

Experimental evidence for within- and cross-seasonal effects of fear on survival and reproduction

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Summary

1. Fear of predation can have non-lethal effects on individuals within a season but whether, and to what extent, these effects carry over into subsequent seasons is not known.
2. Using a replicated seasonal population of the common fruit fly, *Drosophila melanogaster*, we examined both within- and cross-seasonal effects of fear on survival and reproductive output.
3. Compared to controls, flies exposed to the scent of mantid (*Tenodera sinensis*) predators in the non-breeding season had 64% higher mortality, and lost 60% more mass by the end of the non-breeding season and, in the subsequent breeding season, produced 20% fewer offspring that weighed 9% less at maturity. Flies exposed to the scent of mantids in the breeding season did not produce fewer offspring, but their offspring developed faster and weighed less as adults compared to the controls.
4. Our results demonstrate how effects of fear can be manifested both within and across seasons and emphasize the importance of understanding how events throughout the annual cycle influence individual success of animals living in seasonal environments.

Key-words: carry-over effects, *Drosophila melanogaster*, non-consumptive effects, seasonality, top-down effects

Introduction

The fear of predators can have wide-ranging effects on the behaviour, fitness and population dynamics of animals. When animals fear predators, they spend more time being vigilant, less time foraging and less time in higher food-quality, but riskier, habitats (Creel & Christianson 2008; Wirsing & Ripple 2010; Smith, Wang & Wilmers 2015). Such behavioural responses to fear can lead to stress-related physiological changes and have a negative impact on body condition (Preisser & Bolnick 2008; Creel, Winnie & Christianson 2009), which can then influence both reproductive success and survival (Roitberg, Myers & Frazer 1979; Magnhagen 1990; Beckerman, Uriarte & Schmitz 1997; Brown, Laundré & Gurung 1999; Dixon & Agarwala 1999; Li 2002; Preisser, Bolnick & Benard 2005; Eggers *et al.* 2006; Sheriff, Krebs & Boonstra 2009, 2011; Zanette *et al.* 2011). The effects of fear on individuals can also scale up to influence long-term population and community dynamics (Christian 1950; McNamara & Houston

1987; Lima 1998; Laundré, Hernández & Altendorf 2001; Peckarsky *et al.* 2008; Matassa & Trussell 2011; Clinchy, Sheriff & Zanette 2013; Laundré *et al.* 2014), as well as ecosystem function (Schmitz 2008).

Despite the impact of fear on fitness, most studies have been conducted within a season, which means we have a relatively poor understanding of how the effects of fear might carry over to influence the success of individuals in subsequent seasons. Such non-lethal carry-over effects that cross seasonal boundaries have been shown to explain a substantial amount of variance in fitness but they are traditionally linked to bottom-up processes (Harrison *et al.* 2011; O'Connor *et al.* 2014). For example, variation in habitat quality, diet quality and density during the non-breeding period can influence subsequent reproductive success (e.g. Sorensen *et al.* 2009; Morrisette *et al.* 2010; Legagneux *et al.* 2012; Betini, Griswold & Norris 2013a) and even affect population dynamics (Norris & Taylor 2006; Betini, Griswold & Norris 2013a,b). However, top-down processes, such as the non-consumptive effects of predators (McCauley, Rowe & Fortin 2011; Siepielski, Prince & Wang 2014), could also be an important mechanism driving carry-over effects

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and, hence, explaining variation in fitness of animals in seasonal environments. Field experiments, including studies that have spanned multiple seasons, have examined fear in seasonal environments (Ylönen 1989; Sheriff, Krebs & Boonstra 2011; Zanette *et al.* 2011). However, we are unaware of studies that demonstrate that fear can create cross-seasonal carry-over effects.

We hypothesized that the effects of fear during the non-breeding season would carry over to influence reproductive output the following breeding season due to higher stress and lower net energy gain during feeding. To examine this hypothesis, we subjected *Drosophila melanogaster* to distinct breeding and non-breeding seasons and then experimentally tested how cues from a predator (Chinese praying mantids, *Tenodera sinensis*) during the non-breeding period impacted condition and reproductive output. The mantids cause anti-predator behaviours, including reduced activity, in *Drosophila* (Hurd & Rathet 1986; Parigi *et al.* 2015). Compared to controls with no predators, we predicted that the presence of a predator during the non-breeding period would lead to higher mortality during the non-breeding period. For similar reasons, we also predicted that the presence of a predator during the non-breeding period would lead to lower body mass at the end of the non-breeding period among those flies that did survive. Because female lipid reserves and body mass strongly impact reproductive output in *Drosophila* (Betini *et al.* 2014), we hypothesized that the lower body mass at the end of the non-breeding season would carry over to influence reproductive success. Therefore, we predicted that fecundity, total offspring produced and the weight of those offspring at maturity would be lower for flies exposed to predators in the non-breeding period.

In addition to within- and cross-seasonal effects arising from the non-breeding period, we also examined within-season effects of fear arising from the breeding period. As fear during the larval stages of insects can lower growth rates (Scrimgeour & Culp 1994; Lardner 2000; Dahl & Peckarsky 2002), we predicted that the weight of emerged larvae would be lower when mantids were present during the breeding season compared to controls. Lower growth rates have been associated with both slower and faster development times in other insects (Scrimgeour & Culp 1994; Lardner 2000; Benard 2004), but we hypothesized that development time would be quicker in *Drosophila* exposed to predators during the breeding period because this would lead to less time exposed to predators at the vulnerable larval and pupae stages of growth. Mantids do not directly ingest *Drosophila* larvae (I. Dworkin, pers. obs.), and laboratory-evolved *Drosophila* are unlikely to have encountered mantids in their recent evolutionary history (i.e. >40 years since domestication from wild). Thus, any response is likely to be a generalized anti-predator response rather than a response to a specific predatory threat. For instance, many laboratory animals retain basically unchanged anti-predator responses for hundreds of generations, and some animals, especially insects with

limited cognitive processes, will mount similar anti-predator responses to a wide array of potential threats, even those which are not true threats (Dielenberg & McGregor 2001; Kavaliers & Choleris 2001; Hubbard *et al.* 2004). A competing hypothesis is that, because mantids do not predate larvae, flies develop slower in response to the predator would have higher fitness because they can emerge larger and better capable to rapidly produce eggs under the threat of predation.

Materials and methods

DROSOPHILA MELANOGASTER LINES AND EXPERIMENTAL SEASONALITY

Many animals have distinct stages in their annual cycle ('seasons') that correspond with environmental variation, principally food availability. Here, we consider a system with two seasons defined by whether an animal is breeding or not breeding (Betini, Griswold & Norris 2013a,b), although the principles could be extended to more complex seasonality. As in previous studies simulating seasonality (Betini, Griswold & Norris 2013a,b; Betini *et al.* 2014), we used an outbred population of *Drosophila melanogaster* collected from Dahomey (now Benin) in 1970, which has since been maintained in a cage culture at 25 °C. Prior to the start of experiments, the stock population was maintained on a sugar–yeast diet, a 12 L:12 D light–dark cycle, and a 14-day cycle of non-overlapping generations with 3 days available for breeding each generation.

We simulated seasonality by creating distinct breeding and non-breeding 'seasons', (Fig. 1) following the protocol in Betini *et al.* (2014), Betini, Griswold & Norris (2013a,b). During the 'non-breeding season', adults were placed in an empty vial with access to 0.2 mL of 5% water–sugar solution, for 4 days, which prevented females from producing eggs. During the 'breeding season', flies were placed for 24 h into 10 mL of a dead yeast–agar–sugar medium (1000 mL H₂O, 100 g sucrose, 50 g Fleischmann's yeast, 16 g agar, 8 g C₄H₄KNaO₆, 1 g KH₂PO₄, 0.5 g NaCl, 0.5 g MgCl₂, 0.5 g CaCl₂, 0.5 g Fe₂(SO₄)₃), then adults were discarded, and larvae were allowed to mature to adults (>99% emerged between 9–13 days). Flies were kept at 25 °C, 12 L:12 D cycles, and humidity was between 30 and 50%. The same sized vial (28 × 95 mm) was used for both seasons. Flies are not food-restricted during the non-breeding season as ~95% survive in the 'control' groups. Rather, the female has no source of protein and no laying medium, and so does not produce eggs during the non-breeding season (Betini *et al.* 2014). Likewise, many animals are physiologically incapable of breeding during the non-breeding season, such as migrating songbirds that feed on fruit on the non-breeding grounds and protein-rich insects on the breeding grounds or mosquitoes that require a blood meal to produce many larvae.

Our system is designed to mimic typical conditions in the wild where the breeding season is shorter than the non-breeding season and timed for pulses of food production. For instance, (i) the breeding season for temperate songbirds is roughly 2–3 months, timed to a pulse in insect availability, compared with 6–8 months for the non-breeding season, with a migratory season in between (Greenberg & Marra 2005); (ii) the breeding season (gestation and lactation) of a ground-squirrel is about

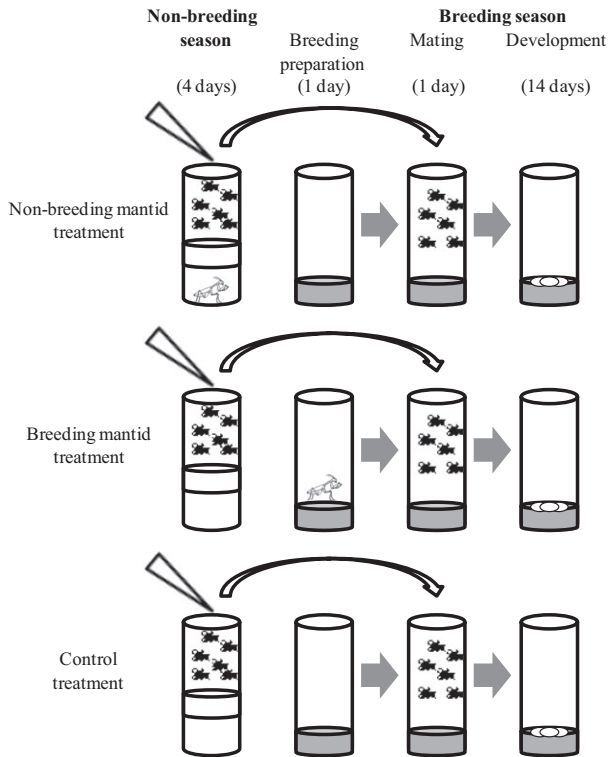


Fig. 1. Schematic diagram of the two treatments and control used in the study. All flies began in the non-breeding season (the slanted triangle at the top of the vial shows the sugar–water solution used to feed the flies during this period) and, after 4 days, were moved to the breeding season (the dark grey at the bottom of the vial represents the dead yeast–agar–sugar medium). The breeding season consisted of 24 h for mating and then 14 days for development of larvae. For the ‘non-breeding mantid treatment’, mantids were placed at the bottom of the vial during the non-breeding season, and for the ‘breeding mantid treatment’, mantids were placed in breeding vials 1 day prior to the start of the breeding season and removed prior to the addition of flies. Larvae emerged between day 8 and 15, with >99% occurring between day 9 and 13 (breeding season days numbered to include 1 day as eggs).

2–3 months timed near peak vegetation growth compared with 9–10 months for the non-breeding season (e.g. Kenagy, Sharbaugh & Nagy 1989; Buck & Barnes 1999); (iii) the breeding season of a mantis is roughly 2 weeks compared with 3 months for the non-breeding season post-hatch (Pohl 1987); and (iv) the breeding season of a monarch butterfly (*Danaus plexippus*) is about 2 weeks compared with 4 weeks for maturation (Oberhauser & Solensky 2004). In addition, within our system, those eggs that are laid later have lower hatching success because the already-emerged larvae eat many of them, mimicking the decline in reproductive success through the breeding season typical of many breeding animals.

FEAR EXPERIMENT

We used Chinese praying mantids (hereafter ‘mantids’) as predators. We tested *Drosophila* with single first-instar mantid that had been feeding on adult *Drosophila* for their entire lives prior to the start of the experiment. To control for age and body size, we

only used individual *Drosophila* between 1 and 3 days old that came from parents that bred at low density. To obtain these offspring, we selected males and females from the stock population and placed 20 individuals (50:50 sex ratio) in separate 28 × 95 mm vials ($n = 50$ vials) with 10 mL of fresh food for 24 h (day 0). It takes a minimum of 8 days for offspring to develop. On day 9, we discarded all offspring and used only individuals that emerged between days 10 and 12. We then combined these offspring and randomly grouped 10 males and 10 females into one of three treatments: ‘control’ ($n = 40$), ‘non-breeding mantid’ ($n = 37$) and ‘breeding mantid’ ($n = 41$).

All treatments began at the start of the non-breeding season and finished at the end of the breeding season. After 4 days in the non-breeding season, we moved the survivors to the breeding season, but controlled density by combining all survivors from each treatment at the end of the non-breeding period and then placed 8 males and 8 females in each breeding vial. Fewer flies were used for the breeding season than the non-breeding season because ~10% of flies died in the non-breeding season and others were frozen and weighed (see below). Because of this, we also had a slightly smaller number of replicates per treatment (‘breeding mantid’ treatment: $n = 36$ replicate vials; ‘non-breeding mantid’ treatment: $n = 37$ replicate vials; ‘control’: $n = 38$ replicate vials) for analyses involving breeding season variables (see below for description of variables).

Non-breeding mantid treatment

For the ‘non-breeding mantid’ treatment, immediately prior to the commencement of the non-breeding season, we placed a first-instar mantid at the bottom of the non-breeding season vial and placed a sponge between the mantid and the flies (Fig. 1). This prevented the mantid from ascending the vial and the flies from seeing the mantid. After 4 days, the mantid and flies were removed and placed into the breeding season. The breeding season was identical between the non-breeding mantid and control treatments (Fig. 1).

Breeding mantid treatment

For the ‘breeding mantid’ treatment, the non-breeding season was identical to the control. After 4 days, the flies were transferred into the breeding season. For the breeding mantid treatment, a sponge was placed in an empty non-breeding vial and the mantid was added in the breeding season vial with fresh fly food 24 h before the survivors from the non-breeding season were transferred to the breeding period (Fig. 1). The breeding season was shorter than the non-breeding season as we attempted to create an ecologically relevant experimental system and breeding seasons are shorter than non-breeding seasons for many wild animals. Consequently, the flies in the breeding season were exposed to the predator for a shorter period than the flies in the non-breeding season, which could also be true for wild animals with longer non-breeding seasons than breeding seasons. A longer breeding season would be unlikely to change the impact of the predator on fecundity as ~80% of eggs are laid within 6 h after the females are exposed to the medium and larvae hatch after 24 h and, as they grow, consume eggs that are subsequently laid. The ‘control’ followed the same protocols as the two treatments (see also next section) but did not have a mantid added in either season (Fig. 1).

Change in body mass and timing of emergence

To investigate the changes in body mass over the non-breeding season, we weighed individual flies from each treatment that were selected before ($n \sim 30$) and after ($n \sim 30$) the non-breeding season. To weigh the flies, we anesthetized them on a CO₂ pad that gently passed carbon dioxide over the flies. Flies were chosen for weighing following anesthetization by mixing all flies and selecting the flies that ended closest to the edge of the CO₂ pad. Flies were frozen for 2 days until there were weighed (Mettler Toledo XP26 scale, Columbus, OH, USA; precision = 0.001 mg; hereafter termed 'body mass'). We counted the number of dead flies at the end of the non-breeding season and termed 'non-breeding mortality' as the proportion that did not survive over the non-breeding season. To investigate the effects of the treatment on reproductive output, we used 'fecundity' (eggs produced) and 'total number of offspring emerged'. We also measured the 'timing of emergence', which we considered as the proportion of total offspring emerged on day 9 (fewer than 0.1% of flies emerged prior to day 9, so this was considered the first day of emergence). We examined three other variables to quantify the stage at which variation in development occurred. We counted the number of eggs every 6 h (excluding 12 h darkness) for the first 24 h (Chippindale *et al.* 1998) and we recorded the number of larvae and pupae every day until all pupae had been produced (day 9). Six hours after the females entered the breeding period, >85% eggs in all treatments had been laid, and so we focused on the value at 6 h when comparing the timing of lay among treatments. We defined 'speed of laying' as the proportion of eggs laid before 6 h, 'speed of hatch' as the proportion of replicates where eggs had hatched at 24 h and 'speed of early development' as the number of pupae present on day 4.

STATISTICAL ANALYSIS

We used one-tailed *t*-tests to compare the effects of the treatment ('non-breeding mantid', 'breeding mantid') with the controls. We conducted *t*-tests for non-breeding mortality, timing of lay, timing of early development and timing of emergence. Means presented in the test are with \pm SE. We tested for normality (Shapiro–Wilks test) and homogeneity of variance (Levene's test) prior to using parametric tests. For those variables with multiple measures per vial (changes in body mass before and after the non-breeding season, fecundity, mass of female offspring 2 days after emergence), we used a linear mixed model with vial as a random effect. All analyses were conducted in R 2.15.1 (R Core Team 2013) with statistical significance set at $\alpha < 0.05$. To avoid the potential for a type I error, we used sequential Bonferroni's corrections separately within each experimental set-up ('non-breeding mantid' and 'breeding mantid'). Because type II errors may be highly prevalent in behavioural ecology (Nakagawa 2004), we assess as 'marginally significant' values that are statistically significant without, but not statistically significant with, the sequential Bonferroni's correction.

Results

There was no difference in body mass among treatments at the start of the non-breeding season (females: $F_{2,63} = 0.33$, $P = 0.72$; males: $F_{2,63} = 0.43$, $P = 0.65$; Fig. 2a). Consequently, flies appeared to be equivalent in size at the onset of the experiments.

NON-BREEDING SEASON EFFECTS OF FEAR

By the end of the non-breeding season, body mass was 60% lower for flies in the 'non-breeding mantid' treatment than in the control (males: $t_{56} = 2.57$, $P = 0.01$; females: $t_{54} = 3.30$, $P = 0.002$; Fig. 2a), suggesting that flies in the 'non-breeding mantid' treatment were in poorer condition upon entering the breeding season. A similar pattern was seen in non-breeding mortality. Non-breeding mortality was 64% higher in the 'non-breeding mantid' treatment compared to the control ($t_{75} = 2.97$, $P = 0.004$; Fig. 2b). There was clear evidence that the non-lethal effect of fear carried over to the breeding season. Both fecundity ($t_{75} = 3.46$, $P < 0.001$; Fig. 2c) and the total number of offspring emerged ($t_{75} = 3.35$, $P = 0.001$; Fig. 2d) were lower for flies in the 'non-breeding mantid' treatment than the control.

There was no difference in the speed of laying (non-breeding mantid: $86 \pm 5\%$; control: $87 \pm 3\%$; $t_{75} = 0.11$, $P = 0.91$) or speed of hatch (none had hatched from either treatment after 24 h) between the 'non-breeding mantid' and control treatments. Likewise, there was no difference in the speed of early development (control: 13.0 ± 1.9 pupae present on day 4; non-breeding mantid: 8.5 ± 1.0 ; $t_{75} = 1.62$, $P = 0.11$) and the timing of emergence ($t_{75} = 1.60$, $P = 0.11$; Fig. 2e) between the control and 'non-breeding mantid' treatment. Finally, the mass of female offspring 2 days after hatch was marginally significantly lower in the 'non-breeding mantid' treatment than the control ($t_{58} = 2.21$, $P = 0.03$; Fig. 2f).

BREEDING SEASON EFFECTS OF FEAR

Body mass at the end of the non-breeding season (males: $t_{56} = 0.34$, $P = 0.78$; females: $t_{54} = 0.96$, $P = 0.28$; Fig. 2a) and survival during the non-breeding season ($t_{74} = 0.11$, $P = 0.90$; Fig. 2b) were similar between the 'breeding mantid' treatment and control, which had been treated the same up to the end of the non-breeding season. There was also no evidence of a within-season effect of fear on reproductive output: flies in the 'breeding mantid treatment' had similar levels of fecundity ($t_{74} = 1.29$, $P = 0.20$; Fig. 2c) and total number of offspring emerged ($t_{74} = 1.23$, $P = 0.22$; Fig. 2d) compared to the control.

Despite the lack of evidence for a within-season effect of fear on fecundity or number of offspring, there was evidence for a within-season effect on the timing of reproduction and size of offspring. There was no difference in the speed of laying between the 'breeding mantid' treatment and control (breeding mantid: $88 \pm 4\%$; control: $87 \pm 3\%$; $t_{74} = 0.20$, $P = 0.84$), but the speed of hatch was earlier in the 'breeding mantid' treatment (Fisher's exact test: $P < 0.001$). Twenty-four hours after the start of the breeding season, larvae had begun emerging in 19% (7/36) of the 'breeding mantid' treatment replicates, but none had emerged in the control. Furthermore, both the speed of early development (breeding mantid:

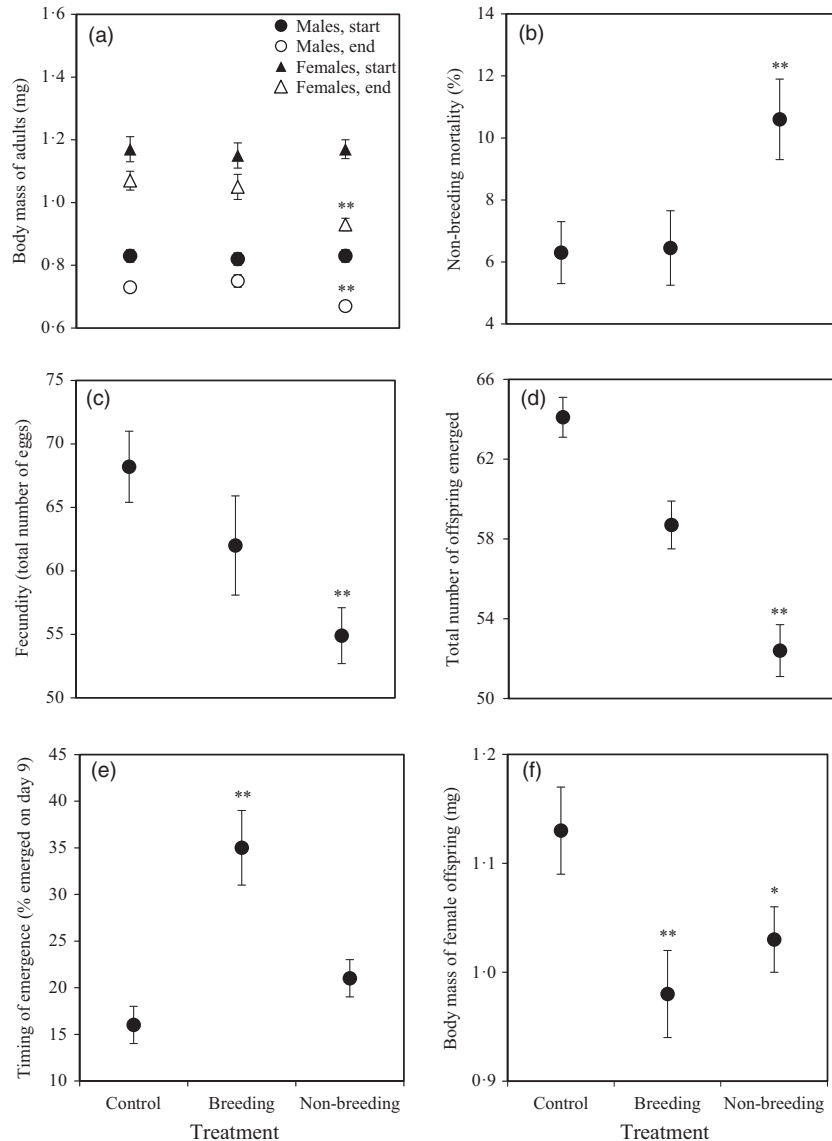


Fig. 2. Mean (\pm SE) of (a) body mass of adults at the start and end of the non-breeding season (b) mortality during the non-breeding season, (c) fecundity (total number of eggs laid), (d) total number of offspring emerged, (e) the timing of emergence and (f) the body mass of offspring in relation to the three treatments: without mantid cues ('control'), with mantid cues during in the breeding season ('breeding mantid') and with mantid cues during the non-breeding ('non-breeding mantid') season. Treatments with a double asterisk are significantly different from the control ($\alpha = 0.05$ with sequential Bonferroni's correction) while treatments with a single asterisk are marginally significantly different from the control ($\alpha = 0.05$ without sequential Bonferroni's correction).

19.5 ± 1.9 pupae present on day 4; control: 13.0 ± 1.9 ; $t_{74} = 2.71$, $P = 0.008$) and the timing of emergence ($t_{74} = 3.20$, $P = 0.002$; Fig. 2e) were earlier in the 'breeding mantid' treatment than the control. Finally, the mass of female offspring 2 days after hatch was lower in the 'breeding mantid' than the control ($t_{58} = 3.15$, $P = 0.002$; Fig. 2f).

Discussion

THE EFFECT OF FEAR IN THE NON-BREEDING SEASON

Our results provide evidence that the presence of a predator can not only have important consequences on individuals within a season but also carry over to impact fitness in subsequent seasons. The presence of a hidden mantid during the non-breeding season increased mortality and decreased body mass over the non-breeding period when

compared to controls. Although we have no direct behavioural evidence, these results from the non-breeding season suggest that individuals were likely more stressed and may have spent less time actively foraging when exposed to the mantid cue, possibly because they were more vigilant, or may have assimilated food less effectively (Duvall & Williams 1995; Lima 1998; Stoks 2001). Importantly, among those that survived the non-breeding season, flies exposed to the mantid cue had lower fecundity, as well as fewer and lighter offspring the following breeding season, providing experimental evidence that fear of predators is a mechanism that can drive seasonal carry-over effects.

We have argued that the presence of the mantid during the non-breeding period caused flies to feed less or assimilate less food due to stress and, therefore, be in poorer condition by the time they entered the breeding season (Boonstra *et al.* 1998; Stoks 2001; McPeck 2004). In particular, under the threat of predation, animals are not as efficient at converting their food into their own biomass

because stress affects metabolic processes (e.g. Duvall & Williams 1995; Stoks 2001; McPeck 2004). However, an alternative explanation, given that we did not use a longitudinal approach and the same individuals were not weighed at the beginning and end of the non-breeding period, is that there was selection for smaller flies over this time period (Betini *et al.* 2014). We argue that such selection is unlikely for two reasons. First, we attempted to control for initial size of the flies entering the non-breeding season, which means that there was likely little variation for selection to act upon. Secondly, the average weight of a female fly at the start of the non-breeding season was 1.17 mg (Fig. 2a) but, by the end of the non-breeding season in the 'non-breeding mantid' treatment, <6% of female flies were above 1.17 mg (see DRYAD Data Appendix) despite only ~10% non-breeding mortality (Fig. 2b). Even if all of the 10% heaviest female flies died in the sample of flies weighed at the start of the non-breeding season, then average weight would only decrease by 0.06 mg, whereas we observed a decrease of 0.24 mg between the start and end of the non-breeding season and a difference of 0.14 mg between the non-breeding mantid and control treatments at the end of the non-breeding season. The same is likely to be true for males. There were only four of 30 males that weighed <0.75 mg at the start of the non-breeding season while only five of 30 males weighed more than 0.75 mg at the end of the non-breeding season. Given that mortality averaged about 10%, clearly differential mortality of male flies cannot explain the large difference in body mass over the non-breeding season and individual flies must have lost mass. We, therefore, argue that the difference in wet weight primarily represents individual mass loss rather than differential selection for small flies. Indeed, mortality is low across all treatments, and differences in responses likely represented within-individual changes. Thus, although our study was not strictly longitudinal, it seems unlikely that differential mortality affected our results.

Whereas most studies focus on a single season, usually breeding (Marra *et al.* 2015), our study adds to the growing consensus that seasonal carry-over effects can impact fitness and population dynamics (e.g. Morrisette *et al.* 2010; Legagneux *et al.* 2012; Betini, Griswold & Norris 2013a,b). Whereas other authors have suggested that reduced female body mass due to fear in the non-breeding season can cause reduced body condition at the onset of breeding and a reduction in fecundity, we are the first to directly demonstrate this effect experimentally. For instance, a classic example of fear impacting population size is the snowshoe hare (*Lepus americanus*), whose population cycles are partly linked to female stress during high-predator years, which leads to reduced reproductive output (Stefan & Krebs 2001; Sheriff, Krebs & Boonstra 2009, 2011). Our experiments imply that perceived predation risk in the previous winter may be as, or even more, important than perceived risk at the time of breeding because of the delayed, negative effects fear can have on

condition. As a second example, many birds have declined over the past 50 years during a period of increasing predator (raptor) populations in response to reduced toxic contamination. Increased falcon populations in the non-breeding season disturb large flocks of shorebirds, which have been linked to the observed decline in shorebird population size (Lank *et al.* 2003; Ydenberg *et al.* 2004). It has been suggested that the primary vital rate linking predator presence with shorebird declines is through a direct effect of increase mortality on the non-breeding grounds where predators are present in increasing numbers (Lank *et al.* 2003; Ydenberg *et al.* 2004). However, it is also possible that, as our results show, an increase in predators during the non-breeding period may affect condition and carry over to influence reproductive success. In migratory birds, the full effects of fear may, therefore, only be realized thousands of kilometres away on the breeding grounds. Across a wide range of taxa, fear is a potential mechanism for cross seasonal carry-over effects to shape fitness and ultimately population dynamics.

THE EFFECT OF FEAR IN THE BREEDING SEASON

Rather than having a direct influence on reproductive output, the mantid cue in the breeding season caused females to produce larvae that developed faster than controls – as occurs in some other invertebrates (e.g. Benard 2004). Lighter offspring emerging from the non-breeding treatment were likely produced because adults were breeding in poor condition as there is strong evidence in *Drosophila* and other insects that larger females produce larger eggs and that larger eggs develop into larger adults (e.g. Reavey 1992; Fox 1993, 1994; Azevedo, Partridge & French 1997). In contrast, the lighter offspring emerging from the breeding treatment were produced by females in similar condition to the females in the control treatment and presumably resulted from the faster development time. This is also true for other species. For instance, female condition often does not influence offspring condition in snowshoe hares (e.g. Sheriff, Krebs & Boonstra 2009, 2011). The quicker development time was not because females laid sooner, as there was no difference in laying times, or because larvae developed quicker at later stages, as the difference in timing of cocooning was similar to the difference in larval emergence time. Rather, we suggest that the quicker development was either due to direct sensing of the mantid in the environment at the egg or early larvae stage, or due to a maternal effect, perhaps via stress hormones, on development. In several species, larvae that develop in the presence of predators have lower growth rates and smaller adult body sizes (Scrimgeour & Culp 1994; Stoks 2001; Benard 2004). In some cases, lower adult body size is associated with lower growth rates due to the mobilization of resources to create protective armour or appendages (Lardner 2000; Dahl & Peckarsky 2002). As this does not apply to *Drosophila*, we argue that faster growth rates during development are an adaptive

response to reduce the amount of time at the egg and larval stage. However, this response appears to come with a potential cost in the form of smaller adult body size.

Interestingly, although we did not measure this directly, the within-season effect arising from the presence of the mantid in the breeding season is also likely to cause a carry-over effect into subsequent life stages. In the context of our simulated seasonal environment, this carry-over effect would have a positive effect on survival, as smaller flies have higher rates of survival during the non-breeding period (Betini *et al.* 2014). However, there is likely also a negative carry-over effect of fear in the following breeding period because smaller flies are known to have lower reproductive rates (Mueller & Joshi 2000). Lower reproductive rates of *Drosophila* dampened fluctuations in population size between seasons, creating more stable long-term dynamics (Betini *et al.* 2014).

Regardless, the faster development and lower offspring size implies that there are possibly beneficial maternal effects derived from predator-induced stress. There is growing awareness that environmental stressors, including predators, can induce mothers to adaptively programme their offspring's stress axis (Meylan & Clobert 2005; Meylan, Miles & Clobert 2012; Love, McGowan & Sheriff 2013; Sheriff & Love 2013). In our case, in response to mantid cues, female flies may have increased the stress axis of their offspring, leading to more rapid growth. In a stressful, high-predator environment, an elevated stress response may improve survival (Cabezas *et al.* 2007; Sheriff & Love 2013). Consequently, quicker development, lower body mass and a higher stress response may be a beneficial, adaptive response to the predator cue that would enhance fitness in a high-predator environment.

Although we have shown that the breeding mantid treatment produced a smaller effect on reproductive output than the non-breeding mantid treatment, it is possible that this could be because the mantids were actually present during the non-breeding season treatment and therefore release more chemical cues. In contrast, for the breeding mantid treatment, mantids were only present in the vial for 24 h and then removed before the flies entered the vial. However, assuming that the amount of mantid cue is proportional to the surface area of the predator (Kats & Dill 1998), we found no evidence that more mantids (two-first-instar mantids for the treatment instead of one) or larger mantids (third instar) resulted in differences in either the timing of emergence (within-season effect) or the number of offspring emerged (carry-over effect; see electronic supplementary material for details of the experiments). Thus, we suggest that the flies were likely at the maximum threshold response, and therefore, any potential differences in the response of flies between seasonal treatments were likely not due to potential differences in the amount of the predator cue. The duration of cue was longer in the non-breeding than the breeding season, which is likely true in most systems as non-breeding

seasons are typically longer than breeding seasons. A breeding season >24 h, however, does not increase fecundity in our study system, as we demonstrated that most eggs are laid in the first 6 h and larvae hatch after ~24 h, and consume most eggs laid after that period.

We provide a simple, albeit artificial, experimental system for examining the non-consumptive effects of predators ('fear') across seasons. Although the main trait distinguishing the breeding from non-breeding season is that the flies were unable to breed in the non-breeding seasons, we believe our system also captured many of the important traits of animals living in seasonal environments in the wild (Betini, Griswold & Norris 2013a,b; Betini *et al.* 2014). Animals living seasonal environments, particularly if they are migratory, are likely to experience very different landscapes of fear among seasons (Peckarsky *et al.* 2008; Sheriff, Krebs & Boonstra 2011; Laundré *et al.* 2014). However, as is the case in our study, the adult breeding season is typically shorter than the non-breeding season, food availability is often higher in the breeding season than the non-breeding season and breeding success usually declines over the course of the breeding season. The presence of a hidden predator (non-breeding season) or predator scent (breeding season) allowed us to examine non-consumptive effects. In wild animals, fear is most pronounced when a scent leads to a prey suspecting a potential ambush (Stauffer & Semlitsch 1993; Kats & Dill 1998; Hartman & Abrahams 2000), and consequently our system is likely typical of many scenarios in the wild.

In conclusion, the presence of a mantid in the non-breeding season impacted fecundity in the breeding season, whereas the presence of a mantid during the breeding season impacted growth rates. Thus, we observed carry-over effects across seasons in adulthood, as well as across development stages. The perception of predation risk itself could be enough to influence population size through carry-over effects. Because the mechanism appeared to be different for the non-breeding mantid and breeding mantid treatment, the net effect of having a predator present in both seasons would likely be additive – but we did not directly test this hypothesis with our experimental design. Regardless, in many species, including non-migratory animals, fear could be an important mechanism that links events across the annual cycle to shape fitness, as well as population dynamics.

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Data accessibility

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.8p0g8> (Elliott et al. 2015).

References

- Azevedo, R.B., Partridge, L. & French, V. (1997) Life-history consequences of egg size in *Drosophila melanogaster*. *American Naturalist*, **150**, 250–282.
- Beckerman, A.P., Uriarte, M. & Schmitz, O.J. (1997) Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. *Proceedings of the National Academy of Sciences*, **94**, 10735–10738.
- Benard, M.F. (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. *Annual Review of Ecology, Evolution and Systematics*, **35**, 651–673.
- Betini, G.S., Griswold, C.K. & Norris, D.R. (2013a) Carry-over effects, sequential density dependence and the dynamics of populations in a seasonal environment. *Proceedings of the Royal Society of London B: Biological Sciences*, **280**, 20130110.
- Betini, G.S., Griswold, C.K. & Norris, D.R. (2013b) Density-mediated carry-over effects explain variation in breeding output across time in a seasonal population. *Biology Letters*, **9**, 20130582.
- Betini, G.S., Griswold, C.K., Prodan, L. & Norris, D.R. (2014) Body size, carry-over effects and survival in a seasonal environment: consequences for population dynamics. *Journal of Animal Ecology*, **83**, 1313–1321.
- Boonstra, R., Hik, D., Singleton, G.R. & Tinnikov, A. (1998) The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs*, **68**, 371–394.
- Brown, J.S., Laundré, J.W. & Gurung, M. (1999) The ecology of fear: optimal foraging, game theory, and trophic interactions. *Journal of Mammalogy*, **80**, 385–399.
- Buck, C.L. & Barnes, B.M. (1999) Annual cycle of body composition and hibernation in free-living arctic ground squirrels. *Journal of Mammalogy*, **80**, 430–442.
- Cabezas, S., Blas, J., Marchant, T.A. & Moreno, S. (2007) Physiological stress levels predict survival probabilities in wild rabbits. *Hormones and Behavior*, **51**, 313–320.
- Chippindale, A.K., Gibbs, A.G., Sheik, M., Yee, K.J., Djawdan, M., Bradley, T.J. et al. (1998) Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. *Evolution*, **52**, 1342–1352.
- Christian, J.J. (1950) The adreno-pituitary system and population cycles in mammals. *Journal of Mammalogy*, **31**, 247–259.
- Clinchy, M., Sheriff, M.J. & Zanette, L.Y. (2013) Predator-induced stress and the ecology of fear. *Functional Ecology*, **27**, 56–65.
- Creel, S. & Christianson, D. (2008) Relationships between direct predation and risk effects. *Trends in Ecology and Evolution*, **23**, 194–201.
- Creel, S., Winnie, J.A. & Christianson, D. (2009) Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. *Proceedings of the National Academy of Sciences*, **106**, 12388–12393.
- Dahl, J. & Peckarsky, B.L. (2002) Induced morphological defenses in the wild: predator effects on a mayfly, *Drunella coloradensis*. *Ecology*, **83**, 1620–1634.
- Dielenberg, R.A. & McGregor, I.S. (2001) Defensive behavior in rats towards predatory odors: a review. *Neuroscience & Biobehavioral Reviews*, **25**, 597–609.
- Dixon, A.F.G. & Agarwala, B.K. (1999) Ladybird-induced life-history changes in aphids. *Proceedings of the Royal Society of London B*, **266**, 1549–1553.
- Duvall, C.J. & Williams, D.D. (1995) Individuality in the growth of stonefly nymphs in response to stress from a predator. *Archiv für Hydrobiologie*, **133**, 273–286.
- Eggers, S., Griesser, M., Nystrand, M. & Ekman, J. (2006) Predation risk induces changes in nest-site selection and clutch size in the Siberian jay. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 701–706.
- Elliott, K.H., Betini, G., Dworkin, I. & Norris, D.R. (2015) Data from: experimental evidence for within- and cross-seasonal effects of fear on survival and reproduction. *Dryad Digital Repository*, <http://dx.doi.org/10.5061/dryad.8p0g8>.
- Fox, C.W. (1993) The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia*, **96**, 139–146.
- Fox, C.W. (1994) The influence of egg size on offspring performance in the seed beetle, *Callosobruchus maculatus*. *Oikos*, **71**, 321–325.
- Greenberg, R. & Marra, P.P. (2005) *Birds of Two Worlds: the Ecology and Evolution of Migration*. John Hopkins University Press, Baltimore, MD, USA.
- Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. & Bearhop, S. (2011) Carry-over effects as drivers of fitness differences in animals. *Journal of Animal Ecology*, **80**, 4–18.
- Hartman, E.J. & Abrahams, M.V. (2000) Sensory compensation and the detection of predators: the interaction between chemical and visual information. *Proceedings of the Royal Society of London B: Biological Sciences*, **267**, 571–575.
- Hubbard, D.T., Blanchard, D.C., Yang, M., Markham, C.M., Gervacio, A., Chun, L. et al. (2004) Development of defensive behavior and conditioning to cat odor in the rat. *Physiology & Behavior*, **80**, 525–530.
- Hurd, L.E. & Rathet, I.H. (1986) Functional response and success in juvenile mantids. *Ecology*, **67**, 163–167.
- Kats, L.B. & Dill, L.M. (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience*, **5**, 361–394.
- Kavaliers, M. & Choleris, E. (2001) Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neuroscience & Biobehavioral Reviews*, **25**, 577–586.
- Kenagy, G.J., Sharbaugh, S.M. & Nagy, K.A. (1989) Annual cycle of energy and time expenditure in a golden-mantled ground squirrel population. *Oecologia*, **78**, 269–282.
- Lank, D.B., Butler, R.W., Ireland, J. & Ydenberg, R.C. (2003) Effects of predation danger on migration strategies of sandpipers. *Oikos*, **103**, 303–319.
- Lardner, B. (2000) Morphological and life history responses to predators in larvae of seven anurans. *Oikos*, **88**, 169–180.
- Laundré, J.W., Hernández, L. & Altendorf, K.B. (2001) Wolves, elk, and bison: reestablishing the “landscape of fear” in Yellowstone National Park, USA. *Canadian Journal of Zoology*, **79**, 1401–1409.
- Laundré, J.W., Hernández, L., Medina, P.L., Campanella, A., López-Portillo, J., González-Romero, A. et al. (2014) The landscape of fear: the missing link to understand top-down and bottom-up controls of prey abundance? *Ecology*, **95**, 1141–1152.
- Legagneux, P., Fast, P.L., Gauthier, G. & Bêty, J. (2012) Manipulating individual state during migration provides evidence for carry-over effects modulated by environmental conditions. *Proceedings of the Royal Society of London B: Biological Sciences*, **279**, 876–883.
- Li, D. (2002) Hatching responses of subsocial spitting spiders to predation risk. *Proceedings of the Royal Society of London B: Biological Sciences*, **269**, 2155–2161.
- Lima, S. (1998) Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Advances in the Study of Behavior*, **27**, 215–290.
- Love, O.P., McGowan, P.O. & Sheriff, M.J. (2013) Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. *Functional Ecology*, **27**, 81–92.
- Magnhagen, C. (1990) Reproduction under predation risk in the sand goby, *Pomatoschistus minutus*, and the black goby, *Gobius niger*: the effect of age and longevity. *Behavioral Ecology and Sociobiology*, **26**, 331–335.
- Marra, P.P., Cohen, E.B., Loss, S.R., Rutter, J.E. & Tonra, C.M. (2015) A call for full annual cycle research in animal ecology. *Biology Letters*, **11**, 20150552.
- Matassa, C.M. & Trussell, G.C. (2011) Landscape of fear influences the relative importance of consumptive and nonconsumptive predator effects. *Ecology*, **92**, 2258–2266.
- McCauley, S.J., Rowe, L. & Fortin, M.J. (2011) The deadly effects of ‘non-lethal’ predators. *Ecology*, **92**, 2043–2048.
- McNamara, J.M. & Houston, A.I. (1987) Starvation and predation as factors limiting population size. *Ecology*, **68**, 1515–1519.
- McPeck, M.A. (2004) The growth/predation risk trade-off: so what is the mechanism? *American Naturalist*, **163**, E88–E111.
- Meylan, S. & Clobert, J. (2005) Is corticosterone-mediated phenotype development adaptive? Maternal corticosterone treatment enhances survival in male lizards. *Hormones and Behavior*, **48**, 44–52.
- Meylan, S., Miles, D.B. & Clobert, J. (2012) Hormonally mediated maternal effects, individual strategy and global change. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **367**, 1647–1664.
- Morrisette, M., Bêty, J., Gauthier, G., Reed, A. & Lefebvre, J. (2010) Climate, trophic interactions, density dependence and carry-over effects on

- the population productivity of a migratory Arctic herbivorous bird. *Oikos*, **119**, 1181–1191.
- Mueller, L.D. & Joshi, A. (2000) *Stability in Model Populations*, Vol. 31. Princeton University Press, Princeton, NJ, USA.
- Nakagawa, S. (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behavioral Ecology*, **15**, 1044–1045.
- Norris, D.R. & Taylor, C.M. (2006) Predicting the consequences of carry-over effects for migratory populations. *Biology Letters*, **2**, 148–151.
- Oberhauser, K.S. & Solensky, M.J. (2004) *Monarch Butterfly Biology & Conservation*. Cornell University Press, Ithaca, NY, USA.
- O'Connor, C.M., Norris, D.R., Crossin, G.T. & Cooke, S.J. (2014) Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere*, **5**, art28.
- Parigi, A., Porter, C., Cermak, M., Pitchers, W.R. & Dworkin, I. (2015) How predator hunting-modes affect prey behaviour: capture deterrence in *Drosophila melanogaster*. *bioRxiv* <http://biorxiv.org/content/early/2014/11/19/010330>
- Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbegg, B. *et al.* (2008) Revisiting the classics: considering non-consumptive effects in textbook examples of predator-prey interactions. *Ecology*, **89**, 2416–2425.
- Pohl, R. (1987) *The Praying Mantid*. Raintree Books, New York, NY, USA.
- Preisser, E.L. & Bolnick, D.I. (2008) The many faces of fear: comparing the pathways and impacts of nonconsumptive predator effects on prey populations. *PLoS ONE*, **3**, e2465.
- Preisser, E., Bolnick, D. & Benard, M. (2005) The high cost of fear: behavioral effects dominate predator-prey interactions. *Ecology*, **86**, 501–509.
- R Core Team. 2013. *R: A Language and Environment For Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Reavey, D. (1992) Egg size, first instar behaviour and the ecology of Lepidoptera. *Journal of Zoology*, **227**, 277–297.
- Roitberg, B.D., Myers, J.H. & Frazer, B.D. (1979) The influence of predators on the movement of apterous pea aphids between plants. *Journal of Animal Ecology*, **48**, 111–122.
- Schmitz, O.J. (2008) Effects of predator hunting mode on grassland ecosystem function. *Science*, **319**, 952–954.
- Scrimgeour, G.J. & Culp, J.M. (1994) Foraging and evading predators: the effect of predator species on a behavioural trade-off by a lotic mayfly. *Oikos*, **69**, 71–79.
- Sheriff, M.J., Krebs, C.J. & Boonstra, R. (2009) The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology*, **78**, 1249–1258.
- Sheriff, M.J., Krebs, C.J. & Boonstra, R. (2011) From process to pattern: how fluctuating predation risk impacts the stress axis of snowshoe hares during the 10-year cycle. *Oecologia*, **166**, 593–605.
- Sheriff, M.J. & Love, O.P. (2013) Determining the adaptive potential of maternal stress. *Ecology Letters*, **16**, 271–280.
- Siepielski, A.M., Prince, G. & Wang, J. (2014) Non-consumptive predator induced phenotypic selection. *Evolution*, **68**, 696–704.
- Smith, J.A., Wang, Y. & Wilms, C.C. (2015) Top carnivores increase their kill rates on prey as a response to human-induced fear. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20142711.
- Sorensen, M.C., Hipfner, J.M., Kyser, T.K. & Norris, D.R. (2009) Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. *Journal of Animal Ecology*, **78**, 460–467.
- Stauffer, H.P. & Semlitsch, R.D. (1993) Effects of visual, chemical and tactile cues of fish on the behavioural responses of tadpoles. *Animal Behaviour*, **46**, 355–364.
- Stefan, C.I. & Krebs, C.J. (2001) Reproductive changes in a cyclic population of snowshoe hares. *Canadian Journal of Zoology*, **79**, 2101–2108.
- Stoks, R. (2001) Food stress and predator-induced stress shape developmental performance in a damselfly. *Oecologia*, **127**, 222–229.
- Wirsing, A.J. & Ripple, W.J. (2010) A comparison of shark and wolf research reveals similar behavioral responses by prey. *Frontiers in Ecology and the Environment*, **9**, 335–341.
- Ydenberg, R.C., Butler, R.W., Lank, D.B., Smith, B.D. & Ireland, J. (2004) Western sandpipers have altered migration tactics as peregrine falcon populations have recovered. *Proceedings of the Royal Society of London B*, **271**, 1263–1269.
- Ylönen, H. (1989) Weasels *Mustela nivalis* suppress reproduction in cyclic bank voles *Clethrionomys glareolus*. *Oikos*, **55**, 138–140.
- Zanette, L.Y., White, A.F., Allen, M.C. & Clinchy, M. (2011) Perceived predation risk reduces the number of offspring songbirds produce per year. *Science*, **334**, 1398–1401.

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Supporting Information

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

Data S1. Testing the effect of the amount of mantid cue.