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Growth, development, and life-history strategies in an unpredictable environment: case study of a rare hoverfly *Blera fallax* (Diptera, Syrphidae)

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Abstract. 1. Development in organisms can vary in response to fluctuating environments. In holometabolous insects, variation in adult phenotypic traits is strongly influenced by growth conditions experienced by larvae. The main aim of this study was to assess how much environmental insight can be gained from analysis of the phenotypic changes in an insect's life history parameters in response to realistic food limitations.

2. This investigation was motivated by a need for more information about the developmental requirements of the endangered pine hoverfly *Blera fallax* (Linnaeus) (Diptera, Syrphidae) in Scotland. *Blera fallax* depends on a scarce and often ephemeral habitat, rot holes of Scots pine *Pinus sylvestris* L. stumps. We studied how rearing conditions affected growth in captive larvae, and compared these responses with a wild population.

3. The growth curve observed in the field was similar to that in resource-limited, lab conditions, suggesting that resources are limiting in nature. The effects of resource availability on development time and body size depended on sex. Adult females were larger but had more variable size at maturity compared with males. In contrast, males typically were not smaller in resource-limited conditions, but rather continued to develop for another year. Between 2% and 20% of larvae extended development over 2 years regardless of growth conditions, perhaps indicating a semivoltine strategy to circumvent extinction during years with a low breeding success.

4. These results identify life history traits that may be important for other saproxylic Diptera in rot holes, and organisms that experience food restrictions during growth.

Key words. *Ex-situ* conservation, larval growth, *Pinus sylvestris*, rot hole, semivoltine, starvation.

Introduction

Organisms are capable of adaptively moderating physiological processes (such as growth) to correspond to certain environmental variables (Taylor, 1981; Schmidt-Nielsen, 1997; Blanckenhorn, 1999). One such process involves coping with unpredictable food supplies via trade-offs between growth and longevity (Blanckenhorn, 1998; Stillwell *et al.,* 2010; Hou *et al.,* 2011). In holometabolous insects, growth is restricted to the larval stages (Abrams *et al.,* 1996) and in seasonal environments, larvae may diapause which interrupts growth (Smith & Smith, 2006). Diapause exhibits plasticity according to resource conditions (Abrams *et al.,* 1996). Typically, a trade-off exists between spending more time in the larval stage (to increase adult size and thereby, increase mating success or fecundity Dmitriew, 2011) and adult longevity (which, because of extended larval development, may be restricted especially in seasonal environments). Often size has a closer association with fitness than development time does, so many animals delay sexual maturation to increase in size despite the costs (Reznick, 1990). However, when conditions are poor individuals may sometimes initiate diapause to make the 'best of a bad job': their eventual fitness may still be lower than animals that developed to adulthood more rapidly. The relationships between environmental resources, growth, and diapause remain poorly understood for many taxa, especially rare non-model organisms.

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Fig. 1. *Blera fallax* at the adult (a) and larval stage (b), and an example of the habitat of the larval stage: a rot hole in the surface of a pine stump (c).

In this paper, we investigate growth in larvae of the pine hoverfly, *Blera fallax* (Linnaeus) (Diptera, Syrphidae) (Fig. 1). *Blera fallax* is listed in the UK red data book as category 1 (endangered), it is a biodiversity action plan priority species and is 1 of 32 species in the Scottish Natural heritage species action framework list (Scottish Natural Heritage, 2007). *Blera fallax* is saproxylic with larvae developing on microbes associated with decaying whitewood in water pockets or rot holes of stumps of Scots Pine, *Pinus sylvestris* L. (Fig. 1) and larvae face winter at least once in their development (Rotheray & MacGowan, 2000). To conserve this species, one strategy under scrutiny is creating an artificial breeding habitat at historical sites into which captive-bred larvae are released (Rotheray, 2010, 2012). Providing additional rot-holes should improve the numbers of breeding sites and bring habitats in line with continental forests (e.g. in Finland), where rot-holes are more numerous. For a supplementing breeding habitat to be successful, we need to know what factors affect larval fitness. We, therefore, investigated growth trajectories of larvae in response to manipulated conditions, including characteristics of the larval substrate and the presence and intensity of intra-specific competition. In addition, rot hole inhabiting syrphid larvae occasionally suffer from filamentous fungal growth on the integument, with likely negative consequences for survival and health. We tested the effect of applying Nipagen, a standard antifungal agent in experiments involving *Drosophila* (e.g. Tinsley *et al.,* 2006) on growth and survival of *B. fallax* larvae.

In rot hole mosquito larvae, the effects of competition can be moderated by Allee effects i.e. larval activity promotes bacterial growth through grazing, which means that larvae with low densities of inter-specific competitors outperform those living in isolation (Kaufman *et al.,* 1999). However, most studies find competition has a negative effect on filter-feeding larvae (Knight *et al.,* 2004). Our investigation concerns how substrate conditions affect larval development and the relative importance of intra-specific competition versus Allee effects on larval fitness. We altered growth conditions and density for larvae and measured the response in terms of larval growth, fat deposition, survival, development time, and pupal and adult size. Furthermore, because *B. fallax* larvae exploit a fluctuating and heterogeneous source of food, we expect adjustments to the timing and occurrence of diapause according to the available resources. We expect larval growth to increase with increased substrate surface area to water volume (which may promote microbe density). We further predict that intra-specific competition will constrain larval growth under limited resource conditions. Finally, we expect larvae in sub-optimal conditions to have longer development times, but emerge at comparable sizes to those not so limited, especially in males, owing to presumed advantages of achieving a large size over early emergence (Rotheray, 2012). To the best of our knowledge, this is the first study of its kind to address the effects of food restriction and competition in hoverflies.

Materials and methods

Larvae were collected from a natural population in Strathspey, Scotland, U.K. (57°18′N, 3°39′W) and reared and bred in captivity between October 2008 until November 2010. Larvae were used for three main experiments. Conservation management for *B. fallax* includes making holes in pine stumps and filling them with chips of pine wood and sawdust to imitate similar to natural conditions in rot holes (Rotheray, 2012). Consequently, we first wished to investigate simultaneously the effects of particle size (wood chip/less surface area versus sawdust/greater surface area to water volume) and the application of Nipagen.

In a second experiment, using the now understood optimum conditions for larval growth, we assessed the effects of intra-specific competition in low and high volumes of sawdust and water. From the onset of each experiment until eclosion, we monitored growth and survival at equal time intervals and measured several adult traits upon emergence.

Finally, *B. fallax* appears to be a pine specialist in Scotland, but as they occur in other conifer hosts in Europe, we wanted to know whether alternate species could support larvae, broadening options for future habitat creation, and whether conifer was important for developing larvae by using a common, broadleaf deciduous tree as a third rot hole host. From the onset of each experiment until eclosion, we monitored growth and survival at equal time intervals and measured several adult traits upon emergence. The experiments were run over 2 years.

All lab experiments were carried out using 250-ml glass bottle microcosms with foam stoppers and pine bark ladders ($9 \times 3 \times 1$ cm^3), to allow larvae to adhere to and crawl closer to the surface to breathe. Moss plugs were situated at the mouth of the microcosms for larvae to crawl into upon exiting the water to pupate. Microcosms were filled with pine chips or sawdust and bottled water (Highland Spring Ltd, Blackford, U.K.). These were kept in climate-controlled facilities at temperatures and photoperiods corresponding with those they would experience naturally in North Central Scotland (average max/min 7 °C/3 °C October until March, 17 °C/8 °C April until September: see table 1 in Rotheray, 2013). Larvae were selected based on size (where possible <7 mm body length, which corresponds to the first or early second instar) and randomly assigned to treatments for each experiment.

To measure larval and pupal area and adult traits, we placed individuals on laminated lined paper for scale, captured a digital image, and estimated the two-dimensional area of the larvae and length of adult trait using ImageJ software (Abràmoff *et al.,* 2004). In Experiment 2, after transferring larvae to filter paper to remove excess water, the mass was also measured on a 0.001-g resolution balance. Adult size traits included the length of the thorax from the point at which the neck meets the prothorax to the apex of the scutellum, the length between two wing veins (landmarks 1 and 3 in Milankov *et al.,* 2010). The development time was assessed as the number of days from the start of the experiment until the day of eclosion.

Experiment 1: effect of pine woodchip size and Nipagen

A replicate (×40) experiment included the following four treatments: sawdust (hereafter abbreviated as treatment S) (50 ml), chips (treatment C) (50 cm^3), sawdust and chips (treatment CS) (25 ml + 25 cm^3), and sawdust (50 ml) plus 0.5 ml (0.35%) Nipagen (treatment NS), with one larva inhabiting each microcosm. We chose substrate volumes based on data from a previous study (Rotheray, 2013). Five millilitres of water were added to each microcosm every few months to replace losses owing to evaporation. We took 17 larval area measurements between 30 August 2009 and 14 July 2010. The measurement interval was increased to 30 days between November 2009 and January 2010, but otherwise measurements were taken at 15-day intervals.

Blera fallax larvae are almost transparent during growth and development, but they become opaque with white fat as they overwinter or pupate. We predicted that in good conditions fat development would occur sooner, leading to early diapause. Fat deposition over time was assessed photometrically (for C, CS, S, and NS treatments) by estimating the proportion of the total surface area of larvae under which the fat body extended.

Larval growth field comparison. We collected field growth data to study the 'natural' *B. fallax* larval growth trajectory over time. Between July and November 2009, 27 artificially bored pine rot holes containing *B. fallax* larvae were located in the field. These were visited five times in 30-day intervals, and all larvae located in the stumps were photographed and measured for the larval area. No manipulation was carried out in the field, and as a result of the large number of confounding variables, particularly the inability to track individual larvae, formal statistical comparisons between field data and *in situ* experiments were not possible. Instead, we visually compared the patterns of change in morphology in the field with those observed in each of our lab experimental treatments.

Experiment 2: effect of intra-specific competition

A replicated (×20) $2 \times 2 \times 3$ full factorial experiment was carried out in 2010 with a new generation of *B. fallax* larvae, which included two substrate levels (low = 40 ml and high = 80 ml pine sawdust), two water levels (low = 70 ml and high = 140 ml), and three larval densities (1, 2 and 3 larvae) (Table S1). We were unable to explore higher densities of competitors because of the limited number of larvae available, however, a previous study demonstrated that 40 ml of sawdust is the minimum substrate volume required for optimum growth of a single larva (Rotheray, 2013). To track possible maternal effects, larvae sharing a microcosm were from the same brood, i.e. were siblings through the maternal line, from 1 of 13 dams. Four weight and area measurements were taken at 15-day intervals between 18 September and 10 November 2010.

Experiment 3: effect of tree species

The effect of tree species on larval growth was studied in a replicate (×20) experiment including 50 ml of sawdust per microcosm of Pine *P. sylvestris*, Birch *Betula pubescens* Ehrh. and Spruce *Picea abies* L. One larva was added to each microcosm and three area and weight measurements were taken at 15-day intervals from September until November 2010.

Statistical analysis

All statistical analyses were carried out using R statistical software (version 2.13.1) (R Team, 2011).

Fitting growth curves. We used the R packages nlme (Pinheiro *et al.,* 2015) and nlstools (Baty *et al.,* 2015) to fit von Bertalanffy (vB) growth functions with parameters L0, size at hatching/birth; L∞, mean asymptotic size, and K, a growth rate constant. Please see Appendix S1 for a detailed description of these, and this analysis. This approach was used to compare larval growth across treatments in Experiments 1, 2, and 3. In addition, the data in each treatment were split by sex to assess sex differences in growth. We recorded both mass and larval area in the second experiment, but as the two variables were strongly collinear ($R = 0.97$), and the model outcome did not depend on the response used, we report only models based on larval mass below for simplicity.

Fat deposition over time. The fat deposition was a binomial response (the proportion of larval area that was opaque). We initially attempted to fit generalised linear models with binomial errors to model this response (using R package lme4; Bates

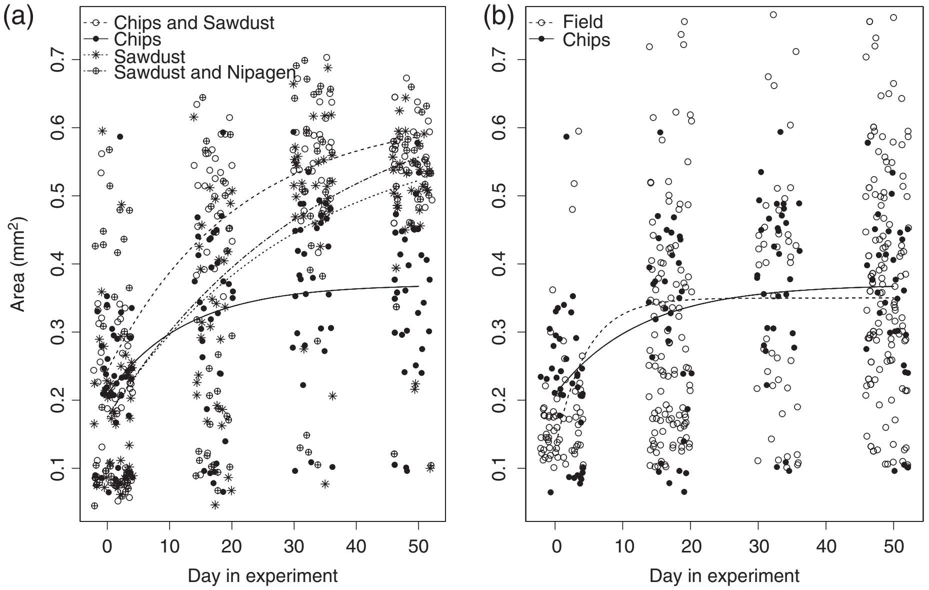

Fig. 2. *Blera fallax* larval growth curves implemented by the von Bertalanffy growth function fitted by non-linear fixed effects models, and 'jittered' point data to illustrate the differences in pine wood treatments (Experiment 1): Chips and Sawdust, Chips, Sawdust, and Sawdust plus Nipagen fungicide treatment (a) and comparing Chips and field growth data (b).

et al., 2014), but we could not get our models to converge on parameter estimates in spite of several attempted transformations. Consequently, we used non-parametric Kruskal–Wallis tests to confirm between-treatment fat deposition differences 2 weeks before (day 49, 20th October) and after (day 230, 29th April) larvae became completely opaque with fat.

Survival analysis. Survival analysis, assessing only pre-adult mortality, was carried out up until day 300 in Experiment 1, and 200 in Experiment 2. In Experiment 2, upon death of a larva, the whole microcosm was removed from the experiment. We fitted survival curves using the non-parametric Cox's proportional hazards model (using R package survival; Therneau, 2015), and compared the output with a parametric model, alternately assuming a constant hazard and a non-constant hazard with Weibull errors (Crawley, 2007). A censoring vector was used because some individuals survived beyond the end of the experiment, and the time of death was unknown for others.

Pupation, emergence, and adult size traits. Chi-square tests were used to assess sex ratios between treatments and over time, and to compare numbers eclosing between treatments. After noting that the fastest developing larvae seemed to have prolonged pupation periods, we tested whether this pattern was significant using a linear regression between the date on which pupation began and the duration of the pupal period.

We used multivariate analysis of variance (MANOVA) to determine the effect of treatment and sex on development time (we computed the inverse of time, 1/day in experiment, to facilitate comparisons with other traits that scale positively with fitness), pupal size ($\sqrt{}$pupal area; square root transformed to convert it to the linear scale shared with other size traits), and adult size traits. Model simplification was carried out using likelihood ratio tests, and sequentially deleting terms (beginning with higher-order interactions) that did not significantly decrease model deviance (Crawley, 2007).

Results

Experiment 1: effect of pine woodchip size and Nipagen

The non-linear model using the vB function parameters would not converge when using the full dataset. Of 40 replicates, 3 each in treatments S (Sawdust) and NS (Nipagen and Sawdust), and 15 in C (Chips) did not appear to grow substantially or did not pupate within the same year as the experiment (Table S2). No slow-growing individuals were found in treatment CS (Chips and Sawdust). Only upon removing these replicates from the analysis was convergence achieved (Fig. 2). The slow-growing individuals were not included in growth or fat deposition analyses but were used to compare adult size traits between years. Below we will refer to the subset of individuals that grew slowly in the first year as semivoltine.

Larval growth. We predicted that larval growth would be adversely affected in the low substrate surface area to water volume treatment i.e. treatment C; as predicted, individuals in treatment C reached a lower mean asymptotic (L∞) size of 0.370 (0.337–0.408 95% confidence interval CI) than those in the S (0.609, 0.512–0.758 CI), NS (0.674, 0.506–1.008 CI), and CS (0.621, 0.575–0.679 CI) treatments (Fig. 2, Table S3). The growth constant (K) was indistinguishable across treatments S (0.033, 0.021–0.046 CI) and NS (0.029, 0.014–0.048 CI), and greater but not significantly in treatment CS (0.048, 0.039–0.058 CI) and significantly greater in C (0.080, 0.056–0.112 CI), (Fig. 2, Table S3). There was also no effect of sex on growth (data not shown).

The mean asymptotic parameter for growth data collected from the field (0.350, 0.331–0.370 CI) was indistinguishable to that for lab treatment C and the growth constant (0.223, 0.152–0.315 CI) was significantly greater than C (Fig. 2, Table S3).

Fat deposition over time. Individuals in treatment C not only appeared to take longer to grow but also to build up fat reserves before winter, and appeared to lose a greater proportion of fat after winter while gaining in size (Figure S1). Two weeks before becoming completely opaque with fat (day 49, 20th October) a lower proportion of body area covered by fat was found in individuals in treatment C compared with a higher proportion of treatments CS ($\chi^2 = 3.75$, d.f. = 1, $P = 0.05$), NS ($\chi^2 = 5.14$, d.f. = 1, $P < 0.05$), and S ($\chi^2 = 6.36$, d.f. = 1, $P < 0.05$). From November until March (days 79–164), fat deposition was 100% in all treatments and feeding appeared to be suspended, indicated by a lack of dark colouration in the gut. Two weeks after winter (day 230, 29th April), again the proportion of fat was lower in individuals in treatment C compared with CS ($\chi^2 = 8.52$, d.f. = 1, $P < 0.005$), NS ($\chi^2 = 18.37$, d.f. = 1, $P < 0.005$), and S ($\chi^2 = 16.4$, d.f. = 1, $P < 0.005$) (Figure S1). Before pupation, fat deposition returned to 100% and the gut cleared indicating cessation of feeding in preparation for pupation.

Survival analysis. While the greatest larval mortality was in treatment CS (0.35, Table S2), we could not detect a statistically significant effect of treatment on survival ($Z = 1.2$, $P > 0.1$, Cox's proportional hazard). Most deaths occurred early in the experiment, and few individuals died after the first 100 days.

Pupation, emergence, and adult size. Seventeen large, opaque individuals (7 CS, 4 NS, and 6 in S treatments) were found exiting the microcosms before winter, a response apparently indicating the completion of development, after which larvae begin searching for a place to pupate. These individuals were returned to microcosms, and moss plugs were provided within which larvae readily came to rest.

Individuals took between 13 and 36 days to develop and eclose depending on when in the year they pupated. The later the onset of pupation, the shorter the subsequent pupal period before eclosion ($r^2 = 0.79$, $F_{1,112} = 420.6$, $P < 0.001$). A significantly greater number of *B. fallax* eclosed in treatments CS (26), NS (25), and S (26) than in C (13, $\chi^2 = 14.9$, d.f. = 3, $P < 0.005$, Table S2).

The sex ratio did not deviate significantly from 50 : 50 (M/F 45/44). In the first week of the emergence period, significantly more males emerged than females (M/F 38/22, $\chi^2 = 8.97$, d.f. = 1, $P < 0.05$), and more females emerged in the final 23 days (M/F 7/22, $\chi^2 = 7.76$, d.f. = 1, $P < 0.05$). Females overall took longer to develop to eclosion (MS = 344.18, $F_{1,88} = 16.49$, $P = 0.0001$). We assessed within treatment sex differences in the development time, which showed the same trend in CS, NS, and S, but no significant difference between males and females in C (Table S4). Individuals in C took longer to develop (Table S1), and significantly fewer eclosed compared with each of the other treatments ($\chi^2 = 3.78$, d.f. = 1, $P = 0.05$, Table S2).

Individuals in treatment C had smaller pupal areas, thoraces and wing lengths, and took longer to develop compared with individuals in treatments CS, NS, and S (MANOVA, $P < 0.005$, Table S4). No difference was found between S, NS, and CS (Table S1 for comparison of means). Female wing and thorax lengths were greater than those of males, and wing length in both males and females was significantly greater in treatments CS, NS, and S than in C (MANOVA, $P < 0.05$, Fig. 3, Tables S1 and S4).

A significant treatment by sex interaction was found for thorax and wing lengths when semivoltine individuals were included in the model. These individuals were smaller compared with those that eclosed in the previous year, and males tended to have greater thorax lengths and longer wings than females, the opposite trend to all treatments (MANOVA, $P < 0.05$, Fig. 3, Tables S1 and S4).

Experiment 2: effect of intra-specific competition

As in Experiment 1, between 2% and 20% of larvae in each treatment did not appear to grow substantially in the first year. The non-linear model using the vB function parameters would not converge with these replicates included in the model. Therefore, 29 of 240 replicates (i.e. microcosms) were removed from the analysis (between 1 and 4 per treatment).

Larval growth. As there were no significant interactions between larval density, water and sawdust level (data not shown), we consider their effects on growth parameters sequentially, below.

We found clear evidence that larval growth was suppressed under competitive conditions. The mean asymptotic size decreased significantly as the number of larvae increased from one larva per microcosm (0.106, 0.091–0.126 CI) to two (0.065, 0.060–0.071 CI) and three (0.051, 0.048–0.054 CI) (Fig. 4, Table S3). The growth constants were lowest in single larva microcosms (0.030, 0.022–0.039 CI) and did not overlap with the confidence region for the growth constant of three (0.071, 0.058–0.085 CI) or two larvae microcosms (0.051, 0.040–0.060 CI) (i.e. the growth curve was flatter associated with a larger asymptotic size compared with other treatments that reached a smaller asymptotic size) (Fig. 4, Table S3).

We found clear evidence that larval growth was suppressed under low water and sawdust conditions. The mean asymptotic size was significantly lower in a low water level (0.061, 0.057–0.064 CI) relative to high water treatments (0.085, 0.076–0.097 CI) (Table S3). The growth constant was greater in low water (0.057, 0.049–0.067 CI) compared with high water (0.036, 0.028–0.044 CI) (Table S3). The mean asymptotic size was significantly lower in low sawdust (0.062, 0.058–0.067 CI) relative to the high sawdust treatment (0.083, 0.076–0.093 CI)

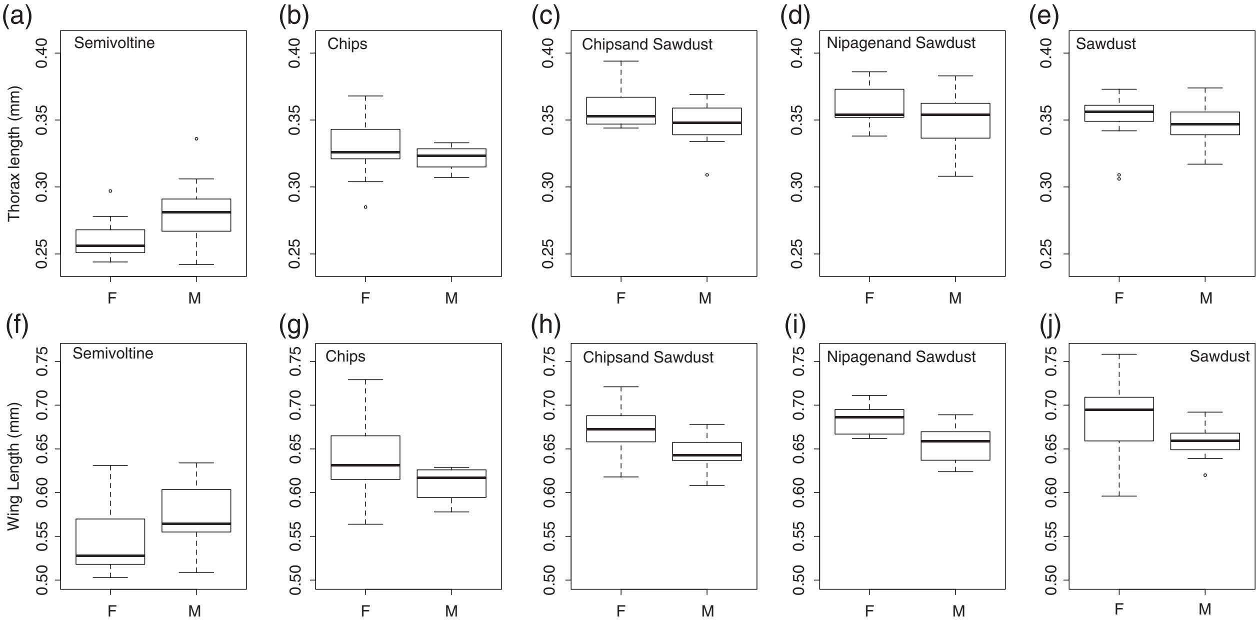

Fig. 3. Boxplots illustrating the difference in thorax (a–e) and wing length (f–j) between males (M) and females (F) in semivoltine individuals (those that took 2 years to develop) and in Chips, Chips and Sawdust, Sawdust and Nipagen, and Sawdust treatments (Experiment 1).

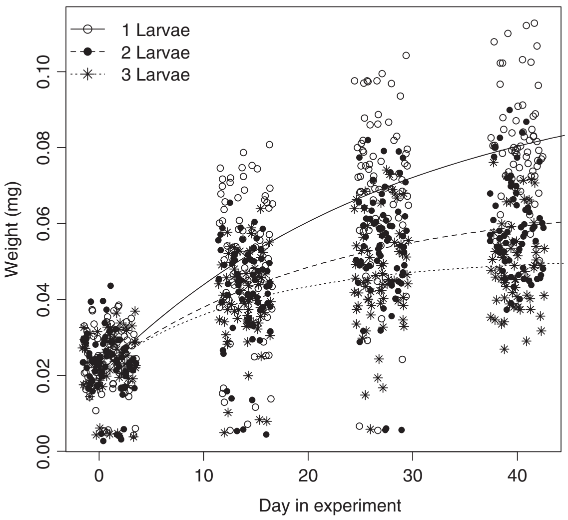

Fig. 4. *Blera fallax* larval growth curves implemented by the von Bertalanffy growth function fitted by non-linear fixed effects models, and 'jittered' points to illustrate the differences in 1, 2, and 3 larval density treatment groups (Experiment 2).

(Table S3). Moreover, the growth constant was greater in low sawdust (0.054, 0.044–0.065 CI) compared with the high sawdust treatment (0.038, 0.030–0.046 CI) (Table S3). Male and female subsets also did not produce different parameter estimates (data not shown).

Survival analysis. Sawdust, water, and larval density all had significant effects on survival (Sawdust, $Z = -2.5$, $P < 0.05$; Water, $Z = 5.8$, $P < 0.005$; Larvae, $Z = -4.3$, $P < 0.005$, Cox's proportional hazard). The greatest mortality was found in the high sawdust, high water, and low larval density treatments (0.55–0.65, Table S2), and the lowest was in the low water, high larval density treatments (0.03–0.15, Table S2).

Pupation, emergence, and adult size. Across treatments, the sex ratio was significantly female biased (M/F 64/102, $\chi^2 = 8.69$, d.f. = 1, $P = 0.003$). A smaller proportion of *B. fallax* individuals eclosed within 1 year in low (0.33) compared with high sawdust treatments (0.47) and low (0.37) compared with high water treatments (0.44), and significantly fewer in high compared with low competition treatments (1 vs. 3 larvae, $\chi^2 = 9.14$, d.f. = 1, $P < 0.005$, Table S2). There were significantly more males emerging (26) than females (18) in the first 2 weeks of emergence (M/F 0.41/0.18, $\chi^2 = 8.97$, d.f. = 1, $P < 0.005$).

The larval density, water, and sawdust level had significant effects on thorax and wing lengths, puparium areas, and times to eclosion, all of which were consistent with effects of these treatments on larval growth. Thorax and wing lengths and pupal areas decreased in increasing larval density and decreasing sawdust and water levels (MANOVA, $P < 0.05$, Table S5). However, the effects of these treatments sometimes depended on sex. Compared with males, a greater negative effect is evident in female thorax and wing length and puparium area in increasing larval density treatments (sex by density interaction $P < 0.05$, Figure S2, Table S5). While male wing lengths did not differ according to sawdust levels, females had significantly longer wings in high sawdust treatments (sex by sawdust interaction $P < 0.05$, Fig. 5, Table S5).

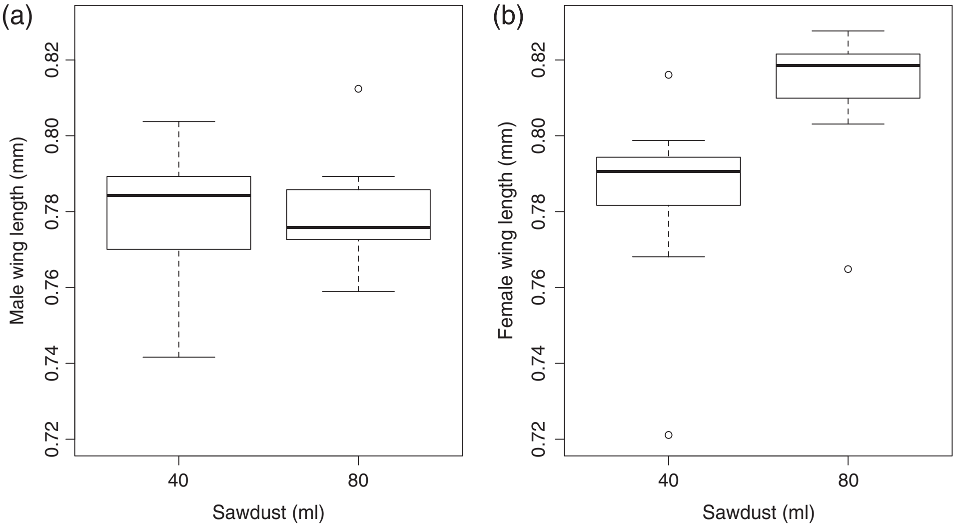

Fig. 5. Boxplots showing different responses of (a) male and (b) female wing length (mm) to sawdust level (larval density = 1) (Experiment 2).

Females took longer to eclose (day in experiment, mean 254 ± 6.5 SD, standard deviation) than males (250 ± 5.2 SD), and females on average were larger than males (Table S1). The development time was significantly shorter for both males and females in high sawdust and water treatments (Tables S1 and S5).

Experiment 3: effect of tree species

Larval growth. The mean asymptotic size was highest for pine (0.119, 0.084–0.192 CI) although this was statistically indistinguishable from the estimate for the birch sawdust treatment (0.089, 0.071–0.125 CI). Asymptotic size was significantly lower in spruce (0.061, 0.051–0.079 CI) than pine and birch (Table S3). Growth constants did not significantly differ across tree species (Table S3). As above, male and female subsets of data did not produce significantly different growth parameters (data not shown).

Survival analysis. Only two individuals died in the phase of the experiment on tree species during which we monitored growth (September to November 2010). Therefore, we did not conduct formal survival analysis for those samples. Survival to eclosion across tree species treatments was determined in June and July 2011, and in total, contrary to prediction significantly more individuals died in the pine treatment (0.55), compared with birch (0.30) and spruce (0.10) ($\chi^2 = 32.10$, d.f. = 2, $P < 0.005$, Table S2).

Pupation, emergence, and adult size. Chi-squared tests show no significant difference between the total number eclosing or the number of males and females in pine (M/F 2/6), birch (M/F 7/3) or spruce treatments (M/F 6/5). Tree species did have a significant effect on thorax length, wing length, and puparium area (MANOVA, $P < 0.05$, Table S5): individuals in the spruce treatment had significantly smaller thorax and wing lengths and pupal areas (Tables S2 and S5). We found no differences in development time between treatments.

Discussion

Rearing conditions had strong effects on growth, development time, and adult size in *B. fallax*. Individuals were larger in treatments with small wood particle size, in greater volumes of substrate and water and at low larval densities. In conditions that do not sustain rapid growth, some individuals responded by extending development into an extra growing season, i.e. they became semivoltine. Males and females appear to resolve the presumed trade-off between development time and size differently, with males prolonging development and females emerging smaller. Surprisingly, individuals grew larger in birch (a species in which *B. fallax* larvae have never been found in nature) than spruce (a host species exploited by *B. fallax* in Europe).

Growth and life history

Depending on resources, larvae grew rapidly at the beginning of their development, but growth then slowed and individuals reached an asymptotic size before winter, a phase that may be governed by seasonal triggers such as photoperiod and temperature signals. Photoperiod is often the main environmental cue in insects (Nylin & Gotthard, 1998). The initial growth trajectory probably reflects the period when all available resources are devoted to juvenile growth (Day & Taylor, 1997). For larvae in good conditions, the asymptotic size parameter in our models defines the mass at eclosion, i.e. no more growth occurs before pupation, whereas for those in less favourable conditions, growth continues after winter.

Voltinism

A survey completed in 2011 showed surviving populations of *B. fallax* consisting solely of large larvae at a similar stage that were developing over 2 years (Rotheray, 2012). This suggests a complete failure of adult breeding, perhaps as a result of cold and wet weather during the adult breeding season. Therefore, a plastic ability to initiate diapause depending on the conditions, or bet hedging involving the production of some slow growing, the semivoltine offspring may be advantageous. In Scottish pine rot holes, risks from predation and retention of the larval habitat may be low. Such conditions may allow for semivoltinism while preserving more important life history traits (Collins, 1980). Theory suggests that when the environment is unpredictable, females could increase the variation among offspring as an adaptive strategy (Plaistow *et al.,* 2006; Marshall *et al.,* 2008; Crean & Marshall, 2009; Monro *et al.,* 2010), or larvae could initiate diapause plastically as a function of condition (Plaistow *et al.,* 2006). Such developmental plasticity has been reported in other insects such as stoneflies and damselflies (Cayrou & Céréghino, 2005) but it has never been formally reported in hoverflies. In addition, this strategy may also reduce direct sibling competition within rot holes, as semivoltine larvae develop little in their first year (Rotheray, 2012, 2013).

Larval growth and the pine rot hole

Individuals grew faster and larger in treatments with sawdust rather than wood chips. The ability of microbes to metabolise wood probably depends on what fraction of the wood is exposed to water, and this is much higher in sawdust treatments relative to wood chips. Growth rates of filter-feeding larvae have been found to increase with the increased area of submerged surfaces (Juliano & Reminger, 1992; Leonard & Juliano, 1995).

Our assessment of growth in the field revealed substantial variation in growth and a greater average rate of growth, which is probably as a result of the variation in conditions larvae are exposed to in the field. The average size achieved before winter diapause was most similar to that in the resource-limited conditions (C) in the lab suggesting that in nature, resources may be limited. Therefore, ensuring rot hole surface area is maximised by, for example providing chips and sawdust, may benefit larval growth in the field. This may be particularly important in artificially created holes that are less structurally complex than natural rot holes.

Increasing larval abundance reduced growth rates, corresponding with studies that show density-dependent limitations on growth in other aquatic dipteran larvae (see Bradshaw & Holzapfel, 1992; Broberg & Bradshaw, 1995). Presumably, larval grazing depletes the food resources available. The accumulation of waste products such as ammonia may be an additional time or density-dependent issue causing further depression in larval growth (Carpenter, 1983). However, it is important to note the limitations of this study. We may not have reached densities where Allee effects could be detected if the effects do not continue linearly. In the field of a 4-year survey of 50 rot holes, larval densities range from 1 to 78 (mean 14) in one rot hole (Rotheray, 2012) so to test these effects further, replicates should include densities up to and in excess of 14 larvae.

Fat deposition

As winter approaches, energy seems to be re-directed from mass accumulation to pre-winter fat storage. Once fat storage is complete, signified by a once translucent body becoming completely opaque with white fat, larvae appear to stop feeding and enter a slow-moving over-winter stasis, which is probably an energy conserving behavioural response (Hart & Bale, 1997). Fat storage is an important basic survival requirement for unproductive periods such as overwintering in many organisms, often involving tradeoffs in which energy has been directed from body and skeletal growth to fat reserves (Morgan & Metcalfe, 2001; Shertzer & Ellner, 2002). The fat deposition was slower in individuals with less favourable conditions; however, by the over-winter period (November to March) all individuals had become 100% opaque.

Individuals that had not completed development before winter (i.e. they continued to gain size after winter) became almost transparent again in spring, which suggests they probably utilised the fat for post-winter growth as well as gaining more energy from continued feeding. Similar findings have been reported in mosquitoes, which take advantage of the rise in temperature after winter to catch up with growth (Bradshaw, 1973). When development is complete, individuals again appear to channel resources into fat storage before pupating.

Effect of tree species

Larvae grow as well in birch as they do in pine, and while growth is significantly inhibited in spruce compared with the other species, the same number of individuals eclosed in spruce as they did in birch, and more than pine within a year. Clearly the larval stage is capable of developing a range of alternative resources. *Blera fallax* has not been found in any other tree species than pine in Scotland, and gravid females are disinclined to oviposit in rotting spruce or birch sawdust (Rotheray, 2012). The specialisation on pine may occur because pine habitat was more available or productive than alternative tree rot holes at some past time, or it may be that our study did not include aspects of fitness that reveal costs of growth in alternative species of hosts, such as subsequent mating success or fecundity.

Larval survival

Larval survival was significantly higher in low levels of water, high levels of sawdust, and high larval densities, and in spruce and birch treatments compared with pine. In all experiments, the treatment levels with the lowest survival were also those in which the growth rate was highest. Physiological costs associated with maximised growth may be the cause, whereby an organism's susceptibility to short-term stresses are increased by higher metabolic demands necessary for faster growth (Blanckenhorn, 1998; Dmitriew & Rowe, 2007; Dmitriew, 2011;

Schäfer *et al.,* 2013). However, the costs of growing slowly could also have been masked by the lab conditions, such as a lack of predators or pathogens. We do not know how important water, sawdust or larval density are for survival in natural conditions and measuring survival in the field is difficult, as larvae often crawl into tight crevices or exit the rot hole completely before winter (E. L. Rotheray, pers. obs.).

Increasing larval density (from 1 to 3 larvae) and the application of the anti-fungal treatment Nipagen did not appear to affect survival. Fungal growth was not directly assessed in the Nipagen experiment, but while fungal growth was apparent in all treatments, there were no noticeable differences in mortality across them. We conclude that while Nipagen does not appear to affect larval growth or survival negatively, its application has no obvious benefit. To the best of our knowledge, this is the first study of its kind to investigate the negative effects of the application of Nipagen.

Development, pupal, and adult size

Individuals reared in low resource conditions had longer development times, lower single year eclosion rates, and were smaller at eclosion. The duration of the pupal stage decreased significantly with the day in the year on which pupation took place, a phenomenon probably triggered by photoperiod (Gotthard, 2008). While it is not known if there are any associated costs in *B. fallax*, in other Dipteran species lower fecundity is associated with a decreased pupal development time (Telles-Romero *et al.*, 2011).

Size may be important to females if it is associated with higher levels of fecundity (Armbruster & Hutchinson, 2002). Females probably allocate more energy than males to reproduction in low resource conditions, thereby exhibiting a stronger response to lower resources. This in turn may explain the switch in a response of semivoltine individuals, where resource levels may have deteriorated over time further reducing the threshold size of females below that of males in these conditions.

Females, which on average are larger than males, appear to vary more substantially in size at eclosion than males, who appear more likely to retain size at the expense of not achieving maturity and having to develop for another year. Eclosing at a smaller size may be compensated by avoiding mortality risks associated with the increased development time, such as increased exposure to predation, and possibly increased over-wintering survival risks. For males, achieving a certain body size may be essential for mating success (Rotheray, 2012), which could explain why males are less likely to emerge smaller in restricted conditions. It often benefits male insects to emerge first, a feature known as protandry, in which males mature their reproductive organs and set up territories in advance of female emergence (Rotheray *et al.,* 2009). This can lead to a smaller size in males or changes in the levels of sexual size dimorphism throughout the season (Nylin & Gotthard, 1998).

The contrasts across the sexes in the propensity to become semivoltine, and the resolution of presumed trade-offs between development and adult size may reflect sex-specific adaptive differences in investment (Dmitriew *et al.*, 2009). However, there are many unresolved questions about the nature of selection on the two sexes, including the direction and intensity of size-associated sexual and fecundity selection (Rotheray, 2012) that will need to be resolved before we can state this with any confidence.

Conservation and animal husbandry for B. fallax

We found clear effects of several aspects of the rearing environment on development time, eclosion, and adult size. Based on these results, the conditions that best enhance fitness for *B. fallax* reared *ex situ* include a minimum sawdust volume of 40 and 140 ml water per larva, rearing in groups of no more than two per microcosm and applying moss plugs for larvae to move into when fully developed. For semivoltine larvae, *ex situ* conditions may require supplementation of new sawdust substrate and water to maximise growth in year two of their development.

Conservation management involves creating habitat for *B. fallax* by boring holes in pine stumps, and the nature of the hole determines the contents and the density (Rotheray, 2012). Increasing the substrate and rot hole surface area or structural complexity when creating habitat for this species will probably benefit larval growth by providing access to more resources. Wild densities can be much higher than those featured in these experiments (suggesting a shortage of suitable rot holes), which could potentially inhibit larval growth or be a source of mortality (Rotheray, 2013). Further studies should investigate the effects of increasing the surface area in rot holes in the field. Monitoring larval growth and survival between July and no later than September allows measurements of the initial growth trajectory, and reduces the chances of larvae exiting the rot hole before growth and survival data collection is complete. Monitoring should also take into account the likely presence of semivoltine individuals.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12269

Appendix S1. Materials and methods.

Table S1. Main *Blera Fallax*-rearing conditions with trait measurements (mean ± standard deviation SD) in treatments; chips, chips and sawdust, Nipagen and sawdust, and sawdust; 2010, semivoltine individuals (those that took 2 years to develop but from the same brood) (Experiment 1); and 1, 2, and 3 larval densities (Experiment 2); low and high sawdust and water treatments; and birch, pine, and spruce sawdust treatments (Experiment 3).

Table S2. Year, treatments, the total number of larvae included in each treatment, the number of male and female adults emerged from each treatment, and percentage dead and pupated within 1 year.

Table S3. Bootstrapped von Bertalanffy parameter estimates and 95% confidence intervals for models of larval mass increase across experimental treatments: chips, chips and sawdust, Nipagen and sawdust and sawdust ($L0 = -10.36$), and field larval growth ($L0 = -2.026$) (Experiment 1); and 1, 2, and 3 larval densities ($L0 = -7.56$), and low and high sawdust and water treatments ($L0 = -7.68$) (Experiment 2); and birch, pine, and spruce sawdust treatments ($L0 = -6.97$) (Experiment 3).

Table S4. Multivariate (MANOVA) hypothesis test results (Experiment 1), between treatments Chips, Sawdust and Nipagen, Chips and Sawdust, and Sawdust and within treatment by sex differences.

Table S5. Multivariate (MANOVA) hypothesis test results investigating the effects of larval density (1/2/3 larvae), sawdust volume (high/low), water volume (high/low), and tree species (pine/birch/spruce) on adult traits (Experiments 2 and 3).

Figure S1. Percentage fat (y-axis) in each time interval representing August 2009 (day 1) until June 2010 (day 230) (x-axis), illustrated in separate graphs for each treatment; Chips, Sawdust and Nipagen, Chips and Sawdust, and Sawdust (Experiment 1).

Figure S2. Boxplots showing the response of male and female thorax (top row), wing length (middle row), and $\sqrt{}$ puparum area (bottom row) (mm) to larval density (Experiment 2).

References

Abràmoff, M.D., Magalhães, P.J. & Ram, S.J. (2004) Image processing with ImageJ. *Biophotonics International*, **11**, 36–42.

Abrams, P.A., Leimar, O., Nylin, S. & Wiklund, C. (1996) The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *American Naturalist*, **147**, 381–395.

Armbruster, P. & Hutchinson, R.A. (2002) Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). *Journal of Medical Entomology*, **39**, 699–704.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2014) *lme4: Linear Mixed-Effects Models using Eigen and S4*. R package, version 1.1-7. [WWW document]. URL http://CRAN.R-project.org/package=lme4 [accessed on 1 January 2012]

Baty, F., Ritz, C., Charles, S., Brutsche, M., Flandrois, J.P. & Delignette-Muller, M.L. (2015) A toolbox for nonlinear regression in R: the package nlstools. *Journal of Statistical Software*, **66**, 1–21.

Blanckenhorn, W.U. (1998) Adaptive phenotypic plasticity in growth, development, and body size in the yellow dung fly. *Evolution*, **52**, 1394–1407.

Blanckenhorn, W.U. (1999) Different growth responses to temperature and resource limitation in three fly species with similar life histories. *Evolutionary Ecology*, **13**, 395–409.

Bradshaw, W.E. (1973) Homeostasis and polymorphism in vernal development of *Chaoborus americanus*. *Ecology*, **54**, 1247–1259.

Bradshaw, W.E. & Holzapfel, C.M. (1992) Resource limitation, habitat segregation, and species interactions of British tree-hole mosquitoes in nature. *Oecologia*, **90**, 227–237.

Broberg, L. & Bradshaw, W.E. (1995) Density-dependent development in *Wyeomyia smithii* (Diptera: Culicidae): intraspecific competition is not the result of interference. *Annals of the Entomological Society of America*, **88**, 465–470.

Carpenter, S.R. (1983) Resource limitation of larval treehole mosquitoes subsisting on beech detritus. *Ecology*, **64**, 219–223.

Cayrou, J. & Céréghino, R. (2005) Life-cycle phenology of some aquatic insects: implications for pond conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **15**, 559–571.

Collins, N. (1980) Developmental responses to food limitation as indicators of environmental conditions for *Ephydra Cinerea* Jones (Diptera). *Ecology*, **61**, 650–661.

Crawley, M.J. (2007) *The R Book*. Wiley, Chichester, U.K.

Crean, A.J. & Marshall, D.J. (2009) Coping with environmental uncertainty: dynamic bet hedging as a maternal effect. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, **364**, 1087–1096.

Day, T. & Taylor, P.D. (1997) Von Bertalanffy's growth equation should not be used to model age and size at maturity. *The American Naturalist*, **149**, 381–393.

Dmitriew, C.M. (2011) The evolution of growth trajectories: what limits growth rate? *Biological Reviews*, **86**, 97–116.

Dmitriew, C. & Rowe, L. (2007) Effects of early resource limitation and compensatory growth on lifetime fitness in the ladybird beetle (Harmonia axyridis). *Journal of Evolutionary Biology*, **20**, 1298–1310.

Dmitriew, C., Carroll, J., Rowe, L. (2009) Effects of early growth conditions on body composition, allometry, and survival in the ladybird beetle *Harmonia axyridis*. *Canadian Journal of Zoology*, **87**, 175–182.

Gotthard, K. (2008) Adaptive growth decisions in butterflies. *Bioscience*, **58**, 222–230.

Hart, J. & Bale, S. (1997) Factors affecting the freeze tolerance of the hoverfly *Syrphus ribesii* (Diptera: syrphidae). *Journal of Insect Physiology*, **44**, 21–29.

Hou, C., Bolt, K.M. & Bergman, A. (2011) A general model for ontogenetic growth under food restriction. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 2881–2890.

Juliano, S.A. & Reminger, L. (1992) The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: geographic and ontogenetic differences. *Oikos*, **63**, 465–476.

Kaufman, M.G., Walker, E.D., Smith, T.W., Merritt, R.W. & Klug, M.J. (1999) Effects of larval mosquitoes (*Aedes triseriatus*) and stemflow on microbial community dynamics in container habitats. *Applied and Environmental Microbiology*, **65**, 2661–2673.

Knight, T.M., Chase, J.M., Goss, C.W. & Knight, J.J. (2004) Effects of interspecific competition, predation, and their interaction on survival and development time of immature *Anopheles quadrimaculatus*. *Journal of Vector Ecology*, **29**, 277–284.

Leonard, P.M. & Juliano, S.A. (1995) Effect of leaf litter and density on fitness and population performance of the hole mosquito *Aedes triseriatus*. *Ecological Entomology*, **20**, 125–136.

Marshall, D.J., Bonduriansky, R. & Bussière, L.F. (2008) Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. *Ecology*, **89**, 2506–2517.

Milankov, V., Francuski, L., Ludoški, J., Stahls, G. & Vujic, A. (2010) Genetic structure and phenotypic diversity of two northern populations of *Cheilosia aff. longula* (Diptera: Syrphidae) has implications for evolution and conservation. *European Journal of Entomology*, **107**, 305–315.

Monro, K., Sinclair-Taylor, T. & Marshall, D.J. (2010) Selection on offspring size among environments: the roles of environmental quality and variability. *Functional Ecology*, **24**, 676–684.

Morgan, I.J. & Metcalfe, N.B. (2001) Deferred costs of compensatory growth after autumnal food shortage in juvenile salmon. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **268**, 295–301.

Nylin, S. & Gotthard, K. (1998) Plasticity in life-history traits. *Annual Review of Entomology*, **43**, 63–83.

Pinheiro, J., Bates, D. DebRoy, S., Sarkar, D. & R Core Team. (2015) *nlme: Linear and Nonlinear Mixed Effects Models*. R package, version 3.1-120. [WWW document]. URL http://CRAN.R-project.org/package=nlme [accessed on 1 January 2012].

Plaistow, S.J., Lapsley, C.T. & Benton, T.G. (2006) Context-dependent intergenerational effects: the interaction between past and present environments and its effect on population dynamics. *American Naturalist*, **167**, 206–215.

Reznick, D.N. (1990) Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): an experimental evaluation of alternative models of development. *Journal of Evolutionary Biology*, **3**, 185–203.

Rotheray, E.L. (2010) *Restoring the endangered pine hoverfly in the UK*. In: Global Re-introduction Perspective: 2010 (ed. by P. S. Soorae), pp. 21–24. IUCN/SSC Re-introduction Specialist Group & Environmental Agency - Abu Dhabi, Abu Dhabi, UAE.

Rotheray, E.L. (2012) *The ecology and conservation of endangered saproxylic hoverflies (Diptera, Syrphidae) in Scotland*. PhD thesis, University of Stirling, Stirling, U.K.

Rotheray, E.L. (2013) Differences in ecomorphology and microhabitat use of four saproxylic larvae (Diptera, Syrphidae) in Scots pine stump rot holes. *Ecological Entomology*, **38**, 219–229.

Rotheray, G.E. & MacGowan, I. (2000) Status and breeding sites of three presumed endangered Scottish saproxylic syrphids (Diptera, Syrphidae). *Journal of Insect Conservation*, **4**, 215–223.

Rotheray, E.L., MacGowan, I., Rotheray, G.E., Sears, J. & Elliott, A. (2009) The conservation requirements of an endangered hoverfly, *Hammerschmidtia ferruginea* (Diptera, Syrphidae) in the UK. *Journal of Insect Conservation*, **13**, 569–574.

R Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0 [WWW document]. URL http://www.R-project.org.

Schäfer, M.A., Berger, D., Jochmann, R., Blanckenhorn, W.U. & Bussiere, L.F. (2013) The development plasticity and functional significance of an additional sperm storage compartment in female yellow dung flies. *Functional Ecology*, **27**, 1392–1402.

Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*. Cambridge University Press, Cambridge, U.K.

Scottish Natural Heritage (2007) *A Five Year Species Action Frame-Work: Making a Difference for Scotland's Species*. Scottish Natural Heritage Publications, Perth, Australia.

Shertzer, K.W. & Ellner, S.P. (2002) State-dependent energy allocation in variable environments: life history evolution of a rotifer. *Ecology*, **83**, 2181–2193.

Smith, R.L. & Smith, T.M. (2006) *Elements of Ecology*. Benjamin Cummings, Boston, Massachusetts.

Stillwell, R.C., Blanckenhorn, W.U., Teder, T., Davidowitz, G. & Fox, C.W. (2010) Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annual Review of Entomology*, **55**, 227–245.

Taylor, F. (1981) Ecology and evolution of physiological time in insects. *American Naturalist*, **117**, 1–23.

Telles-Romero, R., Toledo, J., Hernández, E., Quintero-Fong, J. & Cruz-López, L. (2011) Effect of temperature on pupa development and sexual maturity of laboratory *Anastrepha obliqua* adults. *Bulletin of Entomological Research*, **101**, 565–571.

Therneau, T. (2015) *A Package for Survival Analysis in S*. Version 2.38. [WWW document]. URL http://CRAN.R-project.org/package=survival [accessed on 1 January 2012].

Tinsley, M., Blanford, S. & Jiggins, F. (2006) Genetic variation in *Drosophila melanogaster* pathogen susceptibility. *Parasitology*, **132**, 767–773.

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