this was not the case, it appears that resource modification is not the selective force behind secondary colonisation by *C. rufifacies*.

While resource consumption was not directly measured, observations of the resource before and after colonisation of both species showed that *C. macellaria* consumed most of its food resource prior to pupation. Larvae of *C. rufifacies*, on the other hand, did not consume a substantial amount of the resource, yet were still able to pupate and eclose successfully. This behaviour was even observed in both pure cultures of *C. rufifacies* and those mixed with *C. macellaria*. This difference in resource consumption, along with its maintenance as a secondary coloniser despite apparent selection against such behavior, supports the hypothesis that *C. rufifacies* is a facultative necrophage and a primary predator on patchy carrion resources.

The presence of *C. rufifacies*, and not resource age, impacted fitness of *C. macellaria* (e.g. egg production/female). *Cochliomyia macellaria* had the greatest survival, pupation,

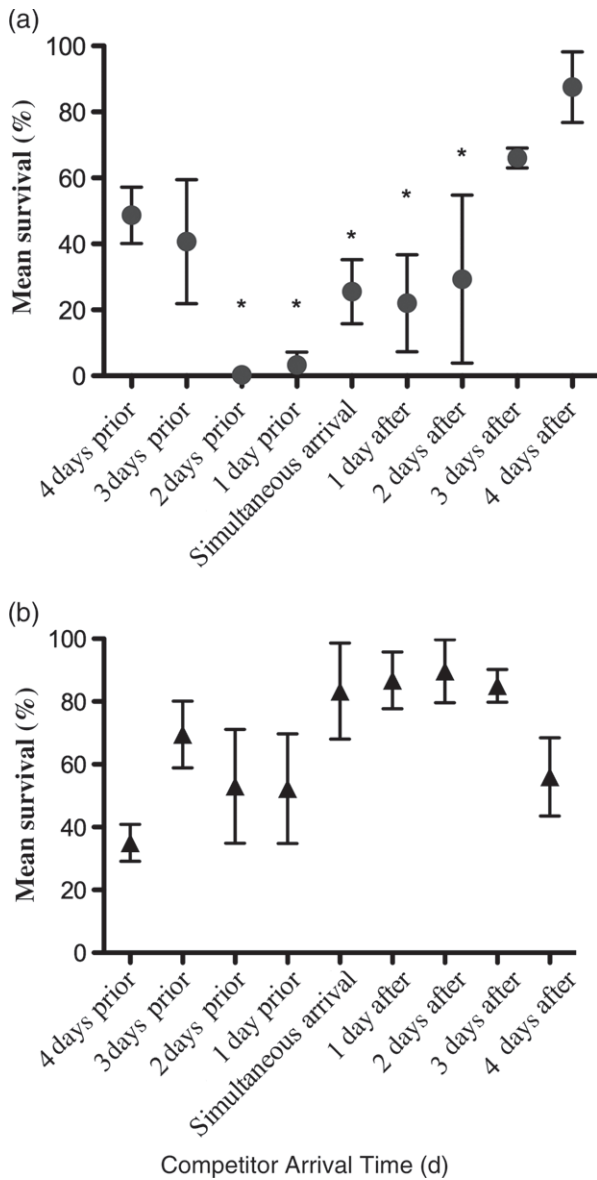


Fig. 2. Mean survival (%) \pm SE of *Cochliomyia macellaria* (a) and *Chrysomya rufifacies* (b) to pupation relative to time of competitor colonisation. *, indicates significant differences from control ($P < 0.05$).

eclosion and fecundity when arriving 4 days before or 4 days after *C. rufifacies*. Arriving 4 days beforehand allowed *C. macellaria* adequate time to reach the pupal stage prior to the onset of competition or potential predation by *C. rufifacies*. Arriving 4 days afterwards allowed *C. macellaria* to inhabit the resource after *C. rufifacies* pupated. Both time frames enabled *C. macellaria* to inhabit 'enemy-free' space without competition or potential predation for enough time to allow completion of development without compromising survivorship, pupal weight, longevity or fecundity.

Close association with *C. rufifacies* was directly correlated with fitness decline in *C. macellaria*. *Chrysomya rufifacies* potentially began predation while *C. macellaria* larvae were

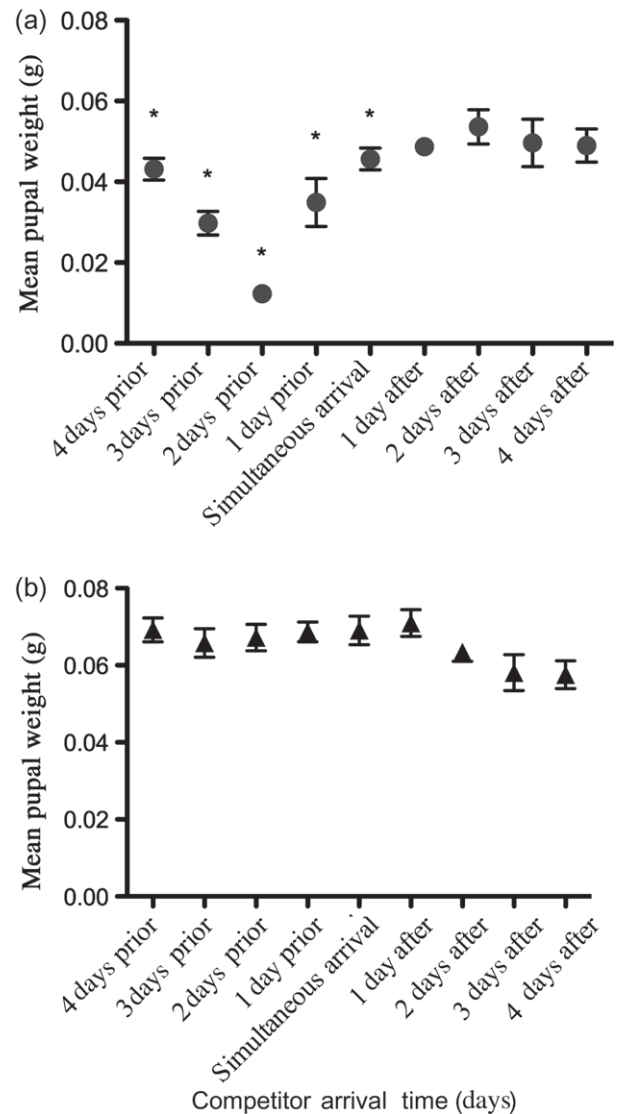


Fig. 3. Mean pupal weight (g) \pm SE of *Cochliomyia macellaria* (a) and *Chrysomya rufifacies* (b) to pupation relative to time of competitor colonisation. *, indicates significant differences from control ($P < 0.05$).

still present, forcing them to leave the resource before reaching the minimum viable weight necessary for successful pupation. *Cochliomyia macellaria* larvae arriving within 2 days after *C. rufifacies* exhibited the most dramatic decrease in survivorship, pupal weight and adult longevity of all the treatments. The 2-day lead time allowed *C. rufifacies* to reach the predeceous second and third instars prior to *C. macellaria* oviposition (Wells & Greenberg, 1992b; Byrd & Butler, 1996a; Sukontason *et al.*, 2004). This resulted in a 98% reduction of *C. macellaria* larvae on the resource; those few larvae that were able to make it to pupation were undersized in comparison to those from the controls and did not eclose.

Chrysomya rufifacies fitness was also impacted by the presence of *C. macellaria*. *Chrysomya rufifacies* colonising 4 days prior to *C. macellaria* exhibited its lowest (35%) survivorship.

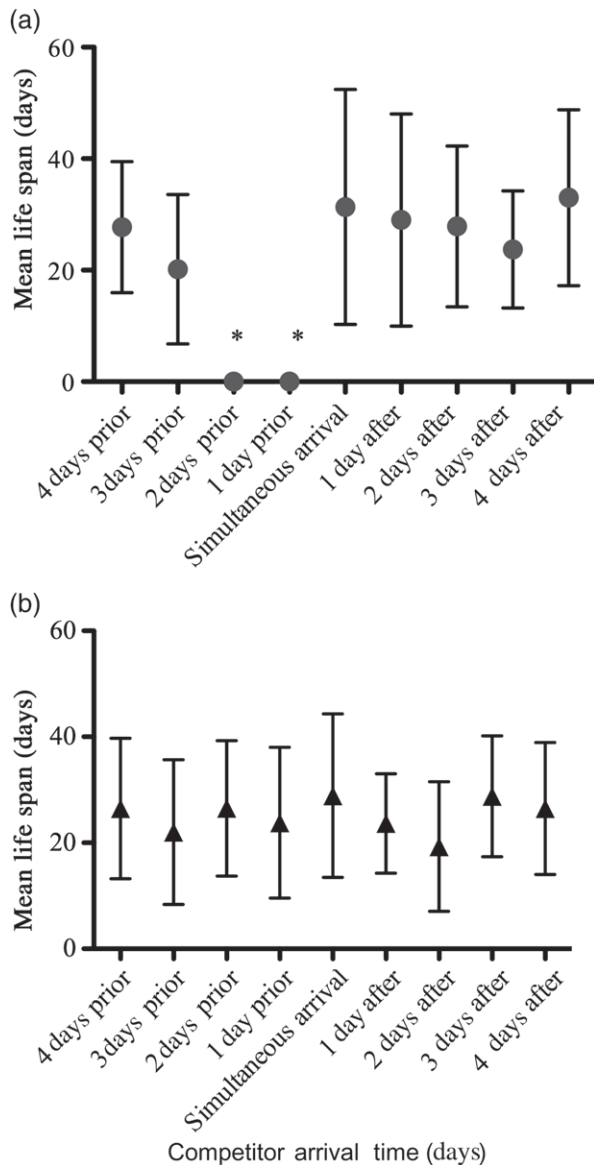


Fig. 4. Mean life span (days) \pm SE of *Cochliomyia macellaria* (a) and *Chrysomya rufffacies* (b) to pupation relative to time of competitor colonisation. *, indicates significant differences from control ($P < 0.05$).

This may be due to two reasons. First, the 4-day lead time allowed *C. rufffacies* to complete its larval stages before *C. macellaria* entered the patch, thus eliminating opportunities for predation on *C. macellaria* larvae. This temporal delay could also have increased the risk of cannibalism due to the lack of prey items (Goodbrod, 1990), and explain the significantly lower survival rates. Secondly, first-instar *C. rufffacies* are known to join interspecific larval masses (Baumgartner, 1993). As first-instar *C. rufffacies* are unable to predate other larvae due in part to their small size, this interspecific aggregation may facilitate efficient feeding on the resource, a hallmark of interspecific larval masses (Rivers *et al.*, 2011). Delayed *Chrysomya rufffacies* colonisation may have kept the larvae from exploiting the interspecific

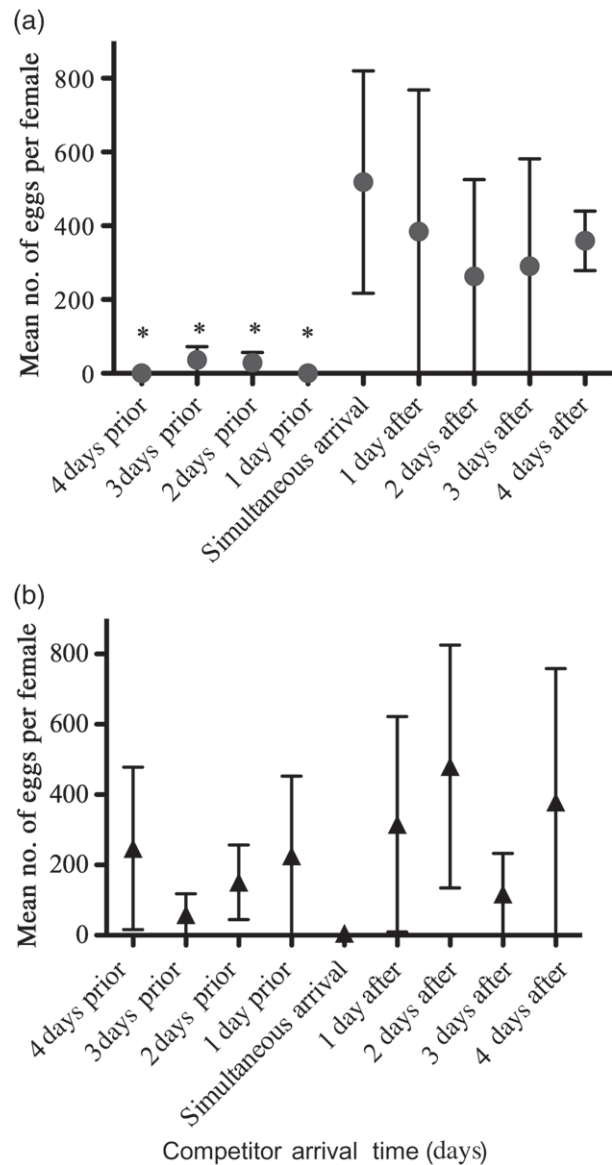


Fig. 5. Mean egg production \pm SE of *Cochliomyia macellaria* (a) and *Chrysomya rufffacies* (b) to pupation relative to time of competitor colonisation. *, indicates significant differences from control ($P < 0.05$).

larval mass, thereby lowering feeding efficiency. Although not observed, this again may have raised the risk of *C. rufffacies* cannibalism and decreased survivorship.

Fitness reduction in *C. macellaria* may also be attributed to non-consumptive effects induced by *C. rufffacies*. Predators influence prey populations both by directly consuming individuals (consumptive effects) and by altering prey behaviour (non-consumptive effects) (Peckarsky *et al.*, 2008; Sih *et al.*, 2010). Non-consumptive effects may impact prey physiology and, in the long run, have a greater consequence than being directly consumed (Dill *et al.*, 2003). Cues released by predatory *C. rufffacies* could result in accelerated development of their larval prey (Aguiar-Coelho & Milward-de-Azevedo,

1998), resulting in smaller adults and thus reducing adult size and fitness of *C. macellaria*. Because *C. ruffifacies* is predaceous in the second and third instars (Wells & Greenberg, 1992a), early-colonising *C. macellaria* only have a limited amount of time to reach critical weight before being potentially consumed. Those taking longer to reach the prepupal stage may be consumed, while those that exhibit shorter developmental times may have a greater chance of reaching adulthood. This process could select for a developmental shift in *C. macellaria* populations that coexist with *C. ruffifacies*, resulting in a significantly faster *C. macellaria* developmental time.

Chrysomya ruffifacies is an intraguild predator of *C. Macellaria*, possibly because it is a weaker competitor with *C. macellaria*. Polis and Myers (1989) suggested that intraguild predation is unique from traditional competition due to the resources gained by the predator, including energy, from the prey. However, it also differs from classical predation, as the behavior reduces exploitation competition (Polis & Myers, 1989). *Chrysomya ruffifacies* larvae take longer to develop completely (Byrd & Butler, 1997) than *C. macellaria* (Byrd & Butler, 1996b; Boatright & Tomberlin, 2010). This difference indicates that *C. macellaria* is potentially more efficient at resource acquisition and utilisation than *C. ruffifacies*. Furthermore, secondary colonisation could be a risky behaviour for *C. ruffifacies* as it increases the likelihood that the resource will become unusable before exploitation by resulting offspring (Beaver, 1984) and increases the risk of interspecific competition (So & Dudgeon, 1990). Despite these possible risks, *C. ruffifacies* continues to exhibit secondary colonisation behaviour, which suggests an evolutionary benefit due to its ability to predate on earlier colonisers.

Adult blow flies can detect volatile organic compounds (VOCs) indicating whose larvae (conspecific or heterospecific) are present prior to contact with the remains (Brundage, 2012; Flores, 2013). This ability is crucial when movement to a resource could be detrimental to the adult (i.e. injury or mortality). Thus, sampling the VOCs allows an assessment from a distance and reduces the need to inspect the resource through contact. We have begun to investigate the role of VOCs in regulating blow fly arrival and colonisation patterns at carrion. Data recently produced in the FLIES Facility indicate that this is the case for *C. macellaria* and *C. ruffifacies*, as the presence of offspring (eggs and larvae) produces VOCs, which impact conspecific and heterospecific attraction and oviposition patterns (Brundage, 2012; Flores, 2013).

Deciphering the ecological interactions between *C. macellaria* and *C. ruffifacies* is important in understanding the impact of an introduced and invasive blow fly species on native blow flies in the Western Hemisphere, and the corresponding decomposition ecology of carrion. These data are also important for forensic entomology. Estimates of carrion age are extrapolated from estimates of the development time of the larvae collected from remains (Pruzan & Bush, 1977) (Greenberg, 1991). This method depends upon extensive knowledge of both the community of decomposers and the larval developmental rates of forensically important flies. A change in either of these parameters could result in inaccurate time of colonisation estimations. Based on this study, carrion lasting less than 4 days in the field

would be more greatly utilised by *C. macellaria* if it were the primary coloniser rather than *C. ruffifacies*, which is the current hypothesis (Bohart, 1951; Norris, 1959). This scenario is important in Texas, as vertebrate carrion the size of an adult human typically has fly activity only during the initial 3–6 days postmortem. After this period of time has elapsed, the remains will have been skeletonised or mummified. Consequently, blow flies colonising remains 4–6 days postmortem potentially will not survive due to lack of resources.

The obvious impact of arrival time on these two species of blow fly implies a mechanism of colonisation regulation. Each species must have the ability to recognize a suitable resource, and colonise such a resource efficiently. An understanding of the mechanisms used by each species to identify proper colonisation windows would help to clarify successional patterns in carrion insects and allow for a deeper understanding of decomposition ecology.

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