




LETTER

Simple signals indicate which period of the annual cycle drives declines in seasonal populations

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Abstract

For declining wild populations, a critical aspect of effective conservation is understanding when and where the causes of decline occur. The primary drivers of decline in migratory and seasonal populations can often be attributed to a specific period of the year. However, generic, broadly applicable indicators of these season-specific drivers of population decline remain elusive. We used a multi-generation experiment to investigate whether habitat loss in either the breeding or non-breeding period generated distinct signatures of population decline. When breeding habitat was reduced, population size remained relatively stable for several generations, before declining precipitously. When non-breeding habitat was reduced, between-season variation in population counts increased relative to control populations, and non-breeding population size declined steadily. Changes in seasonal vital rates and other indicators were predicted by the season in which habitat loss treatment occurred. *Per capita* reproductive output increased when non-breeding habitat was reduced and decreased with breeding habitat reduction, whereas *per capita* non-breeding survival showed the opposite trends. Our results reveal how simple signals inherent in counts and demographics of declining populations can indicate which period of the annual cycle is driving declines.

Keywords

Annual cycle, breeding, conservation, habitat loss, non-breeding, population dynamics, seasonality.

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INTRODUCTION

A diverse array of taxa around the globe have shown rapid and steep population declines in recent years (Barnosky *et al.* 2011; Dirzo *et al.* 2014; Ceballos *et al.* 2015; Young *et al.* 2016). These declines have been attributed to a variety of factors, including habitat loss and degradation (Flockhart *et al.* 2015; Haddad *et al.* 2015), climate change (Pacifi *et al.* 2015), shifting phenology (Both *et al.* 2006; Møller *et al.* 2008) and the introduction of invasive species (Green *et al.* 2015). However, identifying a specific cause or major driver of decline for any given species can be extremely challenging (Hefley *et al.* 2016). One important intermediate step towards identifying population- or species-specific drivers of decline may be to understand which period of the annual cycle is being most impacted.

Virtually all animal populations live in seasonal environments, where the breeding season typically coincides with periods of high resource availability and the non-breeding period with limited resources. Under such regimes, the relative importance of demographic attributes differs between seasons, with the breeding period principally characterized by renewal (reproduction) and the non-breeding period dominated by mortality (Fretwell 1972). These distinct seasonal dynamics might, in turn, produce unique signatures that identify which period of the annual cycle is most likely driving population declines.

The need to identify the period of the annual cycle that is driving population declines is particularly pertinent to migratory species whose seasonal habitats are often separated by

vast geographic distances and whose populations have experienced ongoing global declines (Wilcove & Wikelski 2008; Young *et al.* 2016; Ceballos *et al.* 2017). Several recent examples have demonstrated the insights gained from integrating information across the annual cycle to understand how changes in the environment drive population dynamics, both in the wild (Norris & Marra 2006; Luis *et al.* 2010; Ferreira *et al.* 2016; Woodworth *et al.* 2017; Sutton *et al.* 2019) and in experimental populations (Betini *et al.* 2013a, 2014, 2017). Knowledge of when and where populations are limited has important implications for the targeting of conservation efforts and funding (Sheehy *et al.* 2011). And yet, our understanding of how changes in part of the annual cycle influence overall population trajectories remain limited, and generic indicators of season-specific drivers of decline are not well-established.

In this study, we use a multi-generation seasonal habitat loss experiment to examine whether populations exhibit unique, season-specific signatures of decline when subject to habitat loss in a single part of the annual cycle. We hypothesized that there would be season-specific signatures because, as habitat deteriorates in the non-breeding season, survival will decline during this period due to resource limitation, but population size will rebound as fewer individuals will move to the subsequent breeding period. With increased *per capita* resource availability and reduced competition in the breeding season, individuals will be released from density dependence and *per capita* reproductive output will increase. Conversely, when breeding habitat is lost, *per capita* non-breeding survival should improve because fewer individuals will compete for

non-breeding resources, but populations will not grow via renewal because competition during the breeding season will increase and cause *per capita* reproductive output to decline. In addition to examining the response of population vital rates to habitat loss, we also investigate whether there are unique, season-specific characteristics inherent in breeding population time series that reflect the season in which habitat is lost.

METHODS

Experimental system: seasonalized *Drosophila melanogaster*

Following previous research (Betini *et al.* 2013a, 2013b, 2014, 2015, 2017), we established experimental, seasonal populations with distinct breeding and non-breeding periods. Naturally occurring *Drosophila* populations are not ‘seasonal’ in the sense used here (i.e. mature individuals do not have distinct breeding and non-breeding periods within a single generation; see, e.g. Behrman *et al.* 2015 for a description of seasonality in wild *Drosophila*). However, we established populations of *D. melanogaster* in the laboratory in which individuals experienced bouts of breeding and non-breeding by manipulating the quality of food provided (e.g. Betini *et al.* 2015; Kilgour *et al.* 2018). Prior to initiating the experiment, flies were maintained in an outbred, aseasonal population with a two-week generation time at 25 °C, 40% RH and a 12:12 h light–dark cycle.

Replicate populations were housed in single-use polypropylene vials (28.5 × 95 mm (60.6 cm³); VWR International™, catalog no. 75813-156). To simulate the breeding period, populations of flies were provided a yeast–agar medium (composition: 100 mL H₂O, 100 g C₁₂H₂₂O₁₁, 50 g Fleischmann’s dry active yeast, 16 g agar, 8 g C₄H₄KNaO₆·4H₂O, 1 g KH₂PO₄, 0.5 g NaCl, 0.5 g MgCl₂, 0.5 g CaCl₂, 0.5 g Fe₂(SO₄)₃·xH₂O; Betini *et al.* 2013a), which acted not only as a food source for mating adults, but also as a substrate on which females oviposited and larvae foraged during development. To induce a distinct non-breeding season, mature adults were transferred from their natal (breeding) vial to a second empty vial, where they were provisioned with 5% (w/v) sugar water (Fig. 1). The sugar water provided sufficient energy and hydration for survival at low densities but did not allow the production of

eggs by females nor did it provide a suitable egg-laying substrate (Bownes & Blair 1986; Betini *et al.* 2015).

In each generation (21 days), adults were allowed to breed for 24 h, after which the flies were removed and their eggs were allowed to mature for 16 days. During the maturation period, in order to avoid unintended mortality resulting from breeding medium deterioration (Dey & Joshi 2006), newly emerged adults were collected from the breeding vials on days 12, 14, and 16, and housed in separate holding vials with 5 mL of fresh medium. On day 17, adults were transferred to non-breeding vials where they were provisioned with sugar water twice daily for four days. After the non-breeding period, surviving adults were transferred to new breeding vials to produce the next generation (Fig. 1). Under control conditions, populations were provisioned with 10 mL of yeast–agar medium (breeding) and 0.20 mL·d⁻¹ of 5% (w/v) sugar water (non-breeding).

Season-specific habitat loss experiment

Chronic habitat loss was simulated by systematically reducing the volume of yeast–agar medium (breeding treatments) or sugar water (non-breeding treatments) provided in each subsequent generation. Replicate populations were subjected to habitat reductions in either the breeding or the non-breeding period, but not both. Within each season, populations were exposed to one of two rates of habitat reduction: 10%, and 20% loss per generation. Habitat loss treatments followed an exponential decay, with the volume of food provided in a given generation H_t prescribed as $H_t = H_0(1 - \nu)^t$, where H_0 is the initial volume of food provided (i.e. 10 mL yeast–agar medium in the breeding season and 0.20 mL sugar water per day in the non-breeding season; see above), ν is the rate of decay (i.e. 10% or 20%), and t is the number of generations since treatment commenced (Supporting Information 1). A similar experimental set-up was used by Clements & Ozgul (2016a) to investigate the effects of resource (prey) declines on predator population dynamics.

The experiment consisted of four treatment groups (season of treatment × habitat loss rate), plus controls, with 20 replicates per group ($N = 100$ vials). Replicates were evenly divided over a 5-day period, with each day comprised of a tray with four replicates per treatment ($n_{\text{tray}} = 28$).

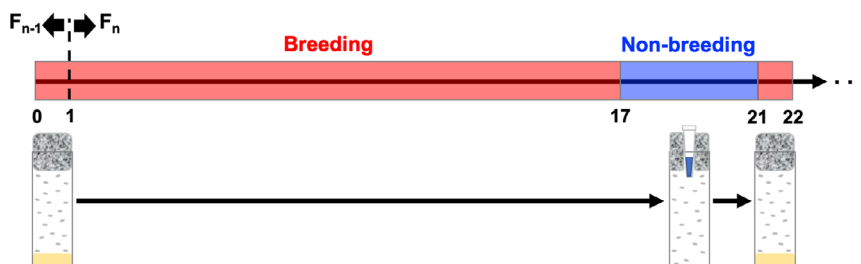


Figure 1 Diagram of the experimental seasonal population set-up. Replicate populations of *Drosophila melanogaster* were maintained under seasonal conditions with non-overlapping generations, where F_n = current generation (offspring) and F_{n-1} = prior generation (parents). Adults were allowed to breed for 24 h and then removed from the breeding vials, where their eggs were left to mature for 16 day. Newly emerged adults were transferred to holding vials on days 12, 14 and 16. After maturation, populations were transferred to the non-breeding season for 4 day, and the survivors were transferred to new breeding vials to produce the next generation.

Populations were initiated using 10 males and 10 females from the stock population (see above). After filtering to exclude replicates that collapsed and were reinitiated during the pre-treatment period (one each from the control and 10% breeding treatments), the dataset includes time series from a total of 98 replicate populations ($n_{\text{treatment}} = 19\text{--}20$). The experiment was initiated in January 2017, with treatments beginning in July 2017 after seven filial generations of control conditions. Habitat reduction treatments were applied over 15 successive generations, with the experiment ending April 2018 (total duration = 486 days).

We estimated changes in population size by counting the number of adult individuals at two stages of the annual cycle: (1) *Non-breeding population size*, which was the number of newly emerged adults as they moved from the breeding to the non-breeding season and represented the maximum population size for a given generation; (2) *Breeding population size*, which was the number of adults after they were removed from the 24 h breeding period and reflected the number of potentially breeding adults (i.e. the number of individuals that survived through the previous non-breeding season).

Metrics to characterize population declines

Beyond describing patterns of population decline, we also sought to characterize patterns of population collapse using a number of metrics. First, we derived two common, season-specific vital rates: (1) *log per capita reproductive output* (i.e. the relative change in population size between the breeding (N_{t-1}) and non-breeding (N_t) population size) was calculated as $r = \ln(N_t/N_{t-1})$; (2) *log per capita non-breeding survival* (i.e. the relative change in non-breeding (N_{t-1}) and breeding (N_t) population sizes) was calculated as $s = \ln(N_t/N_{t-1})$. These vital rates are themselves fundamental to estimates of population growth (i.e. the change in breeding population size across successive generations) but may reveal season-specific responses even in the absence of concomitant changes in growth (Kolb *et al.* 2010; Bellier *et al.* 2018).

In addition to vital rates, we used three metrics commonly used as early warning signals of impending transitions in biological systems (i.e. transitions from population stability to collapse or whole-ecosystem regime shifts; see, e.g. Scheffer *et al.* 2009; Dai *et al.* 2012; Clements & Ozgul 2018). While changes in these metrics are commonly used to identify 'tipping points' preceding population collapse, we use them here to characterize population dynamics and do not treat them as early warning indicators. For each time series, we examined (changes in) the coefficient of variation (c_V ; the degree of dispersion between the data points), skewness (a measure of asymmetry), and autocorrelation (lag-1) of subsequent breeding values. The first two metrics (c_V and skewness) are the second and third statistical moments that describe the shape of a curve or function (the first being the mean), whereas autocorrelation is a measure of the similarity between subsequent observations (Scheffer *et al.* 2009). To calculate these metrics, we used only breeding population sizes as these are the data most commonly available for wild populations, whereas non-breeding population sizes are often unavailable or are more difficult to estimate. See the Supporting Information for a

summary of the complementary results from the non-breeding population sizes (Supporting Information 2). In all cases, these values were first calculated separately for each replicate time series using a sliding window approach (window size = 3) and were then averaged across replicates for each treatment (Burthe *et al.* 2016). The values reported below indicate the mean \pm standard error (SE) for each treatment. Since standard error incorporates sample size, its use allows us to account for the fact that sample sizes decrease through time as replicate populations begin to collapse with the reduction of habitat.

Principal component analysis

We used a principal component analysis (PCA) to account for correlations among the seasonal metrics (*per capita* reproduction, *per capita* non-breeding survival, autocorrelation, coefficient of variation), and to develop a 'composite' of all metrics of interest. Skewness was excluded from the PCA because it did not show distinct patterns contingent on the season in which habitat was reduced. Because a sliding window was required to calculate autocorrelation and c_V , the moving averages of the season-specific vital rates (reproduction and survival) were first re-calculated using a similar approach (window size = 3 generations). Prior to performing the PCA, all variables were scaled and centred within generation to account for the expected divergence of metrics through time.

Statistical analysis

To test predictions related to the season-specific signals hypothesis, we used four separate univariate generalized linear mixed-effects models (GLMMs) in a Bayesian framework (Ellison 2004; Bolker *et al.* 2009): two for survival and two for reproduction, with separate models for each rate of habitat loss (10% and 20%). In each model, we included a two-way interaction terms between the season of treatment (two-level factors: breeding, non-breeding) and generation (integer). Replicate ID was fitted as a random effect. In order to avoid modelling the transitory dynamics of the pre-treatment period (Drake & Griffen 2010), only data from generations in which treatments were applied (generations 8–22) were analysed.

Two univariate GLMMs were performed to examine how habitat reduction treatments affected the behaviour of the first principal component (PC1; see *Principal component analysis* above). These models were similarly parameterized with a two-way interaction between season of habitat reduction and generation and a random effect for replicate ID, and included only data from the treatment period.

We specified the models using uninformed priors and each model consisted of four chains of 104 000 iterations, with a burn-in of 4000 iterations per chain and thinning to every 400th run ($n_{\text{chain}} = 250$; $n_{\text{model}} = 1000$). To confirm model convergence, we consulted \hat{R} values (equal to 1 at convergence) and visually assessed posterior distributions of fitted estimates and caterpillar plots (Bürkner 2017). Effect size estimates were taken from the posterior distribution of model parameters, and 95 percent credible intervals (CI) around the means (β) were made based on the 1000 samples from the model

simulations (Cumming & Finch 2005). We used these credible intervals to evaluate the strength of support for an effect of each parameter (Cohen 1990). We classify estimates for which the 95% CI do not overlap zero as showing ‘strong support’ for an effect; estimates biased away from zero, but for which the 95% CI overlaps zero by up to 15%, as showing ‘moderate support’; and estimates centred about zero as showing ‘no support’ for an effect (see, e.g. Mathot *et al.* 2017). Model fit was estimated with R^2 as the proportion of variance explained (Gelman *et al.* 2018).

All data manipulation and statistical analyses were conducted in the R statistical environment (v. 3.5.0; R Core Development Team 2018). Skewness was calculated using the *skewness* function from the package *eI071* (Myer *et al.* 2018). Bayesian GLMMs were run using the package *brms* (Bürkner 2017). The data used for the analyses have been made publicly available through the Figshare repository: <<https://doi.org/10.6084/m9.figshare.9779177.v1>> (Burant *et al.* 2019b).

RESULTS

Bi-seasonal population dynamics with habitat loss

The bi-seasonal time series, which consisted of two counts per generation (non-breeding and breeding population sizes), revealed distinct patterns of population decline with season-specific habitat loss (Fig. 2a, d, g). After an initial period of increase, control population size in both seasons were stable from generation 5 to 22 (breeding population size = $186 \pm$

$3.48 (\pm \text{SE})$; non-breeding population size = 243 ± 5.15 ; Fig. 2a). In contrast, populations in the habitat reduction treatments declined steadily until all of them went extinct. As expected, populations in the 20% habitat reduction treatments went extinct faster than in the 10% treatments (mean time to extinction for 20%: generation 15 and 16 for breeding and non-breeding habitat loss, respectively; for 10%: generation 22 for both breeding and non-breeding loss; Fig. 2d, g).

With the reduction of breeding habitat, breeding population size remained relatively stable through time before declining precipitously around generation 12 for 20% habitat loss and generation 14 for 10% habitat loss (Fig. 2b, e, h). In contrast, when non-breeding habitat was reduced, breeding population size began to decline immediately with the onset of habitat reduction and the steepness of the decline increased with the rate of loss (Fig. 2b, e, h). Likewise, the trajectories of initial non-breeding population size were dependent on the season of habitat loss (Fig. 2c, f, i). When breeding habitat was reduced, the non-breeding population size at first increased before declining sharply prior to collapse, whereas non-breeding habitat loss resulted in relatively limited change in the initial non-breeding population size (Fig. 2c, f, i).

Season-specific signatures of decline

There was strong support for an interactive effect of the season of treatment and generation on *per capita* reproductive output, such that as the proportion of habitat remaining continued to decline, *per capita* reproductive output of

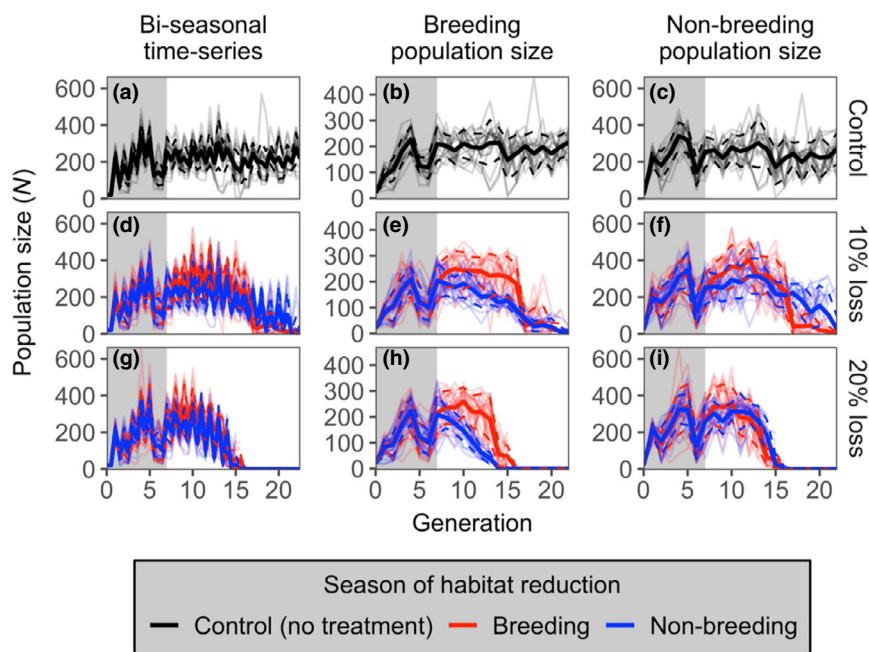


Figure 2 Response of experimental population to season-specific habitat loss. (a, d, g) Bi-seasonal population dynamics. Each generation consists of two population counts: non-breeding population size (i.e. the number of individuals at the start of the non-breeding period; peaks) and breeding population size (i.e. the number of individuals at the start of the breeding season; valleys). (b, e, h) Breeding population size. (c, f, i) Non-breeding population size. Populations were run under control conditions for seven filial generations (shaded grey region) prior to the application of habitat reduction treatments. For all figures, thick solid lines indicate the mean time series for each treatments, with the standard errors indicated with dashed lines, and thin lines indicate individual replicate time series. Sample size $n_{\text{treatment}} = 19 - 20 (N = 98)$.

populations experiencing breeding and non-breeding habitat loss diverged. Consistent with the season-specific signals hypothesis, habitat reduction in the non-breeding season increased *per capita* reproductive output, whereas habitat loss during the breeding season had the opposite effect (Fig. 3a–c). These results were consistent across the two different rates of habitat loss, although the 20% treatment model better explained the variation in *per capita* reproductive output than the 10% model [$R^2_{\text{repr}_{20\%}} = 0.54$, 95% credible interval = (0.48, 0.59) vs. $R^2_{\text{repr}_{10\%}} = 0.31$, 95% CI = (0.24, 0.36)] and also had larger estimates for the interaction between generation and season of habitat reduction (Table 1).

There was also strong support that *per capita* non-breeding survival was affected differently by the habitat reduction treatment (i.e. a two-way interaction between season of treatment and generation; Fig. 3j–l). This suggests that, consistent with our predictions, non-breeding habitat loss resulted in a decrease in *per capita* non-breeding survival, while breeding habitat loss resulted in an increase. As in the *per capita* reproductive output models, effect size estimates and R^2 values were larger for the 20% model ($R^2_{\text{surv}_{20\%}} = 0.76$, 95% CI = (0.74, 0.78)) than for the 10% model ($R^2_{\text{surv}_{10\%}} = 0.41$, 95% CI = (0.35, 0.46); Table 1). Together, models of the

seasonal vital rates indicate that non-breeding habitat loss resulted in a decrease in *per capita* non-breeding survival and a concomitant increase in *per capita* reproduction in the subsequent breeding period, while breeding habitat loss resulted in the opposite trends (Fig. 3a–c, j–l).

The coefficient of variation (c_V) was influenced by the season-specific habitat reduction and increased away from the average control value in the generations preceding populations collapse (Fig. 3d–f). Notably, across the two rates of non-breeding habitat reduction, c_V began to increase only after (2–3 generations) the populations had started to decline (Fig. 3e, f). This indicates that the season in which habitat loss occurs may influence the timing and patterning of changes in c_V .

For control replicates, the average autocorrelation fluctuated around the global mean but was consistently negative throughout the treatment period (Fig. 3m). When habitat was reduced during the breeding period, the behaviour of the autocorrelation was largely similar to the control (i.e. non-directional, stochastic fluctuations; Fig. 3n, o). However, when non-breeding habitat was reduced, lag-1 autocorrelation increased away from the control mean, which suggests that the season of habitat loss influenced changes in intrinsic metrics (Fig. 3n, o).

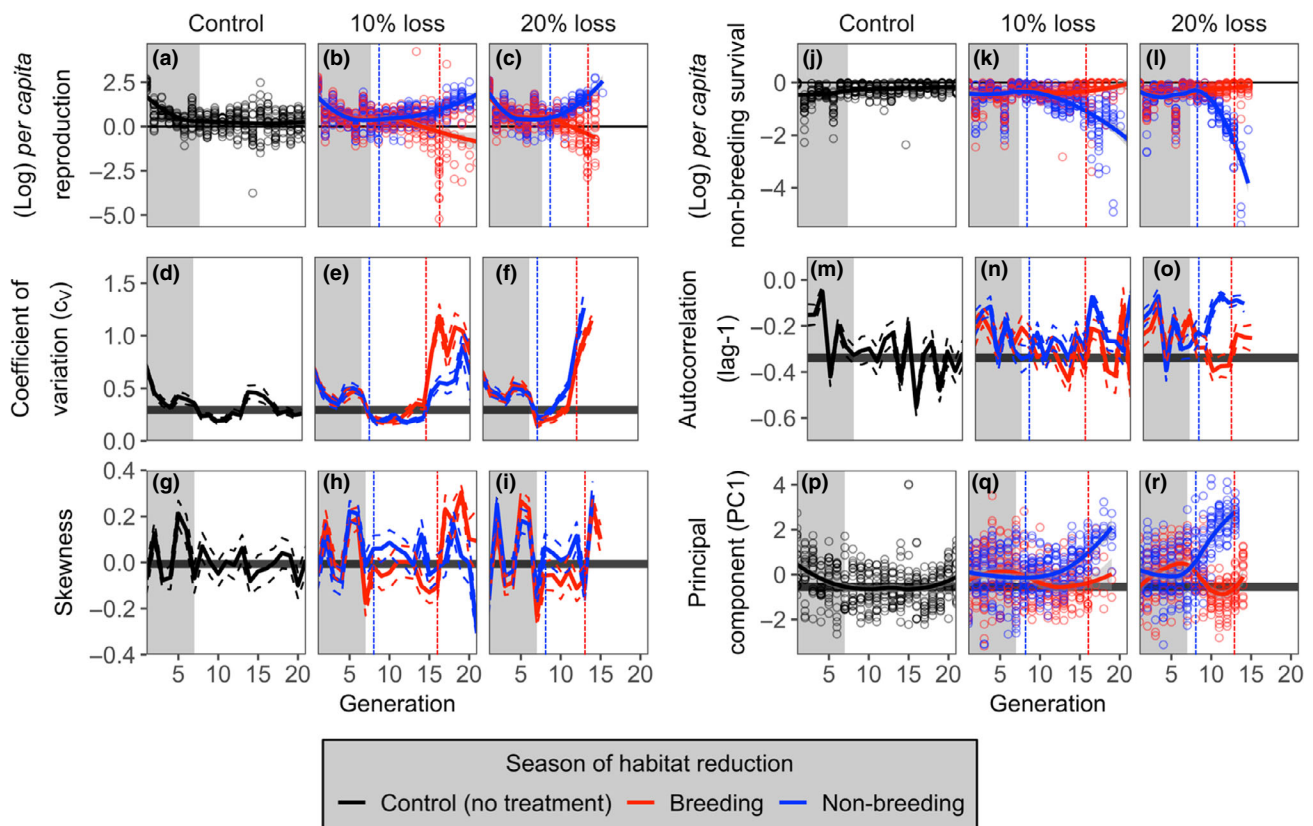


Figure 3 Season-specific patterns in the response of population vital rates and intrinsic metrics. Populations were run under control conditions for seven filial generations (shaded grey region) prior to the application of treatment. Univariate models of vital rates included only data from the treatment period. We also used three intrinsic metrics in time series of breeding population size to characterize changes in population dynamics in response to habitat loss in either the breeding or non-breeding period: coefficient of variation (c_V ; the degree of dispersion between the data points); autocorrelation (lag-1; the level of similarity between subsequent observations in a time series); skewness (a measure of asymmetry). Values were calculated individually for each replicate population using a sliding window (size = 3 generations). Solid lines indicate the average time series for each treatment and dashed lines indicate the standard error. Vertical dashed lines indicate when the breeding population size began to decline with respect to treatment. Sample size $n_{\text{treatment}} = 19 - 20 (N = 98)$.

Finally, principal component analysis reduced the vital rates (survival and reproduction) and intrinsic metrics (autocorrelation and c_V) into a composite variable (PC1; proportion of variance explained = 35.2%), on which the four metrics loaded relatively evenly (magnitude of loadings: range = 0.42–0.55; Supporting Information 3). There was strong support for a positive interactive effect of season of treatment and generation on PC1, such that as habitat loss proceeded the effect of treatment increased ($R^2_{PC1_{20\%}} = 0.64$, 95% CI = (0.59, 0.68); $R^2_{PC1_{10\%}} = 0.34$, 95% CI = (0.27, 0.40); Table 2). Effect size estimates from the models of PC1 were similar to those from the seasonal vital rate models (Tables 1, 2). Our analysis also revealed important differences in the behaviour of PC1 which were dependent on the season of habitat loss: when non-breeding habitat was reduced, PC1 increased away from the control mean, whereas breeding habitat reduction affected relatively little change in the behaviour of PC1 (Fig. 3p–r). Although we here present results derived from breeding population sizes because these are the data most commonly available from wild populations, similar analyses can be conducted using metrics derived from non-breeding counts (Supporting Information 2).

DISCUSSION

Using a multi-generation experiment, we show that season-specific habitat loss influences the way populations decline

and produces unique signals that are indicative of the season of loss. Consistent with our hypothesis, we demonstrate that season-specific habitat loss has unique effects on the behaviour of population vital rates. Reducing habitat availability during the breeding season resulted in a decrease in *per capita* reproductive output and an increase in the *per capita* survival in the subsequent non-breeding season. In contrast, loss of non-breeding habitat led to a decline in *per capita* non-breeding survival and an increase in *per capita* reproductive output, likely a result of lower non-breeding densities.

Previous work has shown that the dynamics of seasonal populations can be strongly driven by environmental conditions in a specific period of the annual cycle (Sæther *et al.* 2000; Sillett *et al.* 2000; Norris *et al.* 2004; Calvert *et al.* 2009; Flockhart *et al.* 2015; Gullett *et al.* 2015; Marra *et al.* 2015b; Rushing *et al.* 2016). For example, Savannah sparrows' (*Passerculus sandwichensis*) population growth during the breeding period has been shown to be limited by temperature effects on non-breeding survival (Woodworth *et al.* 2017). On the other hand, in wood thrush (*Hylocichla mustelina*), estimates of the effects of site-specific habitat loss reveal that the impact of breeding habitat loss on population declines is several times greater than the equivalent loss on the non-breeding grounds (Rushing *et al.* 2016, but see Taylor & Stutchbury 2016). Here we broaden our understanding of the consequence of season-specific effects on population dynamics. Our results

Table 1 Effect size estimates from models of season-specific vital rates

Fixed effects	10% habitat reduction			20% habitat reduction		
	Estimate (β)	95% CI lower	95% CI upper	Estimate (β)	95% CI lower	95% CI upper
Reproductive output models						
(Intercept)	1.437	1.002	1.874	1.828	1.169	2.413
Season (NB)	-2.020	-2.603	-1.428	-3.884	-4.814	-2.982
Generation	-0.108	-0.140	-0.075	-0.174	-0.225	-0.117
Season (NB): generation	0.207	0.165	0.250	0.462	0.376	0.545
Random effects						
Vial ID	0.051	0.002	0.144	0.040	0.002	0.112
Family-specific						
Residual	0.787	0.738	0.840	0.652	0.598	0.713
Non-breeding survival models						
(Intercept)	-0.451	-0.688	-0.183	-0.633	-0.933	-0.337
Season (NB)	1.185	0.843	1.527	3.800	3.298	4.296
Generation	0.009	-0.009	0.026	0.035	0.011	0.062
Season (NB): generation	-0.124	-0.147	-0.100	-0.442	-0.486	-0.396
Random effects						
Vial ID	0.066	0.004	0.139	0.034	0.001	0.092
Family-specific						
Residual	0.476	0.447	0.507	0.387	0.354	0.422

Four separate univariate generalized linear mixed-effects models were run to investigate the effect of the season of habitat loss (breeding = B; non-breeding = NB) on changes in (log) *per capita* reproductive output and (log) *per capita* non-breeding survival through time. For each vital rate, separate models were run for the 10% and 20% habitat reduction treatments (see *Statistical analysis*). Effect size estimates were taken from the posterior distributions of model parameters ($n = 1000$), with uncertainty around the means (β) estimated with 95% credible intervals (CI).

Table 2 Effect size estimates from principal component models

Fixed effects	10% habitat reduction			20% habitat reduction		
	Estimate (β)	95% CI lower	95% CI upper	Estimate (β)	95% CI lower	95% CI upper
(Intercept)	-0.224	-0.772	0.320	-0.155	-1.074	0.769
Season (NB)	-1.497	-2.221	-0.781	-2.74	-4.176	-1.318
Generation	-0.014	-0.058	0.030	-0.036	-0.119	0.047
Season (NB): Generation	0.181	0.125	0.237	0.484	0.354	0.620
Random effects	Estimate (β)	95% CI lower	95% CI upper	Estimate (β)	95% CI lower	95% CI upper
Vial ID	0.152	0.012	0.302	0.139	0.006	0.332
Family-specific	Estimate (σ)	95% CI lower	95% CI upper	Estimate (σ)	95% CI lower	95% CI upper
Residual	0.829	0.769	0.893	0.959	0.876	1.049

A principal component analysis was performed, which included data on four population metrics: *per capita* reproductive output, *per capita* non-breeding survival probability, coefficient of variation, and autocorrelation. The first principal component (PC1) was extracted and two univariate generalized linear mixed-effects models were run to investigate the effect of the season of habitat loss (breeding = B; non-breeding = NB) on changes in PC1 through time. Separate models were run for the 10% and 20% habitat reduction treatments (see *Statistical analysis*). Effect size estimates were taken from the posterior distributions of model parameters ($n = 1000$), with uncertainty around the means (β) estimated with 95 percent credible intervals (CI).

demonstrate that, consistent with some of the findings of studies outlined above, as well as previous work in *Drosophila* (Betini *et al.* 2013a,2013b), changes in environmental conditions in part of the annual cycle can carry over to affect population dynamics in subsequent periods. We further reveal how these carryover effects can generate unique patterns of population decline that are dependent on the season of change.

While the demographic structure of a population is undoubtedly critical to understanding its long-term trajectory and stability, we argue that the season-specific signals presented here should occur irrespective of the specific context of the system. This is because density-dependent mortality in the different seasons drives our results, rather than changes in the age or stage structure of the populations. A large body of literature has focused on the value of considering the age- or stage-structured nature of populations (i.e. the distribution of individuals across different life-history stages within a population, such as the number of juveniles and adults) when investigating the mechanisms of population decline (Liu & Chen 2002; Kendall *et al.* 2011; Fujiwara & Diaz-Lopez 2017). This stage structure is of particular relevance for populations with overlapping generations, where changes in conditions in one part of the annual cycle can differentially affect rates of mortality or reproduction in these different groups. In our experiment, generations are discrete and so differential survival and mortality between adults and their offspring does not occur. In our seasonal *Drosophila* system, only adults occur during the non-breeding period, so there is no stage structure to *per capita* non-breeding survival. Likewise, in the breeding season, we expect reductions in resource availability to only influence the rate at which larvae transition to adult offspring (i.e. larval mortality), and have minimal impact on either (1) egg production, since egg production is dependent on the apparent density of breeders rather than the amount of food available for developing larvae, or (2) adult offspring mortality prior to the non-breeding period, since these adults are housed in separate holding vials before being transferred to non-breeding

vials (see *Experimental system: seasonalized Drosophila melanogaster* in *Methods*).

Along with vital rates, we investigated whether statistical moments (coefficient of variation, autocorrelation, skewness) intrinsic to breeding population counts can be used as signatures of season-specific habitat loss and population declines. While a large body of literature has demonstrated that these signals may act as indicators of population instability and impending collapse (Biggs *et al.* 2009; Drake & Griffen 2010; Dai *et al.* 2012; Clements & Ozgul 2016a, 2016b), we show that their behaviour may be dependent, at least in part, on the timing of habitat loss. While theory on early warning signals holds that autocorrelation should increase as (or before) a population transitions away from stable population size toward collapse, we show that this characteristic increase occurs only with reductions in non-breeding habitat. Although our intention here was to use these metrics to characterize season-specific patterns of decline rather than as predictors of state transitions, these results are in keeping with a number of recent studies that show that the usefulness of *EWS* may be context-dependent (Hastings & Wysham 2010; Perretti & Munch 2012; Burthe *et al.* 2016). Consistent with previous work (Clements & Ozgul 2016a, 2016b), we also show that the strength of the stressor has important consequences for the way in which these putative indicators respond.

Although the timing and strength of habitat loss had effects on both *per capita* reproductive output and *per capita* non-breeding survival, our statistical models explained only part of the variation in vital rates. In addition to changes in vital rates, chronic reductions in resource availability should also be expected to induce a response in other aspects of a population, including the potential for behavioural, phenotypic or even evolutionary change. For example, given the well-characterized relationship between population density and body size in *Drosophila* (with denser populations favouring smaller-bodied individuals; Ashburner & Thompson 1978), we might also predict shifts in body size distribution in response to habitat loss. Perhaps more importantly, season-specific habitat loss is

likely to have different consequences for body size. While larger individuals usually have higher fecundity, the increased metabolic demands of a large body may translate to decreased survival probability during the non-breeding season when resources are limited (Mueller & Joshi 2000; Munch *et al.* 2003; Betini *et al.* 2017; Bonnet *et al.* 2017). Likewise, behavioural adaptation is known to be an important mechanism by which individuals respond to environmental change (Wong & Candolin 2015). In *Drosophila*, population density has been shown to affect the frequency of different foraging strategies (rover *versus* sitter; Sokolowski *et al.* 1997), suggesting that as population size decreases with habitat loss, we might predict concomitant shifts in behaviour.

Our results bring novel insight into the conservation of seasonal and migratory populations that have undergone dramatic declines in recent decades (Wilcove & Wikelski 2008; Young *et al.* 2016). In particular, current efforts to conserve migratory species are often hindered by an absence of complete information on the full annual cycle of these populations, with most taxa studied in only part of the year (typically the breeding period; Marra *et al.* 2015a, but see, e.g. Marra *et al.* 2015b). The capacity to use simple signals inherent in the types of demographic data that are often collected to identify where and when populations are limited represents a fundamental advance in our ability to target scarce conservation efforts and funding. This is perhaps most salient for long-distance migratory populations, whose breeding and non-breeding grounds are separated by vast geographic distances.

Taken together, our results demonstrate the importance of considering the timing (or location) of population limitation within the context of the full annual cycle. By experimentally manipulating the availability of either breeding or non-breeding habitat over multiple generations, we show that when and where habitat is lost has important consequences for the patterns of population decline. Although previous experimental and observational studies have explored how populations can be limited by conditions in only part of the annual cycle, we show that, in the same system, breeding and non-breeding habitat loss induce distinct patterns of decline that are consistent across different rates of loss. These findings are especially pertinent given that the majority of populations exist under seasonal regimes and in light of the ongoing, widespread decline of species threatened by habitat loss and global change.

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AUTHORSHIP

JBB and DRN designed the experiment and GSB was involved in developing the indicators and statistical analysis. JBB conducted the experiment, analysed the data, and wrote the first draft. All authors contributed to revising the manuscript for publication. The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Data available from the Figshare Repository: <https://doi.org/10.6084/m9.figshare.9779177.v1> (Burant *et al.* 2019b).

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SUPPORTING INFORMATION

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