

Early warning indicators of population collapse in a seasonal environment

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Abstract

1. Recent studies have demonstrated that generic statistical signals derived from time series of population abundance and fitness-related traits of individuals can provide reliable indicators of impending shifts in population dynamics. However, how the seasonal timing of environmental stressors influences these early warning indicators is not well understood.
2. The goal of this study was to experimentally assess whether the timing of stressors influences the production, detection and sensitivity of abundance- and trait-based early warning indicators derived from declining populations.
3. In a multi-generation, season-specific habitat loss experiment, we exposed replicate populations of *Drosophila melanogaster* to one of two rates of chronic habitat loss (10% or 20% per generation) in either the breeding or the non-breeding period. We counted population abundance at the beginning of each season, and measured body mass and activity levels in a sample of individuals at the end of each generation.
4. When habitat was lost during the breeding period, declining populations produced signals consistent with those documented in previous studies. Inclusion of trait-based indicators generally improved the detection of impending population collapse. However, when habitat was lost during the non-breeding period, the predictive capacity of these indicators was comparatively diminished.
5. Our results have important implications for interpreting signals in the wild because they suggest that the production and detection of early warning indicators depends on the season in which stressors occur, and that this is likely related to the capacity of populations to respond numerically the following season.

KEYWORDS

activity, body size, *Drosophila melanogaster*, early warning signals, habitat loss, seasonality

1 | INTRODUCTION

Wildlife populations have declined dramatically in recent decades (World Wildlife Fund, 2018), with as many as one million species threatened with extinction by the end of the 21st century (Intergovernmental Science-Policy Platform on Biodiversity & Ecosystem Services, 2019). Major anthropogenic drivers of declines

in wild populations include habitat loss and degradation (Haddad et al., 2015; Iwamura et al., 2013), urbanization (Sol et al., 2014), climate change (Møller et al., 2008; Moritz & Agudo, 2013) and pollution (McCune et al., 2019). As a consequence of these ongoing environmental changes, rapid shifts in or destabilization of population dynamics can lead to population collapse, local extirpation or even global extinction of threatened species. In response to these

threats, much research effort has been directed towards understanding whether these declines are preceded by reliable indicators of impending population collapse (Biggs et al., 2009; Clements & Ozgul, 2016b; Hefley et al., 2016). One avenue of inquiry that has shown promise for detecting and predicting when a population is approaching such a critical transition comes from theory on early warning signals (Scheffer et al., 2009).

Recent work has shown that generic statistical information inherent in times series can be used to evaluate when a population (or ecosystem) is threatened or nearing a tipping point towards extinction (Carpenter et al., 2011; Dai et al., 2012). These statistical signals, or 'leading indicators', are embedded in times series of population abundance and are expected to behave in predictable ways near a tipping point, and, when this is the case, may provide advanced warning of an impending critical transition between alternate states a number of time steps before the transition occurs (hence, *early warning signals*; Scheffer et al., 2009). There exists strong theoretical (Biggs et al., 2009; Boettiger & Hastings, 2012; Kéfi et al., 2013) and empirical evidence (Carpenter et al., 2011; Dai et al., 2012; Drake & Griffen, 2010) to support the predictive capacity of metrics such as the coefficient of variation (cv; i.e. a metric of the change in variability of a system through time), autocorrelation at the first lag (lag-1 acf; i.e. a measure of the similarity of observations at successive time steps) and other related indices (e.g. first autoregressive coefficient, density ratio; Scheffer et al., 2009). The regular behaviour of these leading indicators is a product of the phenomenon of critical slowing down, which indicates a loss of system resilience (Holling, 1973) in response to external forcing by, for example, changes in environmental conditions.

In addition to indicators intrinsic to times series of population abundance, changes in trait dynamics can act as early warning signals of a critical transitions (Baruah et al., 2019; Clements et al., 2017; Clements & Ozgul, 2016a; Spanbauer et al., 2016). Environmental change is known to impact the distribution of traits within a population (van Gils et al., 2016; van der Vinne et al., 2019; Weeks et al., 2020), as a result of either rapid evolution, plasticity or both. Since phenotypic traits, such as morphology and behaviour, and the match between these traits and an organism's environment, are ultimately related to individual survival and reproduction (McNamara & Houston, 2008), shifts in these traits may stabilize or further destabilize population dynamics (Ozgul et al., 2010) and can impact the timing of critical transitions (Baruah et al., 2020). Moreover, because phenotypic traits may also be density-dependent, any change in abundance may be accompanied by concomitant changes in the distribution of traits (Burant et al., 2020). In particular, body size has been identified as a key trait related to the long-term viability and resilience of populations (Olden et al., 2007).

Despite the potential of early warning signals to serve as a tool for understanding and predicting the dynamics of declining populations, how seasonality impacts the detectability and sensitivity of these signals is unknown. Nearly all wild populations persist in seasonal environments, wherein the annual cycle can be broadly divided into two main components: the breeding and non-breeding

periods. In this seasonal context, environmental stressors driving populations to decline can vary between seasons or be localized to only part of the annual cycle (e.g. a population may experience chronic environmental degradation during the breeding period that is not mirrored by similar change during the non-breeding period, or *vice versa*; Calvert et al., 2009). Such seasonality in environmental stressors can make it difficult to identify when and where populations are being driven to decline, although recent evidence suggests that simple demographic signals may reflect the season in which the stressor occurs (Burant et al., 2019). Whether the response of early warning indicators differs depending on the period of the annual cycle in which the stressor occurs remains to be investigated.

In this study, we used a multi-generation seasonal habitat loss experiment in *Drosophila melanogaster* to examine whether leading indicators derived from population counts, as well as traits like body mass and locomotor activity, show unique responses to habitat loss in a single part of the annual cycle. Previous work has shown how body size can be used as an early warning signal of impending regime shifts (Clements & Ozgul, 2016a), and others have urged the inclusion of additional aspects of behaviour and physiology (Burthe et al., 2016). To our knowledge, our present analysis is the first to incorporate a behavioural measure, with locomotor activity predicted to be a major determinant of fitness given its consequences for resource acquisition and reproduction (Gilchrist et al., 1997). In addition, we evaluated whether the season or rate of habitat loss influenced the utility of these leading indicators, as well as composite indicators derived from multiple abundance- and trait-based metrics (see, e.g. Clements & Ozgul, 2016a).

In a previous paper, Burant et al. (2019) showed that the pattern of population decline and change in demographic vital rates were predicted by the season in which habitat loss occurred, suggesting that vital rates themselves may be useful in identifying the season driving a population to decline. Here, we hypothesized that whether a suite of indicators acted as early warning signals of an impending population collapse would be dependent on the season of habitat loss because the capacity of populations to respond to environmental change and the way in which populations decline differs depending on the season in which habitat is lost (Burant et al., 2019). When non-breeding habitat is lost, we hypothesized that populations would rebound the following breeding period as a result of density-dependent reproduction, and that this rebound would dampen the production of signals in breeding time series. In contrast, when breeding habitat is lost, there is no similar compensatory mechanism by which populations can rebound since no reproduction occurs during the non-breeding period (Burant et al., 2019). Following this rationale, we predicted that, when habitat was lost during the non-breeding period, the abundance and trait time series would produce fewer and less sensitive early warning signals compared to when habitat was lost during the breeding period. Previous work has also demonstrated how the rate or strength of forcing can influence the production and detection of early warning signals, with faster rates of environmental change resulting in signal production only after the system has passed its tipping point (Clements & Ozgul, 2016a,

2016b). Based on this, we predicted that as the strength of habitat loss increased, the detectability of early warning signals would decrease, irrespective of the season of loss.

2 | MATERIALS AND METHODS

2.1 | Experimental system: Seasonal populations of *Drosophila*

Data were collected as part of the same experiment described in Burant et al. (2019), in which replicate populations of *Drosophila melanogaster* were established with distinct breeding and non-breeding periods. Seasonal populations were generated from an outbred, aseasonal stock population maintained at 25°C, 40% RH and a 12:12 hr light-dark cycle, with a 2-week generation time. In these populations, which were housed in polypropylene vials (28.5 mm × 95 mm; 60.6 cm³; VWR International™, catalogue no. 75813-156), sequential bouts of breeding and non-breeding were enforced by manipulating the quality of food provisioned (Betini et al., 2013a, 2013b).

During the experiment, seasonal populations had a 21-day generation ('annual cycle'), in which parental and offspring generations did not overlap. At the beginning of each generation, adult flies were allowed to breed for 24 hr before they were removed, and their offspring were left to develop for 16 days. As offspring eclosed as adult flies, they were collected and transferred to a separate holding vial containing 5 ml of fresh yeast-agar medium on days 12, 14 and 16.

On day 17, all adult offspring were transferred to non-breeding vials, where they remained for 4 days before the survivors were transferred to new breeding vials.

During the breeding period, populations of flies were fed a yeast-agar medium (see recipe in Burant et al., 2019). This medium, which is the standard substrate for maintaining flies in cage culture, provided not only a source of nutrients for breeding adults but also acted as a substrate on which females deposited their egg and in which larvae developed. In contrast, during the non-breeding period flies were housed in a separate vial, in which they were provided a set volume of 5% (w/v) sugar water dispensed for a small feeding tip at the top of the vial (Figure 1). Unlike the yeast-agar medium, sugar water provided only sufficient energy and hydration for self-maintenance and survival, but did not allow females to produce eggs (due to a lack a protein) nor provide a substrate on which females could oviposit (Betini et al., 2015; Bownes & Blair, 1986). Under control conditions, seasonal populations were supplied with 10 ml of yeast-agar medium during the breeding period and 0.20 ml/day of 5% (w/v) sugar water during the non-breeding period.

2.2 | Season-specific habitat loss treatments

Populations were subjected to one of two types of chronic habitat loss: breeding habitat loss in which each generation saw a reduction in the volume of yeast-agar medium, or non-breeding habitat loss in which each generation saw a reduction in sugar water. In addition to control populations, which did not experience reductions in the

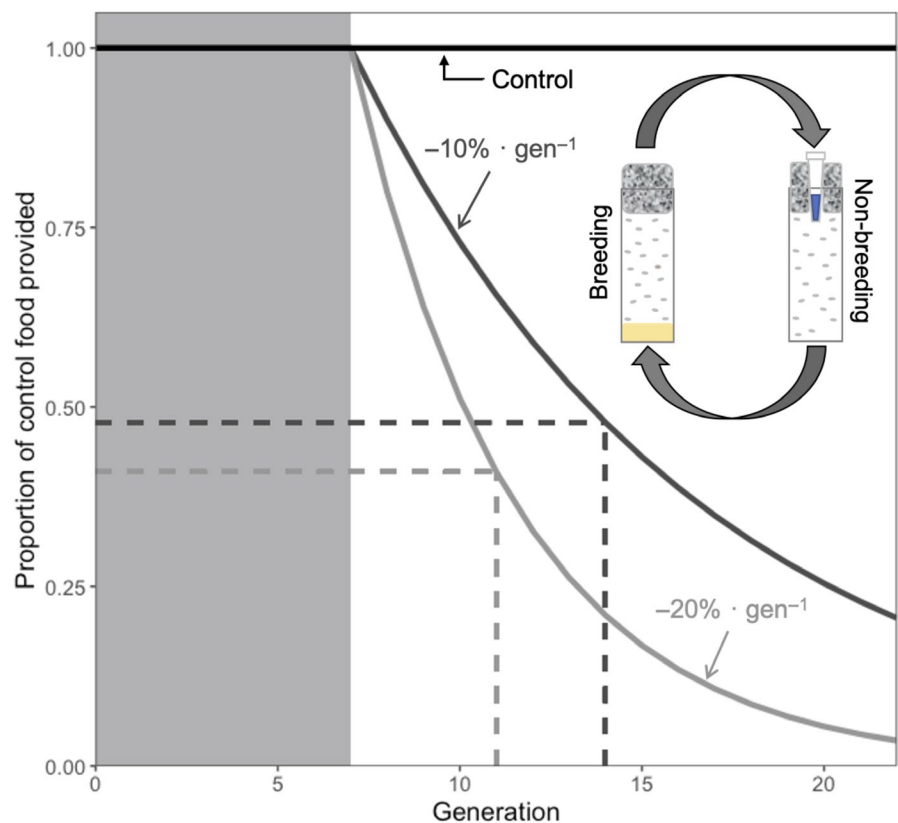


FIGURE 1 Experimental season-specific reductions in habitat. Replicate seasonal populations of fruit flies *Drosophila melanogaster* were exposed to chronic, multi-generation habitat loss in either the breeding or non-breeding season. Populations were maintained under control conditions for seven filial generations prior to the application of treatment (grey shaded area). During the treatment phase (generations 8–22), populations were subjected to either a 10% or 20% reduction in the quantity of food provided during the treatment period in each generation or experienced no reduction in food volume (control)

volumes of food provided in either season, replicates from each type of chronic habitat loss were subjected to one of two rates of loss (10% or 20% per generation; Figure 1). In total, the experiment consisted of four treatments (season of treatment \times rate of habitat loss treatment), plus controls, with 20 replicates per treatment ($N = 100$). To facilitate data collection, replicate populations were split across 5 days with each day comprising a tray of 20 replicates (4 replicates per treatment group). After filtering to exclude replicate populations that did not establish during the pre-treatment period (two each from the control and 10% breeding treatments), the dataset included abundance time series from 96 replicate populations from five treatments groups ($n_{\text{treatment}} = 18\text{--}20$).

Each replicate population was initiated by 10 males and 10 females that were haphazardly sampled from the outbred stock population (see above). All populations were maintained under control conditions (breeding period = 10 ml of yeast-agar medium; non-breeding period = 0.20 ml/day of 5% (w/v) sugar water) for seven filial generations prior to the commencement of treatment. Following the start of habitat loss in generation 8, treatments were applied for 15 generations, with the experiment ending after all treatment populations had collapsed.

2.3 | Bi-seasonal population abundance

In each generation, population size was sampled twice by counting the number of surviving adults. Breeding population size (i.e. the number of potential breeders) was taken as the number of individuals that entered the breeding period, and reflected the number of individuals who survived through the previous non-breeding season. Non-breeding population size was the number of adult offspring at the beginning of the non-breeding period and, therefore, represented the maximum population size in a given generation. We used time series from these two population counts (breeding and non-breeding abundance) to derive potential early warning indicators (see below).

2.4 | Measurement of individual traits

In addition to counting the number of individuals at the beginning of each season, we also sampled two traits, activity level and dry body mass, from a number of individuals in a subset of replicates. Traits were sampled beginning in generation 9 (two generations after the start of treatment), with sampling continued until the source population collapsed. From half of the replicate populations for each treatment ($n = 10$), up to six males per vial (sample size per treatment per generation = 60) were selected for a behavioural assay at the end of the 24-hr breeding period (see above). Measurements were only taken from male flies as selection for activity levels in males was expected to be stronger, with males competing for access to limited resources and mates (Kilgour et al., 2018). Individuals were randomly sampled by first lightly

sedating (but not fully anaesthetizing) the population with CO_2 at the end of the breeding period, then separating the selected males and placing each individual in a closed tube (5 mm \times 65 mm); the remainder of the adults was removed from the vials, frozen at -20°C and saved for counting.

Individuals selected for the behavioural assay were loaded into a *Drosophila* activity monitor (DAM2-5mm; Trikinetics Inc.), which used an infrared beam that bisected each tube to monitor the locomotor activity of the individual contained therein. Limited space in the activity monitor required splitting the flies sampled for activity measurements into three groups, which were measured in series throughout the day. To avoid introducing large amount of among-individual variability by sampling at different times of the day (Chiu et al., 2010), activity measurements were taken between 10:00 and 17:00. Previous data suggested that daily peaks in circadian activity levels occur outside this time interval (G.S. Betini, unpublished data). Individuals were allowed to acclimate to their tube and recover from the effects of light CO_2 for 15 min prior to the start of each trial. During the behavioural assay, as an individual moved from one end of the tube to the other, the number of times it interrupted the infrared beam was autonomously recorded and used as a simple measure of the fly's general activity level (Chiu et al., 2010; Rosato & Kyriacou, 2006). Each individual's behaviour was recorded over a 90-min interval, with activity levels calculated as an hourly rate (i.e. number of crosses per hour). Following the assay, flies were frozen at -20°C and saved for subsequent drying and weighing.

Body mass, which has previously been used as a general measure of body condition and size (Betini et al., 2014; Burant et al., 2020), was measured for the same individuals used in the behavioural assays. Dry masses were taken by first drying the flies at 55°C for 24 hr, before weighing them on an electronic balance (Mettler-Toledo XP26; $d = 0.001$ mg). Since both activity and body mass data were only collected from generations 9 to 21, we interpolated both traits to the start of the experiment to allow us to derive trait-based signals of population collapse (see descriptions of indicators below).

2.5 | Identification of tipping points

For each experimental population, we calculated growth rate as the relative change in abundance at a particular time point (breeding or non-breeding) from one generation to the next. For example, breeding growth rate in generation t was calculated as the breeding population size in generation t divided by the breeding population size in generation $t - 1$. Negative growth or population decline occurs when the growth rate falls below 1. Following Drake and Griffen (2010), loess smoothing was used to establish when the realized growth rate for a given population dropped and remained below 1 prior to population collapse, which was taken as indication that the population had passed through a bifurcation or tipping point. For those populations, the

bifurcation was defined as the first generation in which the realized growth rate fell and remained below 1, and each time series was then truncated to include abundances from generation 2 to the generation in which the bifurcation occurred (Clements & Ozgul, 2016a; Drake & Griffen, 2010). The first generations were excluded to avoid including the initial transitory dynamics as populations grew to carrying capacity (Drake & Griffen, 2010). Replicate populations from the experimental treatments that did not pass through a bifurcation were excluded from subsequent analyses.

To explore false-positive rates, we also assessed control (no habitat loss) replicates for the presence of tipping points. For habitat loss treatment replicates, our definition of tipping point required that realized growth dropped and remained below 1 during the treatment period but also that the population went extinct before the end of the experiment. Because none of the control replicates collapsed during the treatment period, we considered a 'false' tipping point to have occurred if the population was shrinking (i.e. realized growth $r < 1$) in the final generation of the experiment.

2.6 | Abundance-based indicators

Following methods proposed by Drake and Griffen (2010), we derived five leading indicators inherent in abundance time series: (a) autocorrelation at the first lag (acf), (b) first-order autoregressive coefficient (ar1), (c) coefficient of variation (cv), (d) density ratio (dr) and (e) return rate (rr). In all cases leading indicators were calculated on a rolling basis, with the value of an indicator in a particular generation calculated using the data accumulated in the preceding generations. Each leading indicator was then normalized by subtracting the long-run average and dividing by the standard deviation such that the normalized value (\hat{w}_t) of an indicator at time t was equivalent to $(w_t - \bar{w}_{1:t}) / sd(w_{1:t})$.

2.7 | Trait-based indicators

In addition to the five abundance-based indicators described above, we also derived four trait-based indicators for the subset of replicate populations from which individual traits were sampled (see above; $n = 10$): (a) mean locomotor activity level (activity.mean), (b) standard deviation of activity (activity.sd), (c) mean dry body mass (mass.mean) and (d) standard deviation of body mass (mass.sd). Importantly, although the same set of 'classical' leading indicators that were calculated from abundance time series could in principle be derived from time series of phenotypic traits, this is not likely to be informative (Clements & Ozgul, 2016a), and instead we expected density-dependent changes in these traits consistent with previous experiments (Burant et al., 2020; Kilgour et al., 2018). For both mean body mass and activity levels, we predicted indicator values to decrease as treatments experienced

reductions in food availability over time. The variances of these trait values, as measured by standard deviation, were expected to increase due to destabilizing selection favouring disparate trait values.

2.8 | Composite indicators

In addition to assessing the predictive capacity of each leading indicator separately, we were also interested in determining whether composite signals, composed of multiple leading indicators, improved early warning of the impending bifurcation. To do so, composite indicators were calculated by summing the normalized values of the indicators of interest in each generation. Whereas Clements and Ozgul (2016a) calculated all possible pairwise, etc., combinations of leading indicators and assessed which combinations provided improved early warning, here we calculated three composite indicators: (a) all leading indicators derived from abundance time series (comp.count = acf + ar1 + cv + dd + rr), (b) all trait-based leading indicators (comp.trait = activity.mean + activity.sd + mass.mean + mass.sd) and (c) all leading indicators (comp.all). Following Clements and Ozgul (2016a), the normalized values of return rate and mean trait values were multiplied by -1 when used as part of the composite indicators. For return rate, negative values are expected because systems take longer to return to equilibrium as they become unstable while both mass and activity are expected to decline with food availability.

Since we only sampled body mass and activity from individuals at the end of the breeding period (i.e. after adults were removed from the experiment), trait data were only used in conjunction with time series of breeding population abundance. In contrast, abundance-based leading indicators can be derived separately from breeding and non-breeding abundance time series for each population. To avoid using the same trait measurements twice, and because the traits were not measured at the start of the non-breeding period, composite indicators that include trait-based metrics (i.e. comp.trait and comp.all; see definitions above) were only calculated using the breeding abundance time series. In contrast, only abundance-based indicators were calculated for non-breeding time series (see Supporting Information). Typically, information is not available on the demography of seasonal populations throughout the full annual cycle, with populations most often censused in the breeding season (Marra et al., 2015). As such, any application of abundance- or trait-based signals is likely most relevant for data collected during the breeding period, but they should also occur in time series from the non-breeding period.

For each replicate population, leading and composite indicators were considered to act as early warning signals of an impending bifurcation if the value of the indicator deviated from the running average of the indicator by more than one standard deviation in the interval between the application of treatment (generation 8) and the tipping point. Previous work has shown that the strength of the threshold influences the ratio of true

and false positives, and has advocated that the threshold be set based on a considerations such as time-series length (Clements & Ozgul, 2016a). Here, we have selected a 1 *SD* threshold based on the exploratory nature of the analysis, the relatively short length of the experimental time series and the relatively strong rates of environmental forcing.

Finally, a given leading or composite indicator was considered to produce a sensitive signal if it acted as an early warning signal in at least 70% of the replicate populations sampled for each treatment (in a chi-square test, at least 14 of 20 replicates must show a signal for the result to be interpreted as different from chance). Since traits were only sampled in half of the replicates, the sample sizes for abundance- and trait-based indicators differed. For each of the three composite indicators, we performed a series of two-proportion Z-tests (Pan, 2002) to investigate whether the season and rate of habitat loss influenced signal production. We estimated 95% confidence intervals on the difference between the proportions of replicates that produced a signal for each pairwise comparison following Agresti and Caffo (2000). All tests were one-tailed with a significance threshold of $\alpha = 0.05$.

All analyses were conducted in the R statistical environment (v. 3.6.1; R Core Team, 2019). The data used for the analyses are publicly available through the Figshare repository (Burant et al., 2021).

3 | RESULTS

3.1 | Population dynamics

Time series of breeding abundance revealed distinct patterns of population decline depending on whether habitat was lost in either the breeding or non-breeding period (Figure 2a). During the pre-treatment period, when all replicates were maintained under control conditions, populations showed a rapid increase in the number of breeding individuals. In general, although faster rates of habitat loss resulted in more rapid declines, populations exposed to the two different rates of habitat loss (10% and 20% per generation) showed similar dynamics that were contingent on the season in which habitat loss occurred. When breeding habitat was lost,

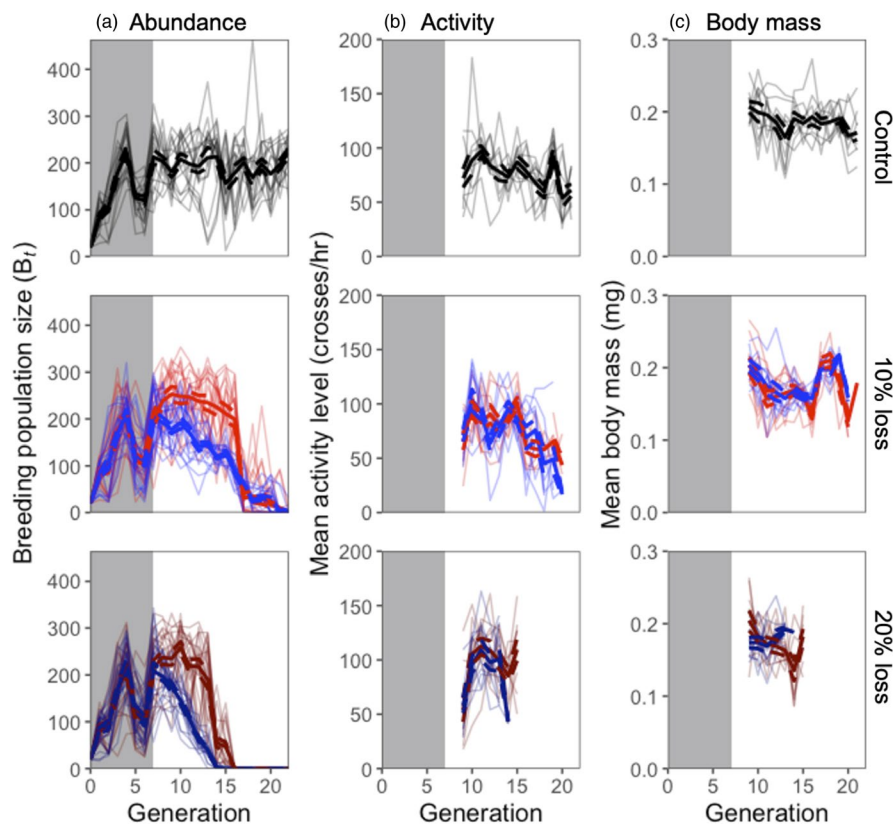


FIGURE 2 Effects of seasonal habitat loss treatments in *Drosophila* populations. (a) Breeding population size through time. Adult abundance was counted twice per generation: the number of adults that survived the previous non-breeding season to reproduce in the next season (breeding population size, B_t), and the total number of adult offspring at the beginning of the non-breeding period (non-breeding population size, NB_t ; see Figure S1). (b) Change in individual activity level through time. For generations 9–21 (two generations after the start of treatment), activity levels were measured for a subset of adults (sample size per treatment per generation = 60 individuals) using *Drosophila* activity monitors (see Section 2.4). (c) Change in body mass through time. Dried weights were taken from the same individuals sampled for activity levels. The shaded grey area corresponds to the pre-treatment period. Line colours indicate the type of treatment: control (black), breeding habitat loss (red) and non-breeding habitat loss (blue). Thick solid lines indicate the average time series for each treatment, with dashed lines showing the standard error. Replicates per treatment = 18–20 (total replicates = 96)

breeding population size remained relatively stable for several generations before declining precipitously in the generations preceding collapse. In contrast, when non-breeding habitat was lost, breeding population size began to decline with the onset of treatment (Figure 2a).

For those populations for which a bifurcation could be identified during the treatment period (number of replicates per treatment: $n_{B10} = 13$, $n_{B20} = 16$, $n_{NB10} = 19$, $n_{NB20} = 20$; see Section 2.5), realized growth rate rapidly declined towards ~ 1 in the initial generations of the pre-treatment period as populations grew towards carrying capacity (Figure 3). During the treatment period, populations in the 20% habitat loss treatment had earlier bifurcations than those in the 10% habitat loss treatment, irrespective of the season of treatment (Figures 2a and 3). That said, the average generation in which the tipping point occurred was generally earlier for populations losing non-breeding habitat (tipping point generation: $\bar{x}_{NB10} = 12.9 \pm 2.9$ ($\pm SD$); $\bar{x}_{NB20} = 10 \pm 0.95$), compared to populations losing breeding habitat ($\bar{x}_{B10} = 14.8 \pm 2.4$; $\bar{x}_{B20} = 12.2 \pm 1.5$; Figure 3). The timing of bifurcations was more variable among populations in 10% treatments than the 20% treatments (Figure 3). In general, similar results were also obtained from time series of non-breeding population size (Figure S2). Among control populations (for which habitat was constant in each season), assessment of realized growth rates at the end of the treatment period showed that 4 of 18 replicates (22%) passed through a bifurcation, which provides a coarse estimate of false-positive rates in tipping point detection.

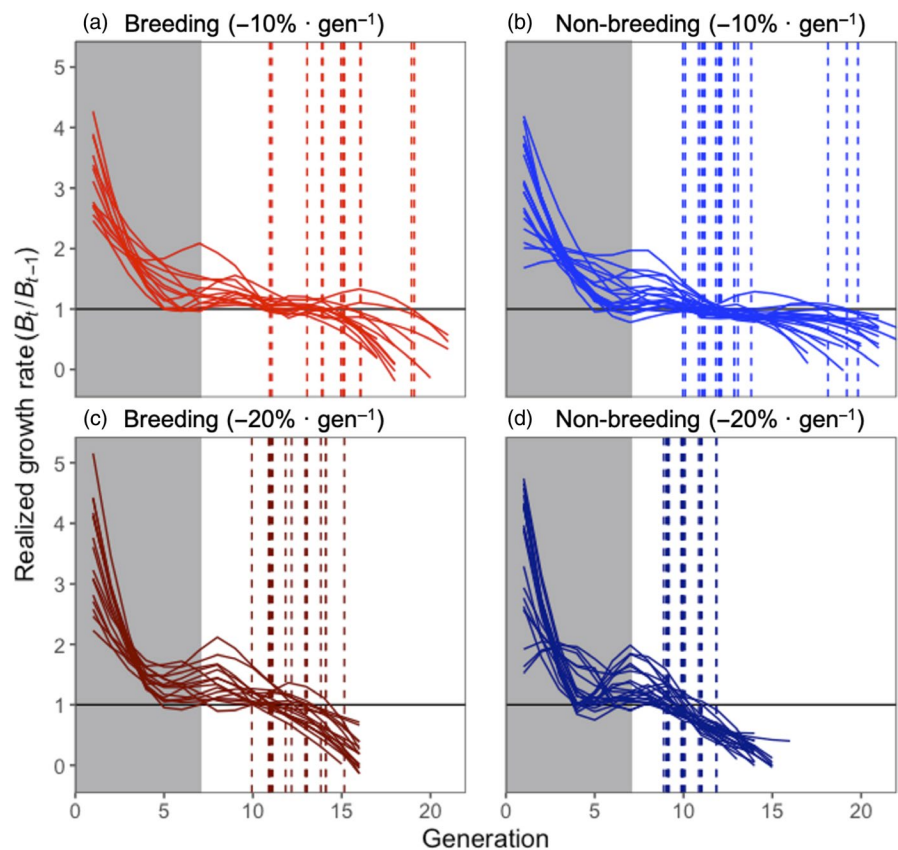
3.2 | Activity levels and body mass

Season-specific habitat loss also impacted how individual body mass and locomotor activity changed through time (Figure 2b,c). For control populations, there was a slight decline in both body mass and activity as the experiment progressed. In the 10% treatment groups, activity declined rapidly with the progression of treatment, likely as a result of a reduction in available energy above what was used for self-maintenance (Figure 2b). In contrast, in the 20% treatments, non-breeding habitat loss resulted in a decrease in activity levels while breeding habitat loss resulted in an increase (Figure 2b). As populations were exposed to habitat loss treatment, individuals also showed an initial decline in body mass followed by an uptick in body mass in the generations preceding population collapse (Figure 2c), potentially a result of density-mediated effects on individual condition.

3.3 | Detection of early warning indicators of population collapse

The performance of abundance-based indicators differed considerably depending on the strength and season in which habitat was lost (Figure 4). In general, abundance-based leading indicators performed more reliably (i.e. were more sensitive) as early warning signals for populations subjected to breeding habitat loss treatments, with three of five indicators indicating an impending transition for

FIGURE 3 Realized growth rates and bifurcations derived from breeding population abundances. Replicate populations were exposed to one of four treatments: (a) 10% breeding habitat loss, (b) 10% non-breeding habitat loss, (c) 20% breeding habitat loss or (d) 20% non-breeding habitat loss. Growth rate in a given generation t was calculated by dividing the breeding population size B_t by the breeding abundance in the previous generation B_{t-1} . A population can be said to have passed through a bifurcation or tipping point when its realized growth rate falls and remains below 1 prior to population collapse. The shaded grey area denotes the pre-treatment period. The dashed lines correspond to the identified tipping point for each replicate population. Only populations that showed a bifurcation and collapsed by the end of the experiment were included in subsequent analyses. Sample size per treatment = 13–20 (total replicates = 68). (See Figure S2 for non-breeding growth rates.)



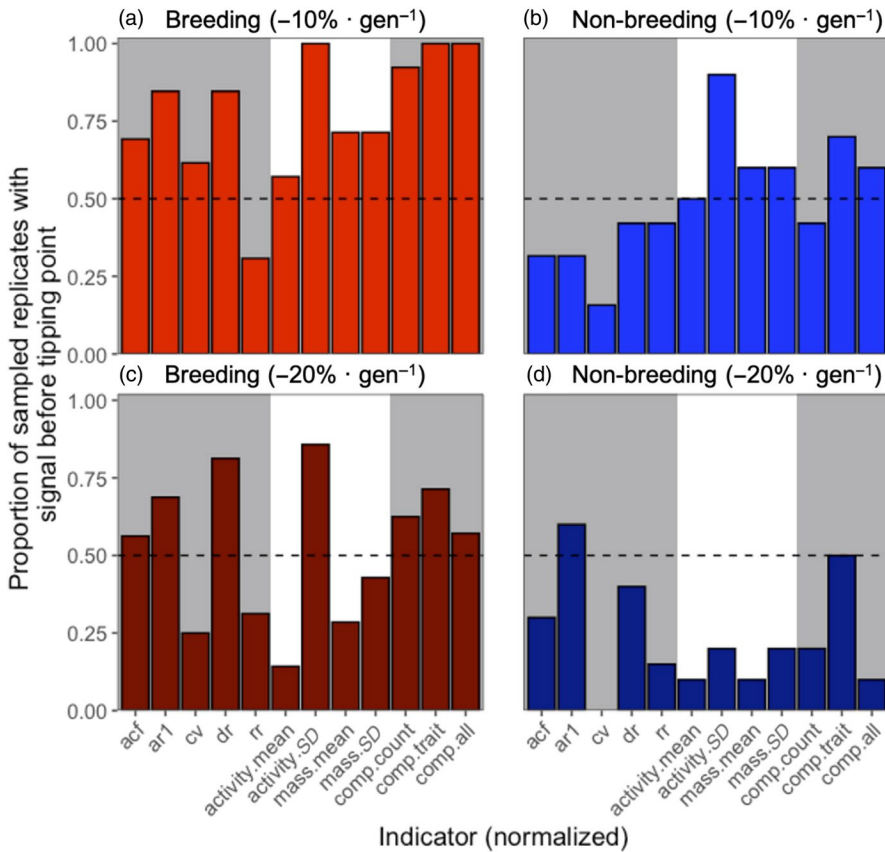


FIGURE 4 Proportion of time series for which leading and composite indicators act as early warning signals. Populations were exposed to one of four treatments: (a) 10% breeding habitat loss, (b) 10% non-breeding habitat loss, (c) 20% breeding habitat loss or (d) 20% non-breeding habitat loss. From each replicate population, we derived a series of indicators intrinsic to the time series of breeding abundance: acf = autocorrelation at the first lag; ar1 = first autoregressive coefficient; cv = coefficient of variation; dr = density ratio; rr = return rate, and four trait-based indicators, namely the average and standard deviations for locomotor activity (activity.mean; activity.sd) and dry body mass (mass.mean; mass.sd). We also calculated three composite indicators, which combined the indicators intrinsic to abundance time series (comp.count), the trait-based indicators (comp.trait) or all leading indicators (comp.all). An indicator served as an early warning signal of an impending bifurcation if the value of the indicator deviated from the running average by more than 1 standard deviation

at least 70% of replicates in the 10% breeding treatment (excluding coefficient of variation (cv); Figure 4a), and two of five for the 20% breeding treatment (excluding cv and return rate (rr); Figure 4c). In contrast, abundance-based indicators were poor predictors of an impending transition when non-breeding habitat was lost (all indicators occurred in <50% of non-breeding habitat loss treatment replicates; Figure 4b,d). The performance of abundance-based indicators derived from non-breeding time series was similar, although the proportion of replicates that showed early warning signals was lower (Figure S3).

Trait-based leading indicators also showed considerable differences in their utility as early warning signals, depending on both the season and strength of habitat loss (Figure 4). The standard deviation in locomotor activity (activity.sd) was the most frequently detected trait-based early warning indicator, and was detected in over 70% of replicate populations in each treatment group except for the 20% non-breeding habitat loss treatment. Two other trait-based indices derived from measurements of individual body mass (mass.mean and mass.sd) functioned as early warning indicators when populations lost breeding or non-breeding habitat, but only for the 10% rate of loss (Figure 4a,b). Detection of trait-based early warning signals was limited in the 20% habitat loss treatments, with none of the four indicators providing early warning signals of population collapse (Figure 4d). Overall, trait-based early warning signals were detected more in populations losing breeding habitat than those losing non-breeding habitat.

In general, composite indicators were good early warning signals of population collapse, with some indication that the inclusion

of trait-based indicators in the composite metrics improved signal detection (Figure 4). The composite indicator comprised of all abundance-based leading indicators (comp.count) showed early warning signs in a majority (>70%) of replicate populations losing breeding habitat (Figure 4a,c), but not in populations that lost non-breeding habitat (see Table 1 for summary of pairwise differences and two-proportion Z-tests; Figure 4b,d). We found evidence that higher rates of breeding habitat loss produced less sensitive abundance-based composite signals, with some support for a similar effect with non-breeding habitat loss (Table 1).

The composite trait-based indicator (comp.trait) appeared to perform better than the abundance-based composite indicator, but again was a poor signal of impending population collapse for the 20% non-breeding treatment (Figure 4d). For the 10% treatments, trait-based composite signals appeared to be more sensitive to breeding habitat loss, but not for the 20% treatments (Table 1; Figure 4). For breeding treatments, but not non-breeding treatments, an increased rate of habitat loss generated fewer early warning signals (Table 1).

Finally, combining all abundance- and trait-based leading indicators into a single composite metric (comp.all) generally provided similar or improved detection of impending population collapse relative to the composite of abundance-based indicators (Figure 4). Notably, the composite of all abundance- and trait-based indicators provided more sensitive early warning for breeding treatments, compared to non-breeding treatments, with fewer signals produced at higher rates of habitat loss (Table 1).

TABLE 1 Pairwise comparisons of the proportion of replicates from each treatment for which composite indicators provided advanced warning of population collapse. The difference in the proportions of sampled replicates from each treatment for which a composite indicator acted as an early warning signal ($\delta\hat{p} \pm 95\%$ confidence interval) was calculated to investigate how the season and rate of habitat loss influenced signal production. In the table: T_1 and T_2 are the two treatments being compared; n_1 and n_2 are the sample sizes from these treatments; \hat{p}_1 and \hat{p}_2 indicate the proportion of sampled replicates that produced a signal for each treatment; Z is the test-statistic calculated from a two-proportion Z-test

Comparison type	T_1	T_2	n_1	n_2	\hat{p}_1	\hat{p}_2	$\delta\hat{p} \pm 95\% \text{ CI}$	Z	p
Composite of abundance-based leading indicators (comp.count)									
Season (10%)	B10	NB10	13	19	0.92	0.42	0.50 ± 0.27	2.87	0.002
Season (20%)	B20	NB20	16	20	0.63	0.20	0.43 ± 0.30	2.63	0.004
Rate (B)	B10	B20	13	16	0.92	0.63	0.30 ± 0.28	1.82	0.034
Rate (NB)	NB10	NB20	19	20	0.42	0.20	0.22 ± 0.28	1.49	0.069
Composite of trait-based leading indicators (comp.trait)									
Season (10%)	B10	NB10	7	10	1.00	0.70	0.30 ± 0.28	1.6	0.055
Season (20%)	B20	NB20	7	10	0.71	0.50	0.21 ± 0.46	0.87	0.19
Rate (B)	B10	B20	7	7	1.00	0.71	0.29 ± 0.33	1.54	0.062
Rate (NB)	NB10	NB20	10	10	0.70	0.50	0.20 ± 0.42	0.91	0.18
Composite of all (abundance- and trait-based) leading indicators (comp.all)									
Season (10%)	B10	NB10	7	10	1.00	0.60	0.40 ± 0.30	1.91	0.056
Season (20%)	B20	NB20	7	10	0.57	0.10	0.47 ± 0.41	2.09	0.037
Rate (B)	B10	B20	7	7	1.00	0.57	0.43 ± 0.37	1.96	0.050
Rate (NB)	NB10	NB20	10	10	0.60	0.10	0.50 ± 0.36	2.34	0.010

3.4 | Time from early warning signal production to the tipping point

In addition to differences in the proportion of replicate populations for which the indicators provided early warning (see above; Figure 4), lead times (the interval between the generation in which an early warning signal was produced and the generation in which the population reached a tipping point) varied with the season and rate of habitat loss. In general, lead times were longer with 10% habitat loss compared to 20%, and were also longer with breeding habitat loss than with non-breeding (Table S1). Lead times from the three composite indicators were similar, although the trait-based composite indicator (comp.trait; lead times: $\bar{x}_{B10} = 3.9 \pm 1.7$ ($\pm SD$); $\bar{x}_{NB10} = 2.7 \pm 1.6$; $\bar{x}_{B20} = 2.2 \pm 1.6$; $\bar{x}_{NB20} = 2.2 \pm 0.45$ generations) typically provided a shorter warning interval than either the composites of abundance-based indicators (comp.count; $\bar{x}_{B10} = 4.5 \pm 3.0$; $\bar{x}_{NB10} = 4.6 \pm 2.1$; $\bar{x}_{B20} = 3.0 \pm 1.3$; $\bar{x}_{NB20} = 1.5 \pm 0.58$ generations) or all the indicators (comp.all; $\bar{x}_{B10} = 4.4 \pm 3.3$; $\bar{x}_{NB10} = 3.0 \pm 2.0$; $\bar{x}_{B20} = 3.0 \pm 0.82$; $\bar{x}_{NB20} = 2.0 \pm 0$ generations; Table S1).

4 | DISCUSSION

Using a multi-generation experiment in which replicate populations were subjected to chronic habitat loss in either the breeding or non-breeding period, we examined how the timing of seasonal stressors influenced the detectability of signals preceding population collapse. Consistent with our hypothesis, we show that the season in which habitat is lost has unique effects on the response of leading

indicators intrinsic to time series of population abundance, as well as indicators derived from the measurement of individual traits. In general, abundance-based indicators derived from breeding population time series provided more sensitive advanced warning of impending collapse for populations losing breeding habitat, compared to those losing non-breeding habitat. This pattern was broadly true for trait-based indicators as well. Composite indicators, derived by combining abundance- and trait-based indicators, generally performed better than the leading indicators by themselves. We further demonstrate that, consistent with our hypothesis and previous studies, leading and composite indicators performed worse with higher levels of habitat loss.

Although body size has been the primary focus of previous studies on trait-based early warning signals (Arkilanian et al., 2020; Baruah et al., 2019; Clements et al., 2017; Clements & Ozgul, 2016a), here we broaden the application of the theory to include a non-morphological trait. By incorporating data on individual locomotor activity, as well as body mass, our results bolster predictions about the utility of other fitness-related traits (e.g. behaviour, phenology) in detecting population declines (Burthe et al., 2016; Clements & Ozgul, 2018). Previous work in our seasonal fruit fly system demonstrated that changes in body size or condition associated with a reduction in food availability can negatively impact per capita reproductive output, thereby impacting fitness and linking individual performance with population-level change (Betini et al., 2013a, 2014; Burant et al., 2020). Likewise, we also anticipated changes in locomotor activity, since behaviour is a common way in which organisms respond to environmental change (Beever et al., 2017; Wong & Candolin, 2015). In this system, changes in density (like

those associated with reductions in food availability) are known to induce behavioural responses (Kilgour et al., 2018), which may be related to either decreased energy expenditure when food is scarce or increased competition for food and space when density is high. Importantly, we only present measures of individual traits in male *Drosophila*, not females, and the sexes are known to differ in their responses to resource limitation (Magwere et al., 2004). Females fruit flies are larger than males and, thus, likely to experience higher basal energetic costs, which may heighten the impact of resource limitation. Likewise, adaptive shifts in body size may be hindered by floor effects in the smaller males. Locomotor activity is likely to be under stronger selection in males due to its importance for resource and mate acquisition. Despite these considerations, we expect that males and females would show similar trait trajectories in response to reductions in food availability. These results support calls for further integration of fitness-related traits in studies of population viability and conservation, and additional work to understand possible difference between sexes.

Consistent with earlier theoretical (Drake & Griffen, 2010) and experimental work (Baruah et al., 2019; Clements & Ozgul, 2016a), we showed that composite metrics composed of multiple abundance- and trait-based indicators have improved predictive capacity. However, our results reveal that whether a composite indicator acted as an early warning signal was, at least in part, dependent on the timing of the stressor driving a population to decline. Composite metrics constructed by combining all abundance-based indicators (including those that do not show early warning signal themselves) performed better, on average, than any of the indicators alone, but only when populations lost breeding habitat. When non-breeding habitat was removed, the abundance-based composite indicator did not provide advanced warning that populations were nearing collapse, irrespective of the treatment strength. With slower rates of habitat loss (10% per generation), incorporating trait-based values generally improved early warning signal production in the composite indicators. This result, while novel in its incorporation of seasonality, aligns with previous evidence that systems can show rapid transitions between stable states in the absence of early warning signals (Boerlijst et al., 2009; Boettiger & Hastings, 2012; Perretti & Munch, 2012).

The discrepancy in signal production between populations subjected to breeding and non-breeding habitat loss may be due to differences in the capacity for populations to respond via changes in population growth and vital rates (Burant et al., 2019). With non-breeding habitat loss, populations rebound in the subsequent breeding period due to density-dependent reproduction, increasing fluctuations and instability between breeding and non-breeding abundances, which may dampen the production of early warning signals in breeding abundance time series. This is not the case with breeding habitat loss because any density-dependent reproduction on the part of breeders is cancelled out by limited food availability for offspring (Burant et al., 2019). The reduced stability with non-breeding habitat loss would similarly hinder advanced detection. Importantly, although there was a general pattern of reduced early warning signal production with non-breeding habitat loss, and no

other studies have considered seasonality in this context, our previous work suggests that other signals, such as changes in vital rates, may also provide indication of which season is driving a population to decline (Burant et al., 2019).

In our experiment, we specified our control and pre-treatment conditions following on previous work in the seasonal fruit fly system (Betini et al., 2013a, 2015). The control conditions were selected based on a series of preliminary trials which suggested that the combination of 10 ml yeast-agar medium (breeding) and 0.2 ml/day sugar water (non-breeding) produced stable, bi-seasonal population dynamics, in which realized growth rates (i.e. B_t/B_{t-1} and NB_t/NB_{t-1}) approach unity (G.S. Betini & D.R. Norris, unpublished data). Despite this, it is possible that seasonal differences in the timing of the onset of population declines following the application of treatment could be in part attributed to differences in the degree or strength of selection imposed. For example, while we argue that the delayed onset of population declines with breeding habitat loss is likely attributable to the effects of density-dependent reproduction (Burant et al., 2019), it is possible that control breeding conditions are simply in greater excess of the absolute food availability required to maintain stable population size than are the control non-breeding conditions. In addition, to measure individual activity levels, we first had to lightly anaesthetize the fruit flies, which is known to have impacts on activity and reproduction (Barron, 2000; van Dijken et al., 1977). Although flies from all replicates were similarly handled (see Section 2.4), it is possible that the breeding and non-breeding treatments (and different rates of loss) differentially influenced the ability of flies to recover from anaesthesia. However, because the populations were first transferred to new breeding vials to reproduce for 24 hr prior to activity measurements, we expect that any differences in activity levels can be attributed to real treatment effects.

In conclusion, our results support a growing body of evidence that indicates that composite and trait-based indicators can provide advanced warning of an impending population decline. We extend existing knowledge on the use and limitations of early warning signals by exploring how seasonal differences in stressors that drive populations to decline influence the detectability of these signals. Our results suggest that it is imperative to consider the timing or location of stressors in the context of annual cycle when using early warning signals (or other predictive tools) to identify populations at risk of local or range-wide extinction. Given that the vast majority of wild populations exist in seasonal regimes, and that many face ongoing anthropogenic threats to their persistence, understanding how populations decline and whether these declines can be detected in the types of demographic and trait data commonly collected in the field is a critical component of conservation efforts.

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AUTHORS' CONTRIBUTIONS

J.B.B. and D.R.N. designed the experiment; G.S.B. was involved in developing the statistical indicators. J.B.B. conducted the experiment; J.B.B. and C.P. collected the phenotypic data. J.B.B. 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 used in the analyses will be made publicly available through the Figshare repository <https://doi.org/10.6084/m9.figshare.13635305> (Burant et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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