Metapopulation extinction risk is increased by environmental stochasticity and assemblage complexity

James C. Bull^{1,2}, Nicola J. Pickup^{1,3}, Brian Pickett¹,
Michael P. Hassell¹ and Michael B. Bonsall^{1,3,*}

¹*Division of Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, UK*

²*Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK*

³*Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*

Extinction risk is a key area of investigation for contemporary ecologists and conservation biologists. Practical conservation efforts for vulnerable species can be considerably enhanced by thoroughly understanding the ecological processes that interact to determine species persistence or extinction. Theory has highlighted the importance of both extrinsic environmental factors and intrinsic demographic processes. In laboratory microcosms, single-species single-habitat patch experimental designs have been widely used to validate the theoretical prediction that environmental heterogeneity can increase extinction risk. Here, we develop on this theme by testing the effects of fluctuating resource levels in experimental multispecies metapopulations. We compare a three-species host–parasitoid assemblage that exhibits apparent competition to the individual pairwise, host–parasitoid interactions. Existing theory is broadly supported for two-species assemblages: environmental stochasticity reduces trophic interaction persistence time, while metapopulation structure increases persistence time. However, with increasing assemblage complexity, the effects of trophic interactions mask environmental impacts and persistence time is further reduced, regardless of resource renewal regime. We relate our findings to recent theory, highlighting the importance of taking into account both intrinsic and extrinsic factors, over a range of spatial scales, in order to understand resource–consumer dynamics.

Keywords: apparent competition; environmental stochasticity; extinction risk; host–parasitoid; microcosm; regulatory process

1. INTRODUCTION

Understanding and quantifying the factors that determine extinction risk is currently one of the most pressing challenges for ecology and conservation science (Lande 1993; Brook *et al.* 2000; McLaughlin *et al.* 2002). Historically, these factors have been broadly classed either as extrinsic, such as density-independent environmental fluctuation, or intrinsic, typically density-dependent demographic processes (Caughley 1994). Stochastic population theory (Caswell 2001; Morris & Doak 2002) and empirical studies (Benton *et al.* 2002; Drake & Lodge 2004; Drake 2005) have shown that both these processes may have profound effects on population dynamics and extinction risk. In addition, there has been growing appreciation that rather than either extrinsic or intrinsic factors predominating in affecting population persistence, environmental heterogeneities may interact in a nonlinear fashion with spatial and temporal demographic processes to affect populations in complex and often unpredictable ways (Chase & Ryberg 2004; Childs *et al.* 2004).

While considerable progress has been made in identifying the regulatory processes that affect natural populations, quantifying and understanding the ways in which these various processes interact is a continuing contemporary problem (Sæther & Engen 2004). While the goal is to understand these processes in natural systems, laboratory microcosm experiments provide an ideal compromise for this type of problem (Bonsall & Hassell 2005), as they allow single variables to be controlled either individually or in concert with others (Huffaker *et al.* 1963; Bonsall & Hassell 1997; Bonsall *et al.* 2002). In addition, replicate populations can be established and analysed with inferences not possible in many field-based studies (Bonsall & Hastings 2004). Recently, much has been learnt about environmental and demographic processes through these types of studies (Holyoak 2000a; Ellner *et al.* 2001; Bonsall & Benmayor 2005). However, currently, laboratory investigations that explicitly investigate the combined roles of both environmental and demographic stochasticity are usually limited to single-species studies (but see Holyoak 2000b).

Previously, we have successfully used insect microcosm assemblages to explore the persistence times and regulatory processes within two-species and simple multispecies predator–prey populations and metapopulations (Bonsall *et al.* 2005; Bull *et al.* 2006). In this study, we extend this

* Author for correspondence (michael.bonsall@zoo.ox.ac.uk).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2006.3691> or via <http://www.journals.royalsoc.ac.uk>.

previous work to investigate the effects of environmental heterogeneity on multispecies metapopulation persistence times in experimental laboratory-based microcosms. We impose a quantified stochastic resource renewal regime and investigate the interactions between this extrinsic source of variation and the resulting demographic effects on a bruchid beetle assemblage in the presence and absence of a shared natural enemy.

Existing theory, supported by experimental studies, predicts that single host species in the absence of predation are significantly more vulnerable to stochastic extinction risks in a fluctuating, rather than constant, environment (Benton *et al.* 2002; Drake & Lodge 2004; Sæther & Engen 2004). We hypothesize that host–parasitoid metapopulations are also less persistent in a fluctuating environment than in one where resource levels, available to host species, are constant. Given that the inclusion of density-dependent demographic stochasticity has been shown to improve prediction of single-species persistence with varying resource levels (Drake 2005), we question whether the nature of demographic regulatory processes is associated with levels of spatio-temporal environmental heterogeneity. Further, it may be the case that intrinsic and extrinsic processes interact to overturn simplistic metapopulation predictions. For example, currently, it might be predicted that the increasing ability of hosts to escape predation should lead to reduced risk of extinction (Ellner *et al.* 2001). We explicitly test this prediction and relate our findings to intrinsic and extrinsic ecological processes.

Previously, we have shown that when species assemblage complexity is increased, other regulatory processes are over-shadowed by the effects of predation (Bonsall *et al.* 2005). Here, by comparing a three-species assemblage (*Callosobruchus maculatus*, *Callosobruchus chinensis*, *Anisopteromalus calandrae*) that exhibits apparent competition to the individual pairwise host–parasitoid interactions, we investigate whether increased pressure from parasitism and indirect trophic interactions reduce assemblage persistence time irrespective of environmental conditions.

2. MATERIAL AND METHODS

(a) Experimental design

Laboratory microcosms were used to explore the hypothesis that stochastic renewal of resources (for the host species) reduces the persistence of host–parasitoid assemblage interactions using the bruchid beetles, *Callosobruchus maculatus* (L.) (Coleoptera: Bruchidae), *C. chinensis* (L.) (Coleoptera: Bruchidae) and the parasitoid, *A. calandrae* (Howard) (Hymenoptera: Pteromalidae). For details of the life histories of these bruchids, see Bellows (1982), and for parasitoid experimental procedures, see Bonsall *et al.* (2002). Briefly, both *C. maculatus* and *C. chinensis* are well-known pests of leguminous crops and have similar life histories that make them particularly suitable for laboratory studies. Eggs are laid on, and adhere to, the surface of a host seed. The first instar larvae burrow directly into the cotyledon of the seed before developing over a period of approximately three weeks under our experimental conditions. Emerged adult seed weevils lay eggs at a declining rate over a period of around one week, before death. Therefore, the generation time of

Figure 1. Diagrammatic plan view of single-patch and four-patch metapopulation experimental design. Single patches comprised plastic boxes, divided into four regions (dividers did not impede insects). Each region was filled with black-eyed beans using a four-week rolling renewal programme such that each week the beans from the oldest quadrant were replaced and no beans remained in patches for more than four weeks. Single patches could be connected (using tubes, denoted by dashed lines), for 2 h per week, to form four-cell (2×2) metapopulations. The figure represents a ‘snap-shot’ of the experimental design for any given week. Patches subject to constant resource renewal had three beans replaced every week. Patches subject to stochastic resource renewal had variable number of beans replaced each week, drawn from a Poisson distribution with a long-term average of three.

bruchids in our system is typically around four weeks. The pteromalid wasp, *A. calandrae*, is a polyphagous ectoparasitoid idiobiont attacking a wide range of stored product insect pests. Larval development time is less than two weeks, with adults living for a further week, under our experimental conditions.

Clear plastic boxes ($73 \times 73 \times 30$ mm) were used as the baseline ‘patch’ for the study. The patches had a hole (4.4 mm diameter) in each of the four sides which could either be either blocked or have lengths (50 mm) of plastic tube inserted to connect patches horizontally into four-patch (2×2) square lattice arrangements (figure 1). A single-layer lattice of these patches was used for treatments that included only one bruchid species. In the apparent competition treatments including both bruchids, patches could be stacked into double-layer systems. In these cases, top- and bottom-layer patches were connected by a 25 mm diameter mesh-covered hole. The mesh (hole size, 2 mm) prevented vertical movement of bruchids, and consequently, any direct interspecific resource competition. However, this tested experimental design did not inhibit the foraging activity of the parasitoid over both bruchid species and allowed an apparent competitive interaction to be established (Bonsall *et al.* 2005; Bull *et al.* 2006).

The host resources used in our study were black-eyed beans (*Vigna unguiculata* (L.) Wapl. (Leguminosae)). Two different bean renewal regimes were followed: a constant resource renewal treatment where three beans were introduced each week (or three beans on each level in double-layer lattices), and a stochastic renewal regime in which the long-term average number of beans was three per week, but each week every individual patch received a number of beans,

drawn from a Poisson distribution with a mean of three, in order to establish a quantified stochastic renewal regime. The Poisson distribution models a count variable where the variance is proportional to the mean and therefore is a simple and appropriate distribution for describing our distribution of resources among patches. Alternative resource distributions, particularly overdispersed distribution of resources, could be informative but were beyond the scope of this study.

The experiments were seeded over a period of four weeks by introducing black-eyed beans, according to the resource renewal regime outlined above, such that the patches were fully stocked with beans by the fourth week—in line with the generation time of the bruchids. Concurrent with the initial seeding with beans, four pairs of bruchids were introduced into every patch each week. In the cases of double-layer lattices, two replicates of each treatment were seeded with four pairs of *C. maculatus* on the upper lattice and four pairs of *C. chinensis* on the lower lattice and two replicates the other way around. No new bruchids were added to any patch after the third week. On the fourth week, the next generation of bruchids emerged from the beans introduced in the first week. Subsequently, beans were replaced following the sequential four-week resource renewal regime, with the oldest beans being replaced from each patch every week. Removed beans were stored for a further four weeks and any newly emerging animals were released back into the appropriate patch.

Three different species combinations were established in separate metapopulations: *C. maculatus* with *A. calandreae*, *C. chinensis* with *A. calandreae* as separate two-species interactions and the full three-species apparent competition interaction (*C. maculatus*–*C. chinensis*–*A. calandreae*). In addition, all species combinations for both resource renewal regimes were replicated in single-patch systems. All resource regime and species combination treatments, including single-patch controls, were replicated four times.

After the host species had become established for several generations without dispersal between patches, dispersal for 2 h each week was initiated. This dispersal regime has previously been shown to result in a significant increase in interaction persistence time compared with unlimited dispersal (Bonsall et al. 2005). Parasitoids were introduced after a further two host generations. Four pairs of wasps were introduced over two consecutive weeks into a single patch in each of the metapopulations. Time-series for all species were obtained by counting both alive and dead insects each week from every patch (dead insects were then removed). All the experiments were undertaken in controlled environmental conditions (30°C, 70% relative humidity, 16 : 8 light : dark cycle).

(b) Host regulatory processes

In order to test the hypothesis that single-patch host populations, subject to either constant or fluctuating resource levels, were viable (self-regulating) in the absence of parasitoids, we fitted separate linear mixed-effects models (Pinheiro & Bates 2000; Bonsall et al. 2002) to time-series of adult *C. maculatus* and *C. chinensis* host alone abundance. The baseline analysis of covariance model was of the form:

$$n_{ijk} = (\beta_{1i} + b_{1ij}) + (\beta_{2i} + b_{2ij})t + \epsilon_{ijk},$$

$$b_{ij} = \begin{bmatrix} b_{1ij} \\ b_{2ij} \end{bmatrix} \sim N(0, \Psi), \quad \epsilon_{ijk} \sim N(0, \sigma^2),$$

where n_{ijk} is the adult host population abundance, subject to i th resource regime categorical factor level ($i=1, 2$), from the j th replicate ($j=1, \dots, 4$) at the k th weekly time point ($k=1, \dots, 36$). The parameters β_{1i} and β_{2i} represent fixed effects of the average value of the individual parameters (intercept and slope) for each resource renewal regime ('constant' and 'stochastic'), and b_{ij} represents deviations between replicates (random effects) in the parameters from each of the resource regime population averages, using time (t) as the explanatory variable. Random effects were assumed to be independent for each of the four replicates. ϵ_{ijk} represents residual errors, which were also assumed to be independent for different replicates and initially independent for each time point. Finally, relaxing this independence of errors between time points, Ψ is the appropriate variance–covariance matrix determined by the specific correlated error structure.

This allowed us to test the significance of constant or stochastic resource renewal as a categorical explanatory variable, covarying with the population trajectory through time while also exploring the contribution of host demographic and stochastic processes by fitting a series of autoregressive–moving average (AR–MA) covariance structures to the time series. We used the Akaike Information Criterion (AIC) to calculate the difference in AIC (Δ_i) between the best-fitting model and competing models. This allowed us to assign AIC weights (w_i), calculated as $w_i = \exp(-\Delta_i/2) / \sum_{i=1}^R \exp(-\Delta_i/2)$ for R competing models, and compare these models using evidence ratios (w_i/w_j), where an evidence ratio of greater than 2.718 indicates a substantial improvement in model fit of model i over model j , being equivalent to a difference in AIC (Δ_i) of greater than 2 (Burnham & Anderson 2002). Significance of fixed effects (differences in host abundance between resource renewal regimes) was tested by model averaging: the sum over all competing models of parameter estimates (and variances) weighted by their respective AIC weights (w_i).

Subsequently, we tested the hypothesis that different density-dependent host self-regulatory processes were associated with constant and stochastic resource renewal. We used conventional population ecological analysis (Royama 1992) by regressing net reproductive rate ($\ln[N_t/N_{t-1}]$) on time-lagged host population size ($\ln[N_{t-1}]$) and included resource regime as a two-level (constant and stochastic) categorical covariate in appropriate generalized linear models. We performed separate analyses using time lags of one week (the temporal scale over which stochastic resource fluctuations occur) and also four weeks (the approximate generation time of bruchid hosts).

(c) Assemblage persistence

We tested the hypotheses that fluctuating resource renewal reduces trophic interaction persistence time, the presence of an apparent competitor reduces interaction persistence time, and interactions persist longer in metapopulations than in single patches. In addition, we investigated the significance of statistical interactions between these three main effects. Metapopulation and single-patch control persistence times for both the pairwise (*C. chinensis*–*A. calandreae*, *C. maculatus*–*A. calandreae*) and apparent competitive (*C. chinensis*–*C. maculatus*–*A. calandreae*) interactions, subject to either constant or stochastic resource renewal, were compared using survival analysis (since surviving replicates were censored, see Cox & Oakes 1984).

Figure 2. Representative time-series from each of the interactions in single patches and metapopulations. Solid line, *C. maculatus*; dashed line, *C. chinensis* and dotted line, *A. calandreae* adult square root abundance. Panels (a–d) *C. chinensis*–*A. calandreae*, (e–h) *C. maculatus*–*A. calandreae*, (i–l) *C. maculatus*–*C. chinensis*–*A. calandreae* apparent competition assemblage. Panels a, c, e, g, i and k received constant resource renewal, and panels b, d, f, h, j and l received stochastic resource renewal. Panels a, b, e, f, i and j show abundance from single patch populations, others are aggregate abundances from four-patch metapopulations.

Alternative survival analysis hazard functions (exponential and Weibull) were compared by calculating the likelihood ratio between the two full models (all fixed effects and interaction terms; Burnham & Anderson 2002). Once the most appropriate hazard function had been identified, model simplification by stepwise deletion of non-significant terms was used to determine the most appropriate minimal adequate model (the model retaining only significant terms).

(d) Spatial scale and density dependence

Having explored the role of environmental stochasticity on host self-regulatory processes in the absence of parasitoids, we tested the hypothesis that changes in host regulatory processes are associated with different levels of spatio-temporal environmental heterogeneity in host–parasitoid metapopulations. Further, we tested whether inclusion of a host apparent competitor affects this result. *Callosobruchus maculatus* was the superior competitor in the apparent competition interaction (Bonsall *et al.* 2005; Bull *et al.* 2006). Therefore, we are best able to explore the effect of environmental stochasticity on density-dependent processes in the presence of parasitoids with these *C. maculatus* metapopulations. The host density-dependent processes operating at both local (patch) and regional (lattice) scales were investigated using both *C. maculatus* and *A. calandreae* two-species metapopulations and the *C. maculatus* hosts under apparent competition in the full three-species assemblages.

As with our host alone time-series (see §2b), density dependence in host regulatory processes was assessed by regressing net reproductive rate on time-lagged host population abundance. Here, we only use time lags of one week as we are most interested in the effects of environmental fluctuations on population abundance. Non-parametric bootstrapping (growth rate resampled 1000 times with

replacement) was used to confirm trends since we did not assume independence of residuals (Efron & Tibshirani 1993). This enabled us to use the regression of net reproductive rate on population size to predict the expected (mean) population growth rate with increasing abundance and to go on to investigate density-dependent effects on deviations from expected population growth rate with appropriate generalized linear models.

In separate generalized linear models for *C. maculatus*–*A. calandreae* assemblages with or without *C. chinensis* as an apparent competitor, the response variable comprised the deviations from the fitted *C. maculatus* net reproductive rate, with population abundance included as a continuous explanatory variable and constant or variable resource renewal included as a categorical covariate. Analysis of co-deviance was then used to assess this statistical model. The gradient of the regression of host net reproductive rate on population density describes the strength of density dependence on population growth rate. By comparing a statistical model including separate gradients for the regression lines at each level of resource renewal (constant and stochastic) with the nested model (which included a common gradient for the regression lines at each resource renewal level), we were able to test for differences in the nature of density-dependent processes between resource renewal regimes.

3. RESULTS

Representative time-series of single patches and metapopulations from each of the pairwise (*C. chinensis*–*A. calandreae* and *C. maculatus*–*A. calandreae*) and apparent competition systems are shown in figure 2.

(a) Host regulatory processes

Initially, we used host alone time-series to compare processes of host self-regulation (via density-dependence)

Figure 3. Host alone net reproductive rate versus one week time-lagged abundance. Solid circle/solid line, constant resource; open circle/dashed line, stochastic resource. (a) *C. maculatus* net reproductive rate regression treatment contrasts: difference between constant and stochastic resource renewal intercepts (s.e.)=0.12 (0.084), $t=1.42$, $p=0.156$; gradient (s.e.)=−0.843 (0.064), $t=13.8$, $p<0.001$. (b) *C. chinensis* net reproductive rate regression treatment contrasts: difference between intercepts (s.e.)=0.228 (0.087), $t=2.62$, $p=0.009$; gradient (s.e.)=−0.801 (0.062), $t=12.9$, $p<0.001$.

under constant or stochastic resource renewal, by fitting appropriate linear mixed-effects models. To compare the goodness of model fit between a series of AR–MA correlation structures on the process of regulation, we fitted all possible combinations of AR and MA temporal autocorrelation, from first to fourth order (host generation times being approximately four weeks). By calculating AIC weights (w_i), we found that, overall, AR–MA correlation structure provided a substantially better fit than uncorrelated errors (*C. maculatus*, $w_{[\text{independent errors}]}$ < 0.0001; *C. chinensis*, $w_{[\text{independent errors}]}$ < 0.0001). Both demographic and environmental stochastic effects (correlated errors) coupled with the regulatory effects of self-regulation affect host beetle abundance. However, the differences in explanatory power between many of the different correlated error structures were relatively small and did not indicate the predominance of any specific time lag. Leading model and evidence ratios for equivalent competing models up to 2.718 (Burnham & Anderson 2002) for *C. maculatus* were the following: leading model—AR[3] MA[2]: $w_i=0.214$; evidence ratios: AR[4] MA[2]=1.04, AR[3] MA[4]=1.20, AR[4] MA[4]=1.41, AR[4]=1.53. Similarly for *C. chinensis*: leading model—AR[4]: $w_i=0.152$, evidence ratios: MA[4]=1.35, MA[3]=1.41, AR[3] MA[2]=1.59, AR[3] MA[1]=1.79, AR[1] MA[3]=1.79, AR[2] MA[3]=1.96, AR[4] MA[1]=2.66.

In the absence of parasitoids, host populations quickly reached an equilibrium size: mean (s.e.) adult abundance: *C. maculatus*—constant resource=13.21 (6.20), stochastic resource=14.11 (11.11); *C. chinensis*—constant resource=24.87 (11.95), stochastic resource=21.65 (13.49). While mean *C. chinensis* abundance is lower in the fluctuating environment than in the constant resource renewal treatment, based on model averaging over all AR–MA covariance structures fitted, the significance of this result is open to interpretation ($t=1.808$, d.f.=283, $p=0.072$). Mean *C. maculatus* abundance between resource renewal regimes

are not significantly different ($t=0.437$, d.f.=283, $p=0.663$) but are substantially lower than for *C. chinensis*. In addition, the coefficients of variation (CV; *C. maculatus*—constant resource=0.47, stochastic resource=0.79; *C. chinensis*—constant resource=0.48, stochastic resource=0.62) for these time-series indicate that there is a greater degree of fluctuation through time for hosts experiencing stochastic resource levels than for those with constant resources.

Exploring the density-dependent processes regulating host populations revealed that multiple lagged population sizes explained a significant amount of observed deviance (one-week (resource renewal time-scale) lag: *C. maculatus*— $F_{1,270}=188.2$, $p<0.001$, *C. chinensis*— $F_{1,270}=158.7$, $p<0.001$; four-week (host generation time) lag: *C. maculatus*— $F_{1,254}=257.7$, $p<0.001$, *C. chinensis*— $F_{1,254}=107.1$, $p<0.001$; figure 3). However, removal of the interaction term describing how population size covaries with resource regime did not result in a significant change in deviance (one-week lag: *C. maculatus*— $F_{1,268}=0.0001$, $p=0.992$, *C. chinensis*— $F_{1,268}=0.1176$, $p=0.732$; four-week lag: *C. maculatus*— $F_{1,252}=0.3473$, $p=0.556$, *C. chinensis*— $F_{1,252}=1.715$, $p=0.192$). We find no evidence for different density-dependent processes between host populations receiving constant or stochastic resource renewal. The significant difference in growth rate between constant and stochastic resource renewal, found in *C. chinensis* alone (one-week lag: $F_{1,269}=6.856$, $p=0.009$; four-week lag: $F_{1,253}=5.036$, $p=0.026$) simply reflects the different population sizes within each treatment for that species. No such differences were found for *C. maculatus* (one-week lag: $F_{1,269}=2.021$, $p=0.156$; four-week lag: $F_{1,253}=1.305$, $p=0.255$). Overall, we find that single-patch host alone populations are self-regulating and persistent in our experimental system.

Figure 4. Box-whisker plots showing the central tendency, distribution, skew and kurtosis of interaction persistence times. (a) Single-patch systems, (b) four-patch metapopulations. Species combinations within each panel are *C. chinensis*-*A. calandreae*, *C. maculatus*-*A. calandreae* and *C. maculatus*-*C. chinensis*-*A. calandreae* 'apparent competition'. Separate replicates of each species combination received constant 'c' or stochastic 's' resource renewal. The central line in each box shows the median time to extinction (in weeks), with the box extending to cover the inter-quartile range and whiskers extending to 1.5 times the inter-quartile range (* denotes that this treatment was censored, with three out of four replicates still persisting once all replicates of other interactions had ended).

(b) Assemblage persistence

Distributions of host-parasitoid persistence times for all replicates across all treatments are shown in figure 4. The mean (with s.e.) persistence times of the single-patch systems with constant resource renewal were the following: *C. maculatus*-*A. calandreae*=10.00 (2.35) weeks; *C. chinensis*-*A. calandreae*=5.75 (1.35) weeks. The full three-species apparent competition interactions persisted for 3.25 (0.48) weeks. In the systems with stochastic resource renewal, persistence times were the following: *C. maculatus*-*A. calandreae*=10.75 (1.84) weeks; *C. chinensis*-*A. calandreae*=4.00 (1.35) weeks and apparent competition=3.00 (0.41) weeks. In all cases, single-patch host populations were rapidly driven extinct by parasitoids.

Compared to single-patch assemblages, persistence time was increased in the four-patch metapopulations. Persistence times in the two-species metapopulations were the following: *C. maculatus*-*A. calandreae*=24.50 (1.50) weeks; *C. chinensis*-*A. calandreae*=13.75 (4.27) weeks. In all cases, *C. chinensis* was the first species driven to extinction through apparent competition. Therefore, the loss of *C. chinensis* determined the end of the apparent competition interaction in 9.00 (1.22) weeks. In all of the two-species metapopulations, stochastic resource renewal reduced persistence time as follows: *C. maculatus*-*A. calandreae*=17.25 (1.89) weeks; *C. chinensis*-*A. calandreae*=9.50 (1.71) weeks. However, in the presence of an apparent competitor, mean persistence time (8.75 (1.31) weeks) was relatively unaffected by stochastic resource renewal compared to the constant resource renewal metapopulations.

Since apparent competition persistence times were defined by extinction of *C. chinensis*, once this species had been driven to extinction in all replicates of all treatments, any remaining *C. maculatus*-*A. calandreae* populations were censored (see asterisk in figure 4b).

With surviving replicates censored, survival analysis shows that the choice of error distribution (exponential, Weibull) resulted in the same fixed effects being significant; however, a Weibull hazard function was found to provide a substantially better fit (difference between exponential and Weibull error distributions: likelihood ratio=54.57, $p<0.001$). Overall, only the main effects were significant, i.e. metapopulations had significantly longer persistence times than single-patch systems ($\chi^2_1=41.7$, $p<0.001$), resource renewal stochasticity was found to reduce persistence times ($\chi^2_1=4.27$, $p=0.039$) and the presence of an apparent competitor significantly decreased persistence times ($\chi^2_2=43.7$, $p<0.001$).

(c) Spatial scale and density dependence

Graphical representation of the effect of host (*C. maculatus*) population size on reproductive rate shows that density-dependent processes operated at both local (patchwise) and regional (metapopulation) scales (figure 5). Further, non-parametric bootstrapped estimates of confidence intervals for these negative slopes indicate differences in the nature of the density-dependent processes between constant and variable resource renewal regimes at the level of the individual patches (local scale: $p<0.05$). However, over whole metapopulations, this difference is considerably less well supported (regional scale: $p<0.20$).

To investigate more subtle differences in density-dependent processes between the local and regional spatial scales, we explored the covariance in population growth rate stochasticity and population density. At the individual patch scale, analysis of co-deviance between fitted net reproductive rate residuals and lagged host population abundance showed a significant interaction between abundance and resource renewal regime ($F_{1,661}=6.427$, $p=0.012$). However, at the metapopulation scale, this was not the case ($F_{1,163}<0.001$, $p=0.985$).

Figure 5. Box-whisker plots showing the central tendency (median) and distribution (with the box extending to cover the inter-quartile range and whiskers extending to 1.5 times the inter-quartile range) of parameter estimates for the gradient of a linear regression of *C. maculatus* net reproductive rate ($\ln[N_t/N_{t-1}]$) on one week time-lagged abundance ($\ln[N_{t-1}]$). The parameter estimates were obtained by non-parametric bootstrapping with 1000 resamplings and p -values below represent the probability that differences between means of each treatment occurred by chance. (a, b) *C. maculatus*-*A. calandrarum* assemblages, (c, d) apparent competition assemblages. (a) Local scale: individual patches within four-patch metapopulations ($p < 0.05$); (b) regional scale: aggregate abundances and population growth rates from four-patch metapopulations ($p < 0.20$); (c) local scale: individual patches within four-patch metapopulations ($p > 0.40$); and (d) regional scale: aggregate abundances and population growth rates from four-patch metapopulations ($p > 0.40$).

In the presence of an apparent competitor, host population growth rate was still density-dependent at both local and regional scales (figure 5), but there was very little support ($p > 0.40$ at both local and regional scales) for differences in density-dependent processes between resource renewal regimes. Furthermore, the analysis of co-deviance between fitted net reproductive rate residuals and lagged host population abundance did not show a significant interaction between abundance and resource renewal regime at either spatial scale (patchwise: $F_{1,441} = 1.639$, $p = 0.201$; lattice-wide: $F_{1,108} = 1.194$, $p = 0.277$).

4. DISCUSSION

The key finding of this study is that the bottom-up effects of increased environmental stochasticity, in the form of fluctuating resource levels, can significantly increase extinction risk in predator-prey metapopulations, supporting our primary hypothesis. In addition, with increasing assemblage complexity (investigated here through the inclusion of an apparent competitor host species), the effects of environmental variations are overwhelmed by the top-down effects of predation and interaction persistence times can be further reduced. Population extinction times are known to depend on a variety of factors including population size, carrying capacity and growth rate (Ludwig 1996). Here, we have demonstrated that the metapopulation dynamic process of recolonization, the role of density-dependent processes and attack by natural enemies all interact to determine the extinction risk. Moreover, we find that the probability of extinction is best described by an increasing risk of instantaneous failure with a Weibull distribution, i.e. a long-tailed distribution, characteristic of stochastic

extinction processes (Mace & Lande 1991; Ludwig 1996). Overall, our finding that multiple ecological and stochastic processes interact to affect extinction risks endorses the questions raised by the recent theoretical (Grimm & Wissell 2004) and single-species empirical (Drake 2005) studies as to the appropriateness of density-independent determinants of extinction.

In the absence of predation, host regulation through resource limitation results in density-dependent net reproduction and population sizes that fluctuate around equilibrium levels (Hanski 1990; Bonsall et al. 2002; Lande et al. 2002; Lindström et al. 2005). Previously, it has been shown that environmental stochasticity can increase single-species extinction rates (Drake & Lodge 2004). In the case of our bruchid beetle host-only populations, environmental stochasticity was not sufficient to drive *C. chinensis* to extinction, but there was a decrease in abundance. When the increase in variation of abundance, through stochastic resource renewal, is also taken into account, this may be seen as an increased risk of extinction through chance events brought about by environmental stochasticity. However, we do not find any evidence for differing strengths of density dependence, operating on net reproductive rate, between constant and variable resource regimes. The inclusion of correlated error structures suggests that the effects of density and random perturbations (through demographic processes) may influence the processes of regulation and persistence. In corroborating this finding, recent results, for instance, on the role of temporal variation and its effects on average abundance in metapopulations (Roy et al. 2005) suggest that differences in covariance structures can affect persistence in spatially explicit systems.

In host–parasitoid assemblages, density dependence was quantified not only through analysis of average net reproductive rates, but also through the relationship between stochastic variations in population growth rate and population abundance. Generally, deviations from expected population sizes may come about through either uncertainty (observation error) or variation (process error; Meir & Fagan 2000; McNamara & Harding 2004). Observation or measurement error is a major problem when using abundance data gathered from natural systems, having been shown to cause false patterns of annual variation in population time-series, distorting statistical tests for direct density dependence (Shenk *et al.* 1998) as well as delayed density dependence (Solow 2001).

In our controlled laboratory system, observation error is likely to be negligible, as all live and dead wasps and beetles were counted. Any deviations may be assumed then to represent variations owing to process error, comprising differences in individual birth and death rates, i.e. demographic stochasticity, and/or resource level fluctuations, i.e. environmental stochasticity. While observed deviations may represent both environmental and demographic stochasticity (which may be density-dependent, see Drake 2005), in the constant resource regimes, environmental fluctuations are minimized. Therefore, any differences in the density-dependent processes affecting population growth rate between constant and variable resource lattices are mediated through fluctuating resource levels.

In the pairwise host–parasitoid assemblages, there are clear differences between the density-dependent processes operating in the constant and variable resource regime treatments. In addition, in the two-species metapopulations, we see an increase in host-only patch occupancy and a decrease in host–parasitoid patch co-occupancy with stochastic resource renewal (see electronic supplementary material—table 1). However, we find a decrease in recolonization rate by hosts and parasitoids (see electronic supplementary material—table 2), as well as in interaction persistence time when resource supply is variable. Despite the appearance of a greater degree of escape from parasitism by hosts in a stochastic environment, there is an increase in extinction risk. This was unexpected. Ellner *et al.* (2001) argued that increased population persistence time in a microcosm metapopulation directly results from reduced average predator success (as prey avoided predation). Therefore, we postulate that our result is brought about by the interaction between the direct effects of parasitism and resulting changes in density-dependent host demographic processes when host resources fluctuate.

Furthermore, in this system, the variations in resource levels apply at the local patch scale and tend to average out over metapopulations. In the two-species assemblages, we find differences in the stochastic processes associated with either constant or fluctuating resource renewal. As expected, these differences, resulting from different resource renewal regimes, are most evident at the local scale, but with some support for similar differences extending out to the regional scale. We have previously shown the importance of spatial scale in metapopulation dynamics, with predation dominating at a local scale and other ecological processes such as the effects of dispersal and self-regulation likely to influence the persistence of

trophic interactions at a metapopulation scale (Bonsall *et al.* 2005). This new result further highlights the importance of investigating ecological processes such as density dependence over the full range of relevant spatial scales.

When an alternative host species is available to the parasitoid, not only are the parasitoid numbers elevated owing to the overall increase in host abundance but the spatial distribution of parasitoids is no longer directed by the location of a single host species. None of the host species are able to escape parasitoid attack through the usual metapopulation processes of asynchronous dynamics and rescue of extinct patches among the habitat (Hanski 1991; Holyoak & Lawler 1996; Hanski 1999). We see a reduction in the proportion of time that hosts spend in patches devoid of parasitoids (see electronic supplementary material—table 1), and the host recolonization rate of patches is substantially reduced compared with their respective two-species host–parasitoid interactions (see electronic supplementary material—table 2). Moreover, we have reported here that the differences in density-dependent processes between constant and fluctuating resource renewal, found in the two-species assemblages, changed with spatial scale. However, these differences are not supported in the apparent competition assemblages at any spatial scale tested. This result indicates that it is not only important to resist inferring roles for ecological processes across spatial scales, but also across levels of assemblage complexity. Overall, with increasing species assemblage complexity, the effects of predation predominate and the effects of density-dependent host regulation brought about by variable resource availability no longer significantly influence metapopulation extinction risk.

Consistent with our previous studies (Bonsall *et al.* 2005; Bull *et al.* 2006), host persistence times, in the presence of natural enemies, differ between host species, with *C. maculatus* being substantially less likely to be driven to extinction than *C. chinensis*. We did not explicitly investigate the reasons for this in the present study. Given that the life histories of the hosts are broadly similar (Bellows 1982), we speculate that differences in host spatial distribution and dispersal are likely to cause differences in persistence between host species. *Callosobruchus maculatus* persisted longer than *C. chinensis* in single patches as well as metapopulations. Therefore, it is particularly the observed tendency for *C. chinensis* adults to aggregate in the system (within rather than across patches)—potentially affecting host intraspecific competition as well as host dispersal and parasitoid avoidance—which may be critical to its increased susceptibility to extinction compared to *C. maculatus*. While intraspecific aggregation across patches is generally thought to promote persistence (Sevenster 1996; Sevenster & van Alphen 1996; Wertheim *et al.* 2000), in the present study, within-patch host aggregation is not likely to result in host–parasitoid interspecific segregation; thus, they escape from parasitism. Since individual parasitoids were observed to move throughout the metapopulation lattices, host aggregation may result in improved detection by parasitoids, thus over-turning any heterogeneity in parasitism underpinning coexistence. In metapopulations, *C. chinensis* extinction risk may be magnified further by a low level of adult dispersal between

patches, thus a potential for rescue effects (Brown & Kodrick-Brown 1977).

However, even in controlled laboratory conditions, there are likely to be many interacting mechanisms, for example immunological (Wertheim *et al.* 2005) and behavioural (Sevenster & van Alphen 1996; van Veen *et al.* 2005), potentially operating and interacting at different spatial and temporal scales to determine vulnerability to parasitism or predation. It may be that the same factors, which allow *C. maculatus* populations to resist parasitoid attack, are instrumental in causing this host species to be the superior competitor under apparent competition. However, our finding that the relative effects of multiple, intrinsic and extrinsic ecological processes change with increasing assemblage complexity and spatial scale again leads us to caution against extrapolating findings from simple resource–consumer interactions to more complex assemblages.

These findings have important implications for the identification of extinction risk in natural communities (Mace & Lande 1991; McLaughlin *et al.* 2002; Lande *et al.* 2003). Extinction risk is associated with environmental heterogeneity (Grenfell *et al.* 1998; Coulson *et al.* 2001; Drake & Lodge 2004); however, with increasing species assemblage complexity, trophic interactions may increasingly out-weigh this effect. These findings also point to areas of existing metapopulation theory which need further investigation; we show that even when, on an average, predator–prey colocalization is reduced over the long term, this may not indicate a reduction in extinction risk. It is necessary to place the local structure of ecological assemblage in the context of the broader spatial scale and alongside reliable data on environmental quality through time and across space. We find that both intrinsic demographic processes and extrinsic environmental factors interact to affect assemblage structure and population extinction risk.

The work was supported by the NERC and The Royal Society. M.B.B. is a Royal Society University Research Fellow.

REFERENCES

- Bellows, T. S. 1982 Analytical models for laboratory populations of *Callosobruchus chinensis* and *C. maculatus* (Coleoptera, Bruchidae). *J. Anim. Ecol.* **51**, 263–287.
- Benton, T. G., Lapsley, C. T. & Beckerman, A. P. 2002 The population response to environmental noise: population size, variance and correlation in an experimental system. *J. Anim. Ecol.* **71**, 320–332. (doi:10.1046/j.1365-2656.2002.00601.x)
- Bonsall, M. B. & Benmayor, R. 2005 Multiple infections alter density dependence in host–pathogen interactions. *J. Anim. Ecol.* **74**, 937–945. (doi:10.1111/j.1365-2656.2005.00991.x)
- Bonsall, M. B. & Hassell, M. P. 1997 Apparent competition structures ecological assemblages. *Nature* **388**, 371–373. (doi:10.1038/41084)
- Bonsall, M. B. & Hassell, M. P. 2005 Understanding ecological concepts: the role of laboratory systems. *Adv. Ecol. Res.* **37**, 1–36. (doi:10.1016/S0065-2504(04)37001-7)
- Bonsall, M. B. & Hastings, A. 2004 Demographic and environmental stochasticity in predator–prey metapopulation dynamics. *J. Anim. Ecol.* **73**, 1043–1055. (doi:10.1111/j.0021-8790.2004.00874.x)
- Bonsall, M. B., French, D. R. & Hassell, M. P. 2002 Metapopulation structures affect persistence of predator–prey interactions. *J. Anim. Ecol.* **71**, 1075–1084. (doi:10.1046/j.1365-2656.2002.00670.x)
- Bonsall, M. B., Bull, J. C., Pickup, N. J. & Hassell, M. P. 2005 Indirect effects and spatial scaling affect the persistence of multispecies metapopulations. *Proc. R. Soc. B* **272**, 1465–1471. (doi:10.1098/rspb.2005.3111)
- Brook, B. W., O'Grady, J. J., Chapman, A. P., Burgman, M. A., Akcakaya, H. R. & Frankham, R. 2000 Predictive accuracy of population viability analysis in conservation biology. *Nature* **404**, 385–387. (doi:10.1038/35006050)
- Brown, J. H. & Kodrick-Brown, A. 1977 Turnover rates in insular biogeography: the effect of immigration on extinction. *Ecology* **58**, 445–449.
- Bull, J. C., Pickup, N. J., Hassell, M. P. & Bonsall, M. B. 2006 Habitat shape, metapopulation processes and the dynamics of multispecies predator–prey interactions. *J. Anim. Ecol.* **75**, 899–907. (doi:10.1111/j.1365-2656.2006.01107.x)
- Burnham, K. P. & Anderson, D. R. 2002 *Model selection and multimodel inference: a practical information-theoretic approach*, 2nd edn. New York, NY: Springer.
- Caswell, H. 2001 *Matrix population models*, 2nd edn. Sunderland, MA: Sinauer Associates, Inc.
- Caughley, G. 1994 Directions in conservation biology. *J. Anim. Ecol.* **63**, 215–244. (doi:10.2307/5542)
- Chase, J. M. & Ryberg, W. A. 2004 Connectivity, scale-dependence, and the productivity–diversity relationship. *Ecol. Lett.* **7**, 676–683. (doi:10.1111/j.1461-0248.2004.00622.x)
- Childs, D. Z., Bonsall, M. B. & Rees, M. 2004 Periodic local disturbance in host–parasitoid metapopulations: host suppression and parasitoid persistence. *J. Theor. Biol.* **227**, 13–23. (doi:10.1016/S0022-5193(03)00293-5)
- Coulson, T., Catchpole, E. A., Albon, S. D., Morgan, B. J. T., Pemberton, J. M., Clutton-Brock, T. H., Crawley, M. J. & Grenfell, B. T. 2001 Age, sex, density, winter weather and population crashes in Soay sheep. *Science* **292**, 1528–1531. (doi:10.1126/science.292.5521.1528)
- Cox, D. R. & Oakes, D. 1984 *Analysis of survival data*. London, UK: Chapman & Hall.
- Drake, J. M. 2005 Density-dependent demographic variation determines extinction rate of experimental populations. *PLoS Biol.* **3**, e222. (doi:10.1371/journal.pbio.0030222)
- Drake, J. M. & Lodge, D. M. 2004 Effects of environmental variation on extinction and establishment. *Ecol. Lett.* **7**, 26–30. (doi:10.1046/j.1461-0248.2003.00546.x)
- Efron, B. & Tibshirani, R. J. 1993 *An introduction to the bootstrap*. Monographs on statistics and applied probability. London, UK: Chapman & Hall.
- Ellner, S. P. *et al.* 2001 Habitat structure and population persistence in an experimental community. *Nature* **412**, 538–543. (doi:10.1038/35087580)
- Grenfell, B. T., Wilson, K., Finkenstädt, B. F., Coulson, T. H., Murray, S., Albon, S. D., Pemberton, J. M., Clutton-Brock, T. H. & Crawley, M. J. 1998 Noise and determinism in synchronized sheep dynamics. *Nature* **394**, 674–677. (doi:10.1038/29291)
- Grimm, V. & Wissell, C. 2004 The intrinsic mean time to extinction. *Oikos* **105**, 501–511. (doi:10.1111/j.0030-1299.2004.12606.x)
- Hanski, I. 1990 Density dependence, regulation and variability in animal populations. *Phil. Trans. R. Soc. B* **330**, 141–150.
- Hanski, I. 1991 Single-species metapopulation dynamics—concepts, models and observations. *Biol. J. Linn. Soc.* **42**, 17–38.
- Hanski, I. 1999 *Metapopulation ecology*. Oxford, UK: Oxford University Press.

- Holyoak, M. 2000a Habitat patch arrangement and metapopulation persistence of predators and prey. *Am. Nat.* **156**, 378–389. (doi:10.1086/303395)
- Holyoak, M. 2000b The effects of nutrient enrichment on predator–prey metapopulation dynamics. *J. Anim. Ecol.* **69**, 985–997. (doi:10.1046/j.1365-2656.2000.00453.x)
- Holyoak, M. & Lawler, S. P. 1996 Persistence of an extinction-prone predator–prey interaction through metapopulation dynamics. *Ecology* **77**, 1867–1879. (doi:10.2307/2265790)
- Huffaker, C. B., Shea, K. P. & Herman, S. G. 1963 Experimental studies on predation: complex dispersion and levels of food in an acarine predator–prey interaction. *Hilgardia* **34**, 305–329.
- Lande, R. 1993 Risks of population extinction from demographic and environmental stochasticity and random catastrophes. *Am. Nat.* **142**, 911–927. (doi:10.1086/285580)
- Lande, R., Engen, S., Sæther, B.-E., Filli, F., Matthysen, E. & Weimerskirch, H. 2002 Estimating density dependence from population time series using demographic theory and life-history data. *Am. Nat.* **159**, 321–327. (doi:10.1086/338988)
- Lande, R., Engen, S. & Sæther, B.-E. 2003 *Stochastic population dynamics in ecology and conservation*. Oxford, UK: Oxford University Press.
- Lindström, Å., Enemar, A., Andersson, G., von Proschwitz, T. & Nyholm, N. E. I. 2005 Density-dependent reproductive output in relation to a drastically varying food supply: getting the density measure right. *Oikos* **110**, 155–163. (doi:10.1111/j.0030-1299.2005.13828.x)
- Ludwig, D. 1996 The distribution of population survival times. *Am. Nat.* **147**, 506–526. (doi:10.1086/285863)
- Mace, G. M. & Lande, R. 1991 Assessing extinction threats: towards a re-evaluation of IUCN species threatened categories. *Conserv. Biol.* **5**, 148–157. (doi:10.1111/j.1523-1739.1991.tb00119.x)
- McLaughlin, J. F., Hellmann, J. J., Boggs, C. L. & Ehrlich, P. R. 2002 Climate change hastens population extinctions. *Proc. Natl Acad. Sci. USA* **99**, 6070–6074. (doi:10.1073/pnas.052131199)
- McNamara, J. M. & Harding, K. C. 2004 Measurement error and estimates of population extinction risk. *Ecol. Lett.* **7**, 16–20. (doi:10.1046/j.1461-0248.2003.00550.x)
- Meir, E. & Fagan, W. F. 2000 Will observation error and biases ruin the use of simple extinction models? *Conserv. Biol.* **14**, 148–154. (doi:10.1046/j.1523-1739.2000.98502.x)
- Morris, W. F. & Doak, D. F. 2002 *Quantitative conservation biology: theory and practice of population viability analysis*. Sunderland, MA: Sinauer Associates, Inc.
- Pinheiro, J. C. & Bates, D. M. 2000 *Mixed-effects models in S and S-Plus*. New York, NY: Springer.
- Roy, M., Holt, R. D. & Barfield, M. 2005 Temporal autocorrelation can enhance the persistence and abundance of metapopulations comprised of coupled sinks. *Am. Nat.* **166**, 246–261. (doi:10.1086/431286)
- Royama, T. 1992 *Analytical population dynamics*. London, UK: Chapman & Hall.
- Sæther, B.-E. & Engen, S. 2004 Stochastic population theory faces reality in the laboratory. *Trends Ecol. Evol.* **19**, 351–353. (doi:10.1016/j.tree.2004.05.003)
- Sevenster, J. G. 1996 Aggregation and coexistence. I. Theory and analysis. *J. Anim. Ecol.* **65**, 297–307. (doi:10.2307/5876)
- Sevenster, J. G. & van Alphen, J. J. M. 1996 Aggregation and coexistence. II. A neotropical *Drosophila* community. *J. Anim. Ecol.* **65**, 308–324.
- Shenk, T. M., White, G. C. & Burnham, K. P. 1998 Sampling variance effects on detecting density dependence from temporal trends in natural populations. *Ecol. Monogr.* **68**, 445–463. (doi:10.2307/2657247)
- Solow, A. R. 2001 Observation error and the detection of delayed density dependence. *Ecology* **82**, 3263–3264. (doi:10.2307/2679850)
- van Veen, F. J. F., van Holland, P. D. & Godfray, H. C. J. 2005 Stable coexistence in insect communities due to density- and trait-mediated indirect effects. *Ecology* **86**, 1382–1389.
- Wertheim, B. G., Sevenster, J. G., Eijs, I. E. M. & van Alphen, J. J. M. 2000 Species diversity in a mycophagous insect community: the case of spatial aggregation vs. resource partitioning. *J. Anim. Ecol.* **69**, 335–351. (doi:10.1046/j.1365-2656.2000.00396.x)
- Wertheim, B. G., Kraaijeveld, A. R., Shuster, E., Blanc, E., Hopkins, M., Pletcher, S. D., Strand, M. R., Partridge, L. & Godfray, H. C. J. 2005 Genome-wide expression in response to parasitoid attack in *Drosophila*. *Genome Biol.* **6**, R94.