

An experimental test of the ecological mechanisms driving density-mediated carry-over effects in a seasonal population

Joseph B. Burant, Aidan Griffin, Gustavo S. Betini, and D. Ryan Norris

Abstract: Carry-over effects occur when past experience influences current individual performance. Although variation in conspecific density in one season has been shown to carry over to influence dynamics in the following season, the proximate ecological mechanisms driving these effects are unknown. One hypothesis is that high density decreases food availability, resulting in poor physiological condition, which in turn compromises performance the next season. Alternatively, high conspecific density could also lead to a high degree of antagonistic interactions, decreasing the amount of time individuals spend foraging. To investigate these hypotheses, we applied a factorial design where both conspecific density and per capita food availability during the non-breeding period were independently manipulated in seasonal populations of common fruit flies (*Drosophila melanogaster* Meigen, 1830). Individual condition at the beginning of the breeding period was influenced by per capita food availability but not density during the previous non-breeding period. In contrast, reproductive output was most strongly influenced by the interaction between per capita food availability and density in the previous non-breeding period, such that populations that experienced high non-breeding densities and low food availability had the lowest reproductive output. However, the strength of this effect was relatively weak. Our results demonstrate how environmental and social conditions in one part of the annual cycle can carry over to influence individual performance in subsequent periods.

Key words: body condition, *Drosophila melanogaster*, food availability, fruit fly, population density, reproductive output, seasonal interactions.

Résumé : Les effets reportés se produisent quand l'expérience passée influence la performance présente des individus. S'il a été démontré que les variations de la densité de conspécifiques durant une saison ont une influence sur la dynamique durant la saison suivante, les mécanismes écologiques proximaux à l'origine de ces effets ne sont pas connus. Une hypothèse veut qu'une forte densité réduise la disponibilité de nourriture, ce qui se traduit par un faible embonpoint qui, lui, compromet la performance durant la saison suivante. Une autre explication serait qu'une forte densité de conspécifiques pourrait aussi mener à un degré élevé d'interactions antagonistes, réduisant du coup le temps affecté par les individus à la quête de nourriture. Afin d'évaluer ces hypothèses, nous avons appliqué un schéma factoriel dans lequel la densité de conspécifiques et la disponibilité de nourriture par individu durant la période internuptiale étaient manipulées de manière indépendante dans des populations saisonnières de drosophiles (*Drosophila melanogaster* Meigen, 1830). L'embonpoint individuel au début de la période de reproduction était influencé par la disponibilité de nourriture par individu, mais non par la densité durant la période internuptiale précédente. En comparaison, la plus forte influence sur l'efficacité de la reproduction était l'interaction entre la disponibilité de nourriture par individu et la densité durant la période internuptiale précédente, l'efficacité de la reproduction étant la plus faible dans les populations caractérisées par une forte densité internuptiale et une faible disponibilité de nourriture. L'intensité de cet effet était toutefois assez faible. Nos résultats démontrent comment des conditions ambiantes et sociales durant une partie du cycle annuel peuvent avoir des effets reportés qui influencent la performance des individus durant des périodes subséquentes. [Traduit par la Rédaction]

Mots-clés : embonpoint, *Drosophila melanogaster*, disponibilité de nourriture, drosophile, densité de population, efficacité de la reproduction, interactions saisonnières.

Introduction

Carry-over effects occur when an individual's past experience explains its current performance (O'Connor et al. 2014). In seasonal environments, there is growing evidence that carry-over effects play an important role in explaining variation in individual success (Norris 2005; Harrison et al. 2011; Betini et al. 2014). For example, in migratory birds, variation in habitat quality and weather during the non-breeding period can impact reproductive performance in the following season (Norris et al. 2004; Costantini et al. 2010; Inger et al. 2010; Duriez et al. 2012; Rockwell et al. 2012).

Similar results have been found in resident bird populations (Robb et al. 2008; Salton et al. 2015), fish (Bunnell et al. 2007), and mammals (Cook et al. 2004; Perryman et al. 2006). In some cases, carry-over effects have been shown to scale up to influence population-level processes, including population growth rate and long-term stability (Van Allen and Rudolf 2013; Liz and Ruiz-Herrera 2016).

In addition to habitat quality, another potential generator of carry-over effects that could have wide-ranging effects is conspecific density. Variation in density over time and between habitats is characteristic of almost all wild populations and the negative

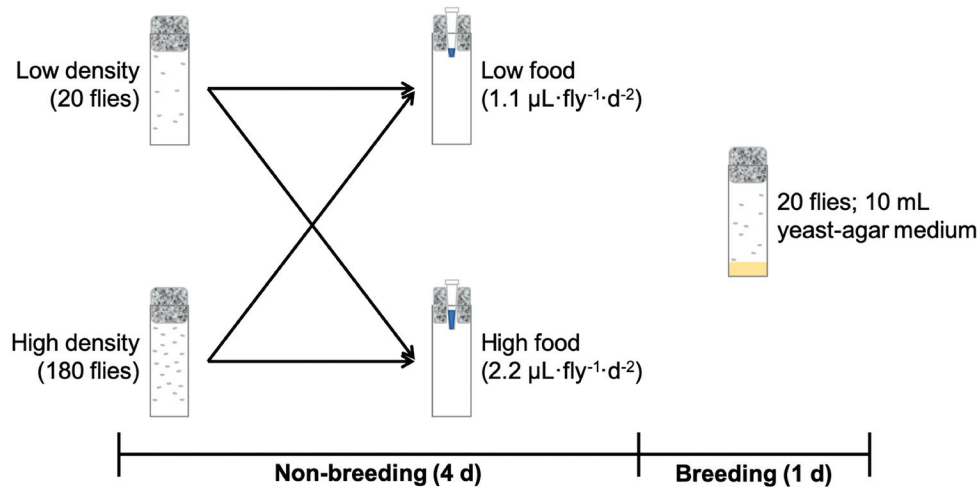
Received 26 November 2019. Accepted 12 March 2020.

J.B. Burant, A. Griffin, and G.S. Betini. Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada.
D.R. Norris. Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada; Nature Conservancy of Canada, 245 Eglinton Avenue East, Suite 410, Toronto, ON M4P 3J1, Canada.

Corresponding author: Joseph B. Burant (email: jburant@uoguelph.ca).

Copyright remains with the author(s) or their institution(s). Permission for reuse (free in most cases) can be obtained from copyright.com.

Fig. 1. Schematic of the treatments and the experimental setup used to simulate seasonality in common fruit fly (*Drosophila melanogaster*) populations. Flies spent 4 days in the non-breeding season, where they were fed 5% (*m/v*) sugar water and exposed to one of four treatments that consisted of a combination of density (20 or 180 flies) and food availability (2.22 or $1.11 \mu\text{L}\cdot\text{fly}^{-1}\cdot\text{d}^{-2}$) manipulations. Those that survived were transferred to the breeding season, where they remained for 24 h. During the breeding season, each replicate was maintained at a population density of 20 flies and was fed 10 mL of a protein-rich food. Colour version online.



effects of increased density on reproductive success and survival within a season have been well documented in a variety of taxa (Åström et al. 1996; Frederiksen and Bregnballe 2000; Szostek et al. 2014). However, changes in density in one part of the annual cycle could also carry over to influence individual success in the following season. Experimental evidence has shown that increased conspecific density during the non-breeding period negatively influences individual condition, which then carries over to affect per capita reproductive output and dispersal during the subsequent breeding period (Betini et al. 2013a, 2013b, 2015). These density-mediated carry-over effects on individuals can also influence the stability of long-term population dynamics and intrinsic rates of population growth (Betini et al. 2013b, 2015).

While it is clear that variation in conspecific density leads to carry-over effects, the specific ecological mechanism that links density with individual condition and reproductive output is still unclear. One possibility is that, given a specific resource quantity, high densities simply reduce per capita resource availability in the non-breeding period season, resulting in poor condition and lower reproductive success the following season (hereafter the resource limitation hypothesis). Previous work has demonstrated that, in many cases, fluctuations in fecundity can be explained by variation in food availability (Robertson and Sang 1944; Harshman et al. 1988; Richardson and Baker 1997; Sorensen et al. 2009; Wells et al. 2016) and associated changes in physiological condition (Lefranc and Bundgaard 2000). Likewise, there is evidence that food availability, independent of density, can act to stimulate aggression in common fruit flies (*Drosophila melanogaster* Meigen, 1830) (Lim et al. 2014). A second possibility is that, by virtue of being at high densities, individuals experience an increase in the number of conspecific interactions, many of which could be antagonistic in nature. In European Starlings (*Sturnus vulgaris* Linnaeus, 1758), for example, experimental elevation of conspecific density resulted in more aggressive interactions between residents and intruders, resulting in a change in foraging behaviour and general activity (Nephew and Romero 2003). In *D. melanogaster*, increased population density results in differential survival of individuals displaying different levels of aggression (Kilgour et al. 2018). Time and energy spent defending against such interactions, regardless of absolute food availability, could alter levels of stored energy available for reproduction the following season (hereafter the overcrowding hypothesis). Of course, these hypotheses are not

mutually exclusive because both mechanisms, in theory, could be operating at the same time.

Here, we examine these hypotheses using a full-factorial experiment in populations of *D. melanogaster* with distinct breeding and non-breeding periods. During the non-breeding period, we manipulated either food availability (high versus low per capita food) or density (high versus low density) and then examined body condition (dry mass) at the beginning of breeding period and total reproductive output during the breeding period. Following the resource limitation hypothesis, we predicted that, compared with the high food treatments, individuals in low food treatments would have lower body mass and that these populations would have lower per capita reproductive output. Following the overcrowding hypothesis, we predicted that, relative to the low-density treatments, individuals in the high-density treatments would have lower body mass and that these populations would have lower per capita reproductive output. Given that these hypotheses are not mutually exclusive, it is possible that there would be interactive effects when both treatments are applied.

Materials and methods

Experimental system

Following previous studies (Betini et al. 2013a, 2013b; Elliott et al. 2016), we used *D. melanogaster* from an outbred stock population collected in Benin in 1970 and maintained in a cage culture at 25 °C prior to the application of the treatments. During the experiment, flies were maintained at 25 °C, 12 h light : 12 h dark photo period, with 30% to 50% relative humidity. In all stages, populations were housed in polypropylene vials of the same size (28 mm × 95 mm; VWR catalogue No. 75813–156).

Prior to experimentation, flies from the aseasonal (i.e., typical constant-breeding conditions) stock population were acclimated to seasonal conditions for one generation. In this acclimatization period and throughout the experiment, seasonality was induced by manipulating the quality of food provided. During the non-breeding period, *D. melanogaster* were fed specific volumes of 5% (*m/v*) sugar water ($0.20 \text{ mL}\cdot\text{d}^{-1}$ during the acclimatization generation) through a small hole in the bottom of a microcentrifuge tube located at the top of the vial (Fig. 1). This method of feeding prevented females from producing and laying eggs because it provided neither the protein needed for egg production nor a

suitable substrate upon which to oviposit (Bownes and Blair 1986; Elliott et al. 2016). The breeding period was simulated by provisioning the flies with 10 mL of a dead yeast–agar medium (see formula in Betini et al. 2013a; Fig. 1). On reintroduction to this protein-rich food source, the ability of females to lay eggs recovers within approximately 12 h (Betini et al. 2013b).

We used a 16-d cycle with discrete generations and two distinct periods (breeding and non-breeding) in each generation, following protocols outlined by Elliott et al. (2016). After a 24 h breeding period, adult flies were removed and their eggs were left to develop over the course of 12 d. We discarded all offspring that emerged prior to day 9 and transferred flies that emerged between days 9 through 12 to holding vials containing 5 mL of fresh yeast–agar medium. This allowed us to control for differences in body size between offspring that emerge under different densities, with the vast majority of flies emerging between 9 and 13 d (Elliott et al. 2016). On day 13, we lightly anesthetized the flies with CO₂ and randomly sorted them into groups with equal numbers of males and females. These groups were then transferred to non-breeding vials where they remained until day 16, after which they were again lightly anesthetized with CO₂, recounted, and transferred to new breeding vials for a 24 h period. During the acclimatization generation, all populations were maintained at low densities (20 flies; 10 males and 10 females) so that all individuals start the experiment in similar body condition. Densities were later adjusted with the application of each treatment (see below).

Food and density treatments

To test the ecological mechanisms through which non-breeding density carries over to influence reproduction, we independently manipulated population density and food availability during the non-breeding period. Following a factorial design, we created a total of four treatments by combining high (180 flies) and low (20 flies) densities with high (2.22 $\mu\text{L}\cdot\text{fly}^{-1}\cdot\text{d}^{-2}$) and low (1.11 $\mu\text{L}\cdot\text{fly}^{-1}\cdot\text{d}^{-2}$) per capita food availability (i.e., low- and high-density populations received 22.2 and 199.8 $\mu\text{L}\cdot\text{d}^{-1}$ under low-food treatments, respectively, and 44.4 and 399.6 $\mu\text{L}\cdot\text{d}^{-1}$ under high-food treatments, respectively). Separating the effects of food availability and density in this way allowed us to determine the potential ecological mechanisms driving carry-over effects in high-density populations (Betini et al. 2013a).

Replicate populations were exposed to one of the four treatments during the non-breeding period, after which we recorded mortality rates and transferred the survivors to breeding vials. We standardized breeding period conditions following the non-breeding treatment so differences in reproductive output could be solely attributed to differences in non-breeding food availability and population density (Betini et al. 2013b). Across all treatments, the conditions during the subsequent breeding period were the same: adult breeding density was maintained at low density (20 flies; 10 males and 10 females) and food availability was high (10 mL yeast–agar medium). For the low-density treatments (LDLF: $n = 9$; LDHF: $n = 12$), this involved combining individuals from two or more replicates to maintain breeding population density at 20 flies. For the high-density treatments (HDLF: $n = 20$; HDHF: $n = 22$), breeding individuals were randomly sampled from the population at the end of the non-breeding period.

Body condition and reproductive output

At the beginning of the breeding period, before the flies were transferred between vials, we measured the body condition of five randomly selected females from each population. Dry mass has been previously used as a metric of body size (Betini et al. 2014), with condition often measured as size-corrected mass (Droney 1998). However, given that the breeding density of flies was maintained across all treatments, we did not anticipate offspring structural size differences among treatments (Ashburner and Thompson 1978). Dry body mass was used as an index of body condition because,

in other species, body mass has provided a good indication of body nutrient contents and was easily and reliably measured (Labocha and Hayes 2011). We used only female flies to preclude the introduction of sex differences in body mass (Carreira et al. 2009) and because reproduction in *D. melanogaster* is female-limited (Morgan 1913). Dry masses were taken by first drying the selected flies for 24 h at 55 °C before weighing them on an electronic balance (Mettler Toledo XP26; readability (scale division value) = 0.001 mg).

To estimate per capita reproductive output at the end of the breeding period, we counted the total number of eggs laid and divided by adult population size. To facilitate counting, we dyed the agar medium with green food colouring to provide contrast to the off-white eggs. We then took macroscopic photos of the medium and used them to determine the egg count. To count the eggs, we divided the vials into six sectors and counted the eggs manually in each sector. A subsequent recount of five random vials was used to estimate repeatability (Pearson's $r = 0.97$), with all counts conducted by the same observer (A.G.).

Statistical analysis

To test the effect of treatment (non-breeding food availability and population density) on body condition, we used a generalized linear mixed model (GLMM; Gaussian) that included a two-way interaction between food availability (two-level factor: low, high) and density (two-level factor: low, high). Since multiple individuals were weighed from each replicate, vial ID was fitted as a random effect. We used a general linear model (GLM) to test the effects of food availability and density on reproductive output. Since per capita reproductive output was estimated as a mean for each replicate, this model only included the interaction between food availability and density and does not include vial ID as a random effect.

For both body condition and reproductive output, 95% confidence intervals (95% CIs) on the mean (β) pairwise effect sizes of independent changes in density and food availability were estimated using bootstrapping (sample size = 5000) as the mean difference between groups (Cohen 1994; Ho et al. 2019). Bootstrapping was performed using randomized resampling with replacement. For both statistical models, we used low density and low food as the reference levels. For the GLMM of body condition, we calculated both marginal R^2 (includes just the fixed effects) and conditional R^2 (includes random and fixed effects) R^2 values as estimates of model fit (Nakagawa and Schielzeth 2013). For all tests, effects were considered significant at $\alpha = 0.05$ threshold.

All analyses were conducted in the R statistical environment version 3.5.0 (R Core Team 2018). The GLMM was fitted using the lme4 package (Bates et al. 2015), with p values estimated using lmerTest (Kuznetsova et al. 2017). Bootstrapped estimates of pairwise treatment effects were taken using the dabestr package (Ho et al. 2019). The results are presented using Gardner–Altman difference estimation plots, which have been advocated as an informative, data-centered tool for visualizing treatment effects (Halsey 2019; Ho et al. 2019). The data used for the analyses are publicly available through the Figshare open access repository at <https://doi.org/10.6084/m9.figshare.12009516.v1> (Burant et al. 2020).

Results

Body condition

Our results provided evidence that only per capita food availability during the non-breeding period, not non-breeding density, influenced body condition of individuals at the beginning of the breeding period (GLMM; marginal $R^2 = 0.18$, conditional $R^2 = 0.32$; Table 1). Consistent with the prediction from the resource limitation hypothesis, increased food availability during the non-breeding period resulted in heavier flies at the start of the breeding period (Figs. 2A–2D). At the beginning of the breeding period, individuals in the low-density and high-food treatment

Table 1. The effects of food availability and population density on common fruit fly (*Drosophila melanogaster*) body mass and per capita reproductive output obtained from linear models.

Dry mass				
Fixed effects	Estimate (β)	Standard error (SE)	<i>t</i>	<i>p</i>
(Intercept)	0.311	0.014	23.04	<0.0001
Density (HD)	-0.016	0.016	-0.98	0.334
Food (HF)	0.048	0.016	2.94	0.005
Density (HD) \times food (HF) interaction	-0.011	0.020	-0.54	0.593
Random effects				
	Estimate (σ^2)	Standard deviation (σ)		
Vial ID	0.0005	0.022		
Residual	0.0024	0.049		
Reproduction				
Fixed effects	Estimate (β)	Standard error (SE)	<i>t</i>	<i>p</i>
(Intercept)	6.61	0.53	12.48	<0.0001
Density (HD)	-1.50	0.64	-2.35	0.022
Food (HF)	-0.76	0.70	-1.09	0.282
Density (HD) \times food (HF) interaction	1.83	0.85	2.14	0.036

Note: In both models, low density (LD) and low (LF) food availability were used as the reference levels.

(LDHF = 0.36 ± 0.06 mg (mean \pm SE)) weighed, on average, 14% more (mean difference between treatments: $\beta = 0.050$ mg and 95% CI = (0.019 mg, 0.077 mg)) than individuals in the low-density and low-food treatment (LDLF = 0.31 ± 0.06 mg; Fig. 2A), whereas individuals in the high-density and high-food treatment (HDHF = 0.33 ± 0.05 mg) weighed, on average, 11% more ($\beta = 0.036$ mg and 95% CI = (0.020 mg, 0.051 mg)) than individuals in the high-density and low-food treatment (HDLF = 0.30 ± 0.04 mg; Fig. 2B).

Consistent with the model results, there was a smaller effect of density on body condition compared with the effect of food availability (Figs. 2A–2D, Table 1). At the beginning of the breeding period, body condition of individuals in the low-density and low-food treatment was, on average, 4% greater than individuals in the high-density and low-food treatment ($\beta = -0.013$ and 95% CI = (-0.043, 0.012); Fig. 2C), whereas individuals in the low-density and high-food treatment were, on average, 8% heavier than individuals in the high-density and high-food treatment ($\beta = -0.027$ and 95% CI = (-0.048, -0.007); Fig. 2D).

Per capita reproductive output

A GLM with non-breeding food availability, population density, and the interaction between the two factors explained 11% of the variation in reproductive output (GLM; $R^2 = 0.11$, $F_{[3,59]} = 2.45$, $p = 0.07$; Table 1). Reproductive output was predicted by the interaction between food and density (Table 1), such that per capita reproductive output was lowest when populations were at high density and low food availability (Table 1, Figs. 3A–3D).

On average, populations subject to the low-density and high-food treatment (LDHF = 5.9 ± 1.5 eggs-individual⁻¹) produced 11% fewer eggs per capita (mean difference between treatments: $\beta = -0.076$ eggs-individual⁻¹ and 95% CI = (-1.90 eggs-individual⁻¹, 0.42 eggs-individual⁻¹)) than those in the low-density and low-food treatment (LDLF = 6.6 ± 1.3 eggs-individual⁻¹; Fig. 3A). In contrast, per capita reproductive output was 17% greater ($\beta = 1.07$ eggs-individual⁻¹ and 95% CI = (0.11 eggs-individual⁻¹, 2.08 eggs-individual⁻¹)) in the high-density and high-food treatment (HDHF = 6.2 ± 1.6 eggs-individual⁻¹) than in the high-density and low-food treatment (LDLF = 5.1 ± 1.8 eggs-individual⁻¹; Fig. 3B). When non-breeding food availability was low, per capita reproductive output was lower at high density ($\beta = -1.50$ eggs-individual⁻¹ and 95% CI = (-2.57 eggs-individual⁻¹, -0.32 eggs-individual⁻¹); Fig. 3C), whereas there was little effect of density when per capita food availability was high ($\beta = 0.34$ eggs-individual⁻¹ and 95% CI = (-0.74 eggs-individual⁻¹, 1.38 eggs-individual⁻¹); Fig. 3D).

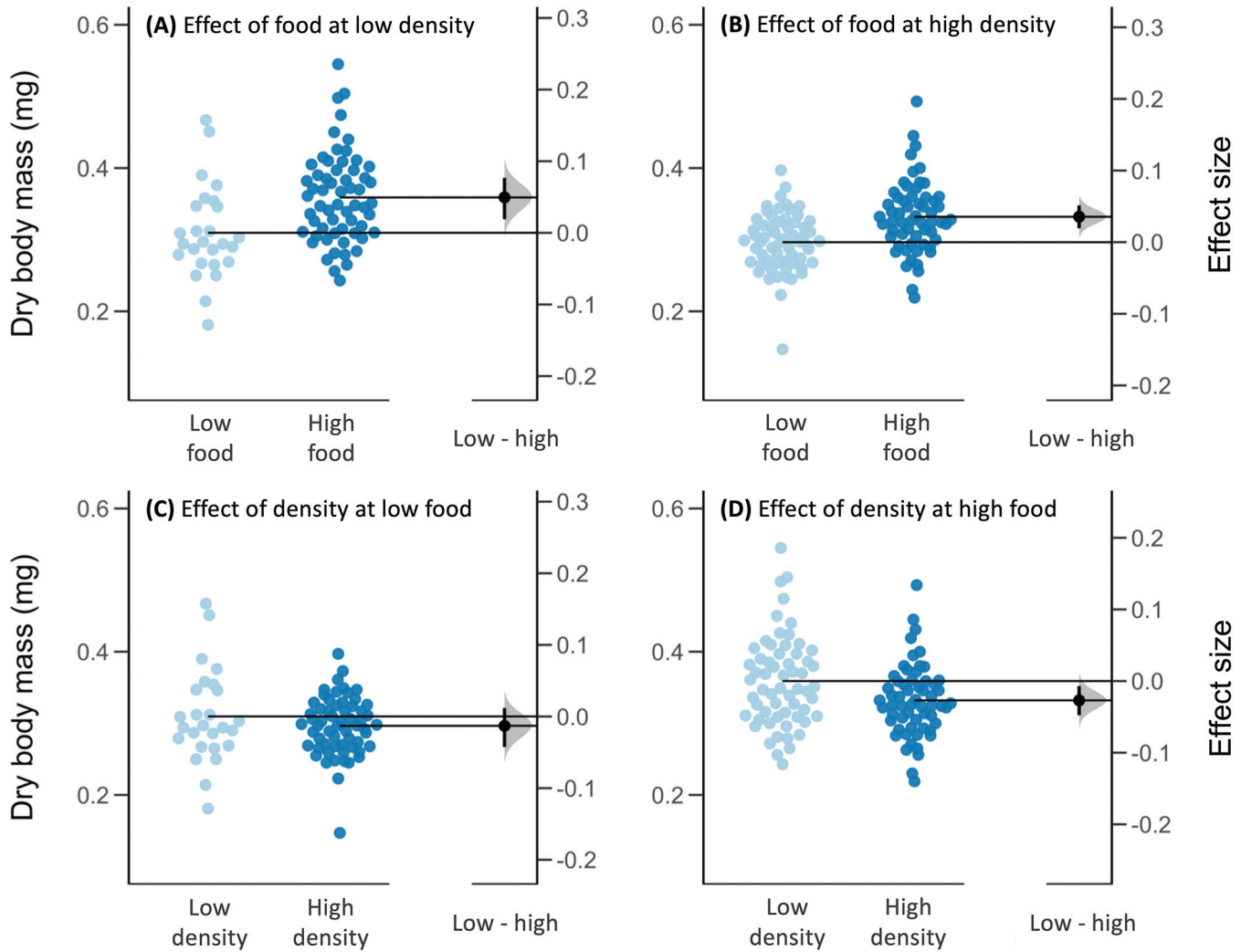
Discussion

Our results indicate that non-breeding food availability has important implications for the condition of individuals at the beginning of the breeding period, and that the interactive effects of non-breeding food availability and population density can generate carry-over effects on subsequent reproductive output. Body condition at the start of the breeding period was worse in individuals fed low per capita volumes of food when compared with individuals fed high per capita volumes. Consistent with the resource limitation hypothesis, any differences in body condition can be attributed to differences in food availability given that similar effects were seen at two different population densities. Upon entering the breeding season, the effects of low food availability on body condition carried over to negatively influence per capita reproductive output, but only for populations exposed to high non-breeding densities. This latter result suggests that with high non-breeding densities, resource limitation drives carry-over effects on reproductive output, whereas when per capita food availability is low, these effects result from overcrowding.

We found a positive effect of per capita food availability on individual body condition (dry mass), which suggests that resource limitation, and not population density per se, acts as the proximate mechanism for driving density-mediated carry-over effects on condition. Whereas previous studies have shown that individual condition decreases with population density (Ashburner and Thompson 1978; Betini et al. 2013a, 2017), these experiments held the total volume of food provided to a population constant and so any change in population density had the effect of increasing or decreasing per capita food availability. Here, we show that when food availability scales with population density, there is no apparent impact on individual condition (but see below). The positive effect of food availability on individual condition is interesting in light of the known positive relationship between body size and fecundity, particularly for female *D. melanogaster* (Robertson 1957; Lefranc and Bundgaard 2000).

Despite the absence of evidence for an effect of non-breeding density on individual condition at the start of the breeding period, the frequency of aggressive interactions between conspecifics is expected to change with density and access to food. While there was no effect of density on body condition when per capita food availability was low, when food availability was high, individuals in high-density populations were in poorer condition than individuals in low-density populations (although this difference was not significant in our statistical models, Fig. 2D provides evidence

Fig. 2. Effects of non-breeding per capita food availability and population density on individual body condition of common fruit flies (*Drosophila melanogaster*) at the beginning of the breeding season. Populations were exposed to one of four treatments that consisted of a combination of low (20 flies; LD) or high (180 flies; HD) density and low ($1.11 \mu\text{L}\cdot\text{fly}^{-1}\cdot\text{d}^{-2}$; LF) or high ($2.22 \mu\text{L}\cdot\text{fly}^{-1}\cdot\text{d}^{-2}$; HF) food availability. Body condition was measured as dry mass (mg) of flies sampled at the beginning of the breeding period. Effect sizes were estimated as the mean pairwise difference (β) between treatments, with 95% confidence intervals (95% CIs) calculated with bootstrapping (see Statistical analysis in the Materials and methods). For each comparison, the low treatment (LD or LF) is shaded in light blue (light grey in print) and the high treatment (HD or HF) is shaded in dark blue (dark grey in print). Colour version online.

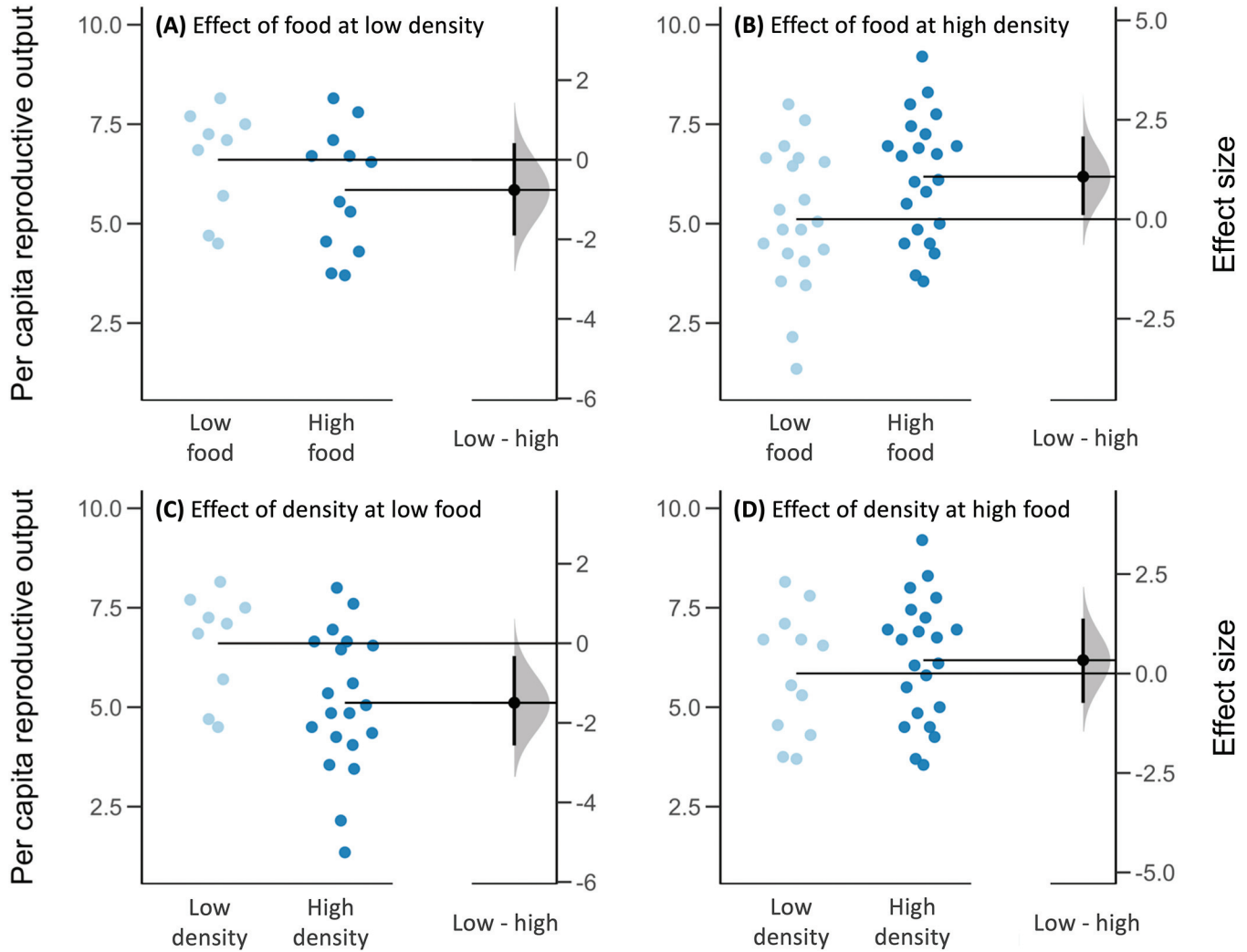


of a negative effect of density with high food availability). This result may indicate that once basic nutritional requirements are satisfied (which may not be the case with low per capita food availability), further differences in condition are driven by density. Previous work has shown that increased aggressive intraspecific interactions can limit foraging intake rates (Goss-Custard et al. 1984; Janson 1985), and that the relative frequency of different foraging strategies can be density-dependent (Sokolowski et al. 1997). Moreover, rates of aggressive interactions and ultimately foraging rates can also be determined by variation in food availability (Hodge et al. 2009; Lim et al. 2014) or group composition (Kilgour et al. 2018).

The interactive effect between food and density on reproductive output was primarily driven by a decrease in reproductive output at high densities when food was limited, while a similar density-mediated effect was not found when food was abundant. One possible explanation for this density-mediated effect of food availability on reproductive output is that non-breeding food availability drives reproductive plasticity and variable investment in reproductive structures (Gage 1995; Bretman et al. 2009; Wigby

et al. 2016). Although we only measured body condition in females, the effects of food availability on condition are likely similar in males (Ashburner and Thompson 1978), and change in reproductive output is likely to be a product of variable investment by both sexes. For example, food limitation has been shown to lead to a decrease in both condition and mating receptivity of female *D. melanogaster* (Churchill et al. 2019). Poor condition, in conjunction with density-dependent reproduction (whereby females produce fewer eggs as density increases; see, e.g., Betini et al. 2013b), results in decreased reproductive output by females. In males, high perceived risk of sperm competition at high densities has been shown to increase ejaculate and testes investment, as well as copulation duration (Bretman et al. 2009; Lizé et al. 2011; Wigby et al. 2016). This may help to explain why, despite having reduced body condition, populations exposed to high non-breeding density had similar per capita reproductive outputs to those exposed to low density, but only when food availability was high. In contrast, when food availability is low, differential investment in reproductive structures in response to changes in density may not be viable since individuals direct these limited resources

Fig. 3. Effects of non-breeding per capita food availability and population density on reproductive output of common fruit fly (*Drosophila melanogaster*) populations in the breeding season. Following 4 days of non-breeding conditions comprising low (20 flies; LD) or high (180 flies; HD) density and low (1.11 $\mu\text{L}\cdot\text{fly}^{-1}\cdot\text{d}^{-2}$; LF) or high (2.22 $\mu\text{L}\cdot\text{fly}^{-1}\cdot\text{d}^{-2}$; HF) food availability, flies were transferred to new breeding vials to reproduce for 24 h (see Food and density treatments in the Materials and methods). Per capita reproductive output was calculated for each vial as the mean number of eggs produced per individual. Effect sizes were estimated as the mean pairwise difference (β) between treatments, with 95% confidence intervals (95% CIs) calculated with bootstrapping (see Statistical analysis in the Materials and methods). For each comparison, the low treatment (LD or LF) is shaded in light blue (light grey in print) and the high treatment (HD or HF) is shaded in dark blue (dark grey in print). Colour version online.



towards self-maintenance, resulting in decreased reproductive performance at high density.

In our experiment, we independently manipulated per capita food availability and population density during the non-breeding period to investigate the origins of carry-over effects on subsequent reproduction. Our choices of low-density (20 flies) and high-density (180 flies) conditions were based on previous work in the seasonal *D. melanogaster* system (Betini et al. 2013a, 2015), which showed that carry-over effects begin to arise at high non-breeding density (Betini et al. 2013b). That said, our experimental design may not have captured the full range of density effects on reproduction, particularly for the larger non-breeding population sizes (>300 flies) that regularly occur in this system (Betini et al. 2017; Burant et al. 2019). In addition, although we manipulated the per capita volume of food provisioned in the non-breeding period, we were unable to control access to the food tip, which is known to change with population density (Kilgour et al. 2020). As a result, our food manipulations may not have reflected the actual volume of food consumed per individual. This is supported by the fact that a doubling of per capita food availability resulted in a mere 11%

14% increase in body mass, and that much of the food remained unconsumed in the high-food treatments (J.B.B., personal observation). These considerations, in conjunction with limited sample sizes for low-density treatments (due to the necessity of aggregating flies from multiple replicates; see Food and density treatments in the Materials and methods), may have limited our ability to assess the effects of low food and low density on condition and reproductive output.

Based on the expected prevalence of density dependence in the wild and the influence of density-mediated carry-over effects on population dynamics (Norris 2005; Morrissette et al. 2010; Van Allen et al. 2010; Betini et al. 2013a; Szostek et al. 2014), our results may be applicable to the conservation and management of a wide range of seasonal taxa. Understanding the mechanisms and drivers of carry-over effects provides an opportunity to mitigate potentially negative carry-over effects in the natural environment before they can impact the dynamics of at-risk populations or impede conservation. With current trends in human development and global climate change, suitable habitats and regional food availability are becoming increasingly scarce and heterogeneous (Both et al.

2006; Calizza et al. 2017). Common approaches to conserving or managing declining populations include the manipulation of food availability (e.g., through supplementation) and (or) density (e.g., through the removal or introduction of individuals), with these approaches varying in their efficacy and feasibility. In the context of density-mediated carry-over effects, and given the difficulties inherent in permanently manipulating local population densities, our results suggest management strategies targeting populations in seasonal environments should focus on maintaining per capita food availability at optimal levels during wintering or non-breeding periods, rather than altering other density-related factors or conditions during the breeding season.

These results bolster the growing body of evidence pointing to the importance of habitat quality and food availability during the non-breeding period. Our results suggest that food limitation is the proximate mechanism that drives carry-over effects when populations are at high densities. Although increased aggressive interactions at high population density are a proximate mechanism that may influence individual condition, such effects might be offset by plasticity in sexual development and behaviour. Our study also points to the importance of events throughout the life of seasonal species for understanding population dynamics and ultimately long-term population stability.

Author contributions

All authors were involved in initial discussions and experimental design. A.G. and J.B.B. conducted the experiment. A.G. wrote the first draft. J.B.B. performed the statistical analysis and prepared later drafts of the manuscript. All authors contributed to revisions.

Conflict of interest statement

The authors declare no competing interests.

Acknowledgements

We thank the two anonymous reviewers who provided valuable feedback that improved the clarity of our work. This research was funded by a Discovery Grant to D.R.N. from the Natural Sciences and Engineering Research Council of Canada. J.B.B. was supported by a Graduate Tuition Scholarship from the University of Guelph and a Queen Elizabeth II Graduate Scholarship in Science and Technology from the Government of Ontario.

References

- Ashburner, M., and Thompson, J.N. 1978. The laboratory culture of *Drosophila*. In *The genetics and biology of Drosophila*. Edited by M. Ashburner and T.R.F. Wright. Academic Press, London, U.K. pp. 1–109.
- Åström, M., Lundberg, P., and Lundberg, S. 1996. Population dynamics with sequential density-dependencies. *Oikos*, **75**(2): 174–181. doi:10.2307/3546241.
- Bates, D., Maechler, M., Bolker, B., and Walker, S. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Software*, **67**(1): 1–48. doi:10.18637/jss.v067.i01.
- Betini, G.S., Griswold, C.K., and Norris, D.R. 2013a. Density-mediated carry-over effects explain variation in breeding output across time in a seasonal population. *Biol. Lett.* **9**(5): 20130582. doi:10.1098/rsbl.2013.0582. PMID:23925837.
- Betini, G.S., Griswold, C.K., and Norris, D.R. 2013b. Carry-over effects, sequential density dependence and the dynamics of populations in a seasonal environment. *Proc. R. Soc. B Biol. Sci.* **280**(1759): 20130110. doi:10.1098/rspb.2013.0110. PMID:23516241.
- Betini, G.S., Griswold, C.K., Prodan, L., and Norris, D.R. 2014. Body size, carry-over effects and survival in seasonal environment: consequences for population dynamics. *J. Anim. Ecol.* **83**(6): 1313–1321. doi:10.1111/1365-2656.12225. PMID:24708450.
- Betini, G.S., Pardy, A., Griswold, C.K., and Norris, D.R. 2015. The role of seasonality and non-lethal carry-over effects on density-dependent dispersal. *Ecosphere*, **6**(12): 1–9. doi:10.1890/ES15-00257.1.
- Betini, G.S., McAdam, A.G., Griswold, C.K., and Norris, D.R. 2017. A fitness trade-off between seasons causes multigenerational cycles in phenotype and population size. *Elife*, **6**: e18770. doi:10.7554/eLife.18770. PMID:28164780.
- Both, C., Bouwhuis, S., Lessells, C.M., and Visser, M.E. 2006. Climate change and population declines in a long-distance migratory bird. *Nature*, **441**(7089): 81–83. doi:10.1038/nature04539. PMID:16672969.
- Bownes, M., and Blair, M. 1986. The effects of a sugar diet and hormones on the expression of the *Drosophila* yolk-protein genes. *J. Insect Physiol.* **32**(5): 493–501. doi:10.1016/0022-1910(86)90011-9.
- Bretman, A., Fricke, C., and Chapman, T. 2009. Plastic responses of male *Drosophila melanogaster* to the level of sperm competition increase reproductive fitness. *Proc. R. Soc. B Biol. Sci.* **276**(1662): 1705–1711. doi:10.1098/rspb.2008.1878. PMID:19324834.
- Bunnell, D.B., Thomas, S.E., and Stein, R.A. 2007. Prey resources before spawning influence gonadal investment of female, but not male, white crappie. *J. Fish Biol.* **70**(6): 1838–1854. doi:10.1111/j.1095-8649.2007.01459.x.
- Burant, J.B., Betini, G.S., and Norris, D.R. 2019. Simple signals indicate which period of the annual cycle drives declines in seasonal populations. *Ecol. Lett.* **22**(12): 1241–1250. doi:10.1111/ele.13393.
- Burant, J.B., Griffin, A., Betini, G.S., and Norris, D.R. 2020. Data from: An experimental test of the ecological mechanisms driving density-mediated carry-over effects in a seasonal population. Available from Figshare repository (posted on 19 March 2020; accessed recently on 29 May 2020). doi:10.6084/m9.figshare.12009516.v1.
- Calizza, E., Costantini, M.L., Careddu, G., and Rossi, L. 2017. Effect of habitat degradation on competition, carrying capacity, and species assemblage stability. *Ecol. Evol.* **7**(15): 5784–5796. doi:10.1002/ece3.2977. PMID:28811883.
- Carreira, V.P., Mensch, J., and Fanara, J.J. 2009. Body size in *Drosophila*: genetic architecture, allometries and sexual dimorphism. *Heredity*, **102**(3): 246–256. doi:10.1038/hdy.2008.117. PMID:19018274.
- Churchill, E.R., Dytham, C., and Thom, M.D.F. 2019. Differing effects of age and starvation on reproductive performance in *Drosophila melanogaster*. *Sci. Rep.* **9**: 2167. doi:10.1038/s41598-019-38843-w. PMID:30770855.
- Cohen, J. 1994. The earth is round ($p < .05$). *Am. Psychol.* **49**(12): 997–1003. doi:10.1037/0003-066X.49.12.997.
- Cook, J.G., Johnson, B.K., Cook, R.C., Riggs, R.A., Delcurto, T., Bryant, L.D., and Irwin, L.L. 2004. Effects of summer-autumn nutrition and parturition date on reproduction and survival of elk. *Wildl. Monogr.* **155**(1): 1–61. doi:10.2193/0084-0173(2004)155[1:EOSNAP]2.0.CO;2.
- Costantini, D., Carello, L., and Dell’Omo, G. 2010. Patterns of covariation among weather conditions, winter North Atlantic Oscillation index and reproductive traits in Mediterranean kestrels. *J. Zool.* **280**(2): 177–184. doi:10.1111/j.1469-7998.2009.00649.x.
- Droney, D.C. 1998. The influence of the nutritional content of the adult male diet on testis mass, body condition and courtship vigour in a Hawaiian *Drosophila*. *Funct. Ecol.* **12**(6): 920–928. doi:10.1046/j.1365-2435.1998.00266.x.
- Duriez, O., Ens, B.J., Choquet, R., Pradel, R., and Klaassen, M. 2012. Comparing the seasonal survival of resident and migratory oystercatchers: carry-over effects of habitat quality and weather conditions. *Oikos*, **121**(6): 862–873. doi:10.1111/j.1600-0706.2012.20326.x.
- Elliott, K.H., Betini, G.S., Dworkin, I., and Norris, D.R. 2016. Experimental evidence for within- and cross-seasonal effects of fear on survival and reproduction. *J. Anim. Ecol.* **85**(2): 507–515. doi:10.1111/1365-2656.12487. PMID:26713420.
- Frederiksen, M., and Bregnballe, T. 2000. Evidence for density-dependent survival in adult cormorants from a combined analysis of recoveries and resightings. *J. Anim. Ecol.* **69**(5): 737–752. doi:10.1046/j.1365-2656.2000.00435.x. PMID:29313997.
- Gage, M.J.G. 1995. Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proc. R. Soc. B Biol. Sci.* **261**(1360): 25–30. doi:10.1098/rspb.1995.0112.
- Goss-Custard, J.D., Clarke, R.T., and Dit Durell, S.E.A.V. 1984. Rates of food intake and aggression of oystercatchers *Haematopus ostralegus* on the most and least preferred mussel *Mytilus edulis* beds of the Exe Estuary. *J. Anim. Ecol.* **53**(1): 233–245. doi:10.2307/4354.
- Halsey, L.G. 2019. The reign of the p -value is over: what alternative analyses could we employ to fill the power vacuum? *Biol. Lett.* **15**(5): 20190174. doi:10.1098/rsbl.2019.0174. PMID:31113309.
- Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R., and Bearhop, S. 2011. Carry-over effects as drivers of fitness differences in animals. *J. Anim. Ecol.* **80**(1): 4–18. doi:10.1111/j.1365-2656.2010.01740.x. PMID:20726924.
- Harshman, L.G., Hoffmann, A.A., and Prout, T. 1988. Environmental effects on remating in *Drosophila melanogaster*. *Evolution*, **42**(2): 312–321. doi:10.1111/j.1558-5646.1988.tb04135.x. PMID:28567838.
- Ho, J., Tumkaya, T., Aryal, S., Choi, H., and Claridge-Chang, A. 2019. Moving beyond P values: data analysis with estimation graphics. *Nat. Methods*, **16**(7): 565–566. doi:10.1038/s41592-019-0470-3. PMID:31217592.
- Hodge, S.J., Thornton, A., Flower, T.P., and Clutton-Brock, T.H. 2009. Food limitation increases aggression in juvenile meerkats. *Behav. Ecol.* **20**(5): 930–935. doi:10.1093/beheco/arp071.
- Inger, R., Harrison, X.A., Ruxton, G.D., Newton, J., Colhoun, K., Gudmundsson, G., et al. 2010. Carry-over effects reveal reproductive costs in a long-distance migrant. *J. Anim. Ecol.* **79**(5): 974–982. doi:10.1111/j.1365-2656.2010.01712.x. PMID:20579179.
- Janson, C. 1985. Aggressive competition and individual food consumption in wild brown capuchin monkeys (*Cebus apella*). *Behav. Ecol. Sociobiol.* **18**(2): 125–138. doi:10.1007/BF00299041.
- Kilgour, R.J., McAdam, A.G., Betini, G.S., and Norris, D.R. 2018. Experimental evidence that density mediates negative frequency-dependent selection on

- aggression. *J. Anim. Ecol.* **87**(4): 1091–1101. doi:10.1111/1365-2656.12813. PMID:29446094.
- Kilgour, R.J., Norris, D.R., and McAdam, A.G. 2020. Carry-over effects of resource competition and social environment on aggression. *Behav. Ecol.* **31**(1): 140–151. doi:10.1093/beheco/arz170.
- Kuznetsova, A., Brockhoff, P.B., and Christensen, R.H.B. 2017. lmerTest package: tests in linear mixed effects models. *J. Stat. Softw.* **82**(13): 1–26. doi:10.18637/jss.v082.i13.
- Labocha, M.K., and Hayes, J.P. 2011. Morphometric indices of body condition in birds: a review. *J. Ornithol.* **153**(1): 1–22. doi:10.1007/s10336-011-0706-1.
- Lefranc, A., and Bundgaard, J. 2000. The influence of male and female body size on copulation duration and fecundity in *Drosophila melanogaster*. *Hereditas*, **132**(3): 243–247. doi:10.1111/j.1601-5223.2000.00243.x. PMID:11075519.
- Lim, R.S., Eyjólfsson, E., Shin, E., Perona, P., and Anderson, D.J. 2014. How food controls aggression in *Drosophila*. *PLoS ONE*, **9**(8): e105626. doi:10.1371/journal.pone.0105626. PMID:25162609.
- Liz, E., and Ruiz-Herrera, A. 2016. Potential impact of carry-over effects in the dynamics and management of seasonal populations. *PLoS ONE*, **11**(5): e0155579. doi:10.1371/journal.pone.0155579. PMID:27171267.
- Lizé, A., Doff, R.J., Smaller, E.A., Lewis, Z., and Hurst, G.D.D. 2011. Perception of male–male competition influences *Drosophila* copulation behaviour even in species where females rarely remate. *Biol. Lett.* **8**(1): 35–38. doi:10.1098/rsbl.2011.0544. PMID:21752815.
- Morgan, T.H. 1913. *Heredity and sex*. Columbia University Press, New York.
- Morrisette, M., Bête, J., Gauthier, G., Reed, A., and Lefebvre, J. 2010. Climate, trophic interactions, density dependence and carry-over effects on the population productivity of a migratory Arctic herbivorous bird. *Oikos*, **119**(7): 1181–1191. doi:10.1111/j.1600-0706.2009.18079.x.
- Nakagawa, S., and Schielzeth, H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**(2): 133–142. doi:10.1111/j.2041-210x.2012.00261.x.
- Nephew, B.C., and Romero, L.M. 2003. Behavioral, physiological, and endocrine responses of starlings to acute increases in density. *Horm. Behav.* **44**(3): 222–232. doi:10.1016/j.yhbeh.2003.06.002. PMID:14609544.
- Norris, D.R. 2005. Carry-over effects and habitat quality in migratory populations. *Oikos*, **109**(1): 178–186. doi:10.1111/j.0030-1299.2005.13671.x.
- Norris, D.R., Marra, P.P., Kyser, T.K., Sherry, T.W., and Ratcliffe, L.M. 2004. Tropical winter habitat limits reproductive success on the temperate breeding grounds in migratory bird. *Proc. R. Soc. B Biol. Sci.* **271**(1534): 59–64. doi:10.1098/rspb.2003.2569. PMID:15002772.
- O'Connor, C.M., Norris, D.R., Crossin, G.T., and Cooke, S.J. 2014. Biological carry-over effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere*, **5**(3): 1–11. doi:10.1890/ES13-00388.1.
- Perryman, W.L., Donahue, M.A., Perkins, P.C., and Reilly, S.B. 2006. Gray whale calf production 1994–2000: are observed fluctuations related to changes in seasonal ice cover? *Mar. Mamm. Sci.* **18**(1): 121–144. doi:10.1111/j.1748-7692.2002.tb01023.x.
- R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from <https://www.R-project.org>.
- Richardson, J.N.L., and Baker, R.L. 1997. Effect of body size and feeding on fecundity in the damselfly *Ischnura verticalis* (Odonata: Coenagrionidae). *Oikos*, **79**(3): 477–483. doi:10.2307/3546890.
- Robb, G.N., McDonald, R.A., Chamberlain, D.E., Reynolds, S.J., Harrison, T.J.E., and Bearhop, S. 2008. Winter feeding of birds increases productivity in the subsequent breeding season. *Biol. Lett.* **4**(2): 220–223. doi:10.1098/rsbl.2007.0622. PMID:18252663.
- Robertson, F.W. 1957. Studies in quantitative inheritance. XI. Genetic and environmental correlation between body size and egg production in *Drosophila melanogaster*. *J. Genet.* **55**(3): 428–443. Available from <https://www.ias.ac.in/article/fulltext/jgen/055/03/0428-0443>.
- Robertson, F.W., and Sang, J.H. 1944. The ecological determinants of population growth in a *Drosophila* culture. I. Fecundity of adult flies. *Proc. R. Soc. B Biol. Sci.* **132**(868): 258–277. doi:10.1098/rspb.1944.0017.
- Rockwell, S.M., Bocetti, C.I., and Marra, P.P. 2012. Carry-over effects of winter climate on spring arrival date and reproductive success in an endangered migratory bird, Kirtland's warbler (*Setophaga kirtlandii*). *Auk*, **129**(4): 744–752. doi:10.1525/auk.2012.12003.
- Salton, M., Saraux, C., Dann, P., and Chiaradia, A. 2015. Carry-over body mass effect from winter to breeding in a resident seabird, the little penguin. *R. Soc. Open Sci.* **2**(1): 140390. doi:10.1098/rsos.140390. PMID:26064587.
- Sokolowski, M.B., Pereira, H.S., and Hughes, K. 1997. Evolution of foraging behavior in *Drosophila* by density-dependent selection. *Proc. Natl. Acad. Sci. U.S.A.* **94**(14): 7373–7377. doi:10.1073/pnas.94.14.7373. PMID:9207098.
- Sorensen, M.C., Hipfner, J.M., Kyser, T.K., and Norris, D.R. 2009. Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. *J. Anim. Ecol.* **78**(2): 460–467. doi:10.1111/j.1365-2656.2008.01492.x. PMID:19021778.
- Szostek, K.L., Becker, P.H., Meyer, B.C., Sudmann, S.R., and Zintl, H. 2014. Colony size and not nest density drives reproductive output in the common tern *Sterna hirundo*. *Ibis*, **156**(1): 48–59. doi:10.1111/ibi.12116.
- Van Allen, B.G., and Rudolf, V.H.W. 2013. Ghosts of habitats past: environmental carry-over effects drive population dynamics in novel habitat. *Am. Nat.* **181**(5): 596–608. doi:10.1086/670127. PMID:23594544.
- Van Allen, B.G., Briggs, V.S., McCoy, M.W., and Vonesh, J.R. 2010. Carry-over effects of the larval environment on post-metamorphic performance in two hylid frogs. *Oecologia*, **164**(4): 891–898. doi:10.1007/s00442-010-1728-8. PMID:20658150.
- Wells, K., O'Hara, R.B., Cooke, B.D., Mutze, G.J., Prowse, T.A.A., and Fordham, D.A. 2016. Environmental effects and individual body condition drive seasonal fecundity of rabbits: identifying acute and lagged processes. *Oecologia*, **181**(3): 853–864. doi:10.1007/s00442-016-3617-2. PMID:27028444.
- Wigby, S., Perry, J.C., Kim, Y., and Sirot, L.K. 2016. Developmental environment mediates male seminal protein investment in *Drosophila melanogaster*. *Funct. Ecol.* **30**(3): 410–419. doi:10.1111/1365-2435.12515. PMID:27546947.