POPULATION DYNAMICS AND ENVIRONMENTAL CHANGE:

WHICH FACTORS COMPLICATE PREDICTION AND INFERENCE?

Jason Ian Griffiths

Department of Animal and Plant Sciences

The University of Sheffield

"What you learn today, for no reason at all, will help you discover all the wonderful secrets of tomorrow."

— **Norton Juster, The Phantom Tollbooth**

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Statement of Contributions

Throughout the thesis the personal pronoun 'we' is used as all chapters were developed, supported or improved by the input and advice of collaborators. Nevertheless, the vast majority of the ideas, modelling, analysis and writing in this thesis are exclusively the candidate's work. Major contributions are listed below and additional contributions noted in the acknowledgements of each chapter.

Chapter 1 and 7 (general introduction and discussion) were written entirely by the candidate, with editorial advice from Dr Dylan Childs.

Chapter 2 is taken directly from a manuscript by Jason I. Griffiths, Prof. Philip Warren & Dr Dylan Childs which was published in Oikos. Concept for the manuscript was developed by the candidate and Dr Dylan Childs. All experimental design, data collection, modelling and writing was done entirely by the candidate, with support with the statistical modelling provided by Dr Dylan Childs. General editorial advice was provided by Prof. Philip Warren and Dr Dylan Childs.

Chapter 3 is in preparation as a manuscript. The initial idea, experimental design, data collection, modelling and writing were all carried out by the candidate. General editorial advice was provided by Dr Dylan Childs.

Chapter 4 is in preparation as a manuscript. The initial idea was developed by the candidate, Dr Dylan Childs and Prof. Owen Petchey. Dr Frank Pennekamp developed laboratory protocols and aided in experimental design. All data collection, modelling and writing was done by the candidate. General editorial advice was provided by Prof. Owen Petchey, Dr. Frank Pennekamp, Prof. Philip Warren and Dr Dylan Childs.

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Published Works by the Author Incorporated into the Thesis

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Declaration by author

This thesis contains original work, and does not contain material previously published or written by other persons, except where due reference has been made in the text. The contribution of collaborators to the conceptualization, data collection, statistical analysis, authorship and editing of this thesis has been clearly stated. The thesis content results from work I have undertaken since starting my research higher degree and includes no work submitted to qualify for any other degree or diploma in any university or other institution. I have clearly stated which parts of the thesis have been submitted for scientific publication and have obtained the required permissions to include this work. I acknowledge that copyright of the thesis content resides with the copyright holder(s) of that material.

Summary

Changes in both the biotic and abiotic environment influence individuals' physiology, morphology and behaviour and influence key ecological rates underpinning population dynamics. Environmental change is ubiquitous in natural systems and is often multifaceted, as multiple aspects of the climate often change simultaneously and the abundance and traits of species in the community are constantly fluctuating. In this thesis, we study the ecological consequences of environmental changes. We identify fundamental factors complicating our understanding of population dynamics and develop analytical tools to reliably infer, from data, the impacts of environmental change on key biological processes.

We present evidence that the impacts of environmental change on population dynamics can be modified by other concurrent environmental changes. Furthermore, the impacts on a focal species will likely be strongly dependent on how the performance of interacting species are affected.

We then show that the addition of predators to an environment can cause prey to become more defended against predation, at a cost of reduced population growth. Such growth-defence trade-offs are expected to drive complex population dynamics.

We demonstrate that our understanding of community dynamics can be improved by identifying how consumption rates vary with changes in morphological or behavioural traits. We identify feedbacks between species' trait and abundance dynamics.

We then provide evidence that environmental warming can modify the impacts of trait change on species interactions. We inferred that this likely resulted from a modified life history strategy or altered resources allocation to growth rather than defence.

Finally, we use simulation studies to assess the reliability of current methods at inferring climate effects on the demography of wild populations. We demonstrate that commonly used approaches perform poorly and also identify a reliable modelling framework.

The findings of this work provided quantitative insights into the impacts of environmental change on the processes driving species' dynamics. It also highlights the role of combined environmental change, trait change and species interaction in complicating the prediction of population dynamics.

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Chapter 1: Introduction

Environmental change

Environmental change is a ubiquitous feature of natural systems, occurring at a range of scales, including inter-annual trends, seasonal cycles, and small scale stochastic variation (Halpern *et al.* 2008; Pachauri *et al.* 2014). Changing environmental conditions influence individuals' physiological and metabolic processes and modify organisms' traits such as size, movement and behaviour (Brown *et al.* 2004; Walther 2010; Sheridan & Bickford 2011). The impacts of environmental change on a species physiological performance can be considered the direct effects of environmental change. However, the environment also influences the performance of interacting species, influencing rates of resource consumption and predation for example (Tylianakis *et al.* 2008; Lindegren *et al.* 2010; Gilman *et al.* 2010; Kordas *et al.* 2011). The environmental impacts resulting from modified interactions between species are known as indirect effects. Both direct and indirect environmental effects may substantially influence the key demographic process, such as birth, growth, maturation and death, which govern changes in species abundance. Consequently, determining how vital rates such as population growth or predation are influenced by environmental change is a key component in understanding community's dynamics, structure and functioning (Harvell *et al.* 2002; Emmerson *et al.* 2004; Post *et al.* 2008; Gilman *et al.* 2010; Kordas *et al.* 2011; O'Connor *et al.* 2009; O'Connor *et al* 2011; O'Connor *et al.* 2012; Griffiths *et al.* 2014). This understanding is necessary for the development of a predictive framework to forecast the ecological consequences of climate change regarding species conservation, sustainable resource management and pest control (Petchey *et al.* 1999; Hughes 2000; Harvell *et al.* 2002; Pounds *et al.* 2006; Post *et al.* 2008; Brook *et al.* 2008; Gilman *et al.* 2010; Kordas *et al.* 2011; Walsh *et al.* 2012; Cahill *et al.* 2013).

Impacts of changes in the abiotic environment

Abiotic environmental changes such as changes in temperature, salinity or precipitation often influence several demographic rates simultaneously (Doney *et al.* 2012; Régnière *et al.* 2012; Jenouvrier 2013). Furthermore, multiple environmental conditions may change concurrently and their impacts may depend on the state of the other variables (Paine *et al.* 1998; Didham *et al.* 2007; Brook *et al.* 2008; Darling & Côté 2008; Laurance & Useche 2009). For example, the

survival of Soay sheep in warm years depends on the concurrent precipitation levels (Coulson *et al.* 2001). Moreover, conditions at a specific time of year can be especially influential upon vital rates (Ireland *et al*. 2004; Luis *et al*. 2010; Jansen *et al*. 2014). For example, warm spring conditions have been linked to earlier breeding and increased clutch and egg sizes in avian populations (Crick 2004; Robinson *et al.* 2007). Extreme weather events and other stochastic factors can also play an important role in determining demographic rates (Descamps *et al.* 2015). Consequently, population dynamics may be very sensitive to environmental shifts and often change in unpredicted ways (Paine *et al.* 1998; Christensen *et al.* 2006; Doak *et al.* 2008). Meta-analyses indicate that combined environmental changes may frequently have non-additive impacts on individual level processes (Crain *et al.* 2008; Darling & Côté 2008), however the long-term population level impacts of this remain largely unknown.

Impacts of changes in the biotic environment: Density and trait dependent ecology

Both the abundance of species within a community and the traits they possess can be considered as biotic components of the environment. It is well understood that key ecological processes are dependent on the density of species within the community (Lande *et al.* 2002; Coulson *et al.* 2008; Bassar *et al.* 2013). For example, the intake rate of a predator generally increases with the density of its prey, up to a point where the consumption rate is limited by the time taken to handle and digest prey (Holling 1965; Dawes & Souza 2013). Importantly, changes in species densities can also drive rapid, ecologically relevant trait change (Endler 1991; Thompson 1998; Hairston *et al.* 2005; Ellner & Becks 2010). Such changes can be driven by phenotypic plasticity, when a single genotype produces different phenotypes under differing environments (Tollrian & Harvell 1999; Agrawal 2001; Fordyce 2006; Cortez 2011). Recently, it has also been realized that rapid evolution can also drive trait change at ecologically relevant time scales when heritable traits are favoured in a genetically variable population (Thompson 1998; Yoshida *et al.* 2004; Hairston *et al.* 2005; Ellner *et al.* 2011; Kasada *et al.*2014).

Trait change in one species can alter interspecific interactions, by modifying feeding rates or other non-consumptive interactions (Peacor & Werner 2001; Bolker *et al.* 2003). An example of such trait-mediated interactions is the inducible morphological or behavioural defence of prey in response to high predation (Schmitz *et al.* 1997; Tollrian & Harvell 1999; Peacor & Werner 2001; Altwegg *et al.* 2006; Hammill *et al.* 2010). Alternatively, prey can respond to

intense predation by altering the timing of life history events, for example reproducing earlier (Spitze 1992; Chivers *et al.* 2001; Travis *et al.* 2014). Importantly, trait driven temporal variation in key ecological rates influences population dynamics (Bolker *et al.* 2003; Preisser *et al.* 2005; Pelletier *et al.* 2007; Harmon *et al.* 2009; Bassar *et al.* 2010; terHorst *et al.* 2010; Turcotte *et al.* 2011). In turn, the resulting changes in species abundances may alter the direction or magnitude of trait selection. This implies the existence of feedbacks between trait dependent ecological rates and density dependent trait change (Pelletier *et al.* 2009; Schoener 2011; Hanski 2012). Feedbacks between species densities and their traits may permit a diversity of community dynamics to occur that would be unexpected from purely ecological theories based on species abundances (Abrams & Matsuda 1997; Yoshida *et al.* 2003, 2007; Pelletier *et al.* 2007; Jones *et al.* 2009; Berg & Ellers 2010; Kishida *et al.* 2010; Becks *et al.* 2010, 2012; Ellner & Becks 2010; Cortez 2011; Schoener 2011; Mougi 2012a; b; Agrawal *et al.* 2013; Cortez & Weitz 2014; Hiltunen *et al.* 2014a). Despite the theoretical literature indicating the potential importance of trait mediated processes in driving species dynamics (Abrams & Matsuda 1997; Ellner & Becks 2010; Cortez & Weitz 2014), the coupling between the dynamics of traits and abundance in real communities is unknown (Kishida *et al.* 2010). It has been proposed that trait and abundance dynamics should be studied in parallel to allow inference of the feedbacks between these variables (Hiltunen *et al.* 2014b).

Ecological inferences in complex environments

The multifaceted nature of environment change can make it difficult to quantify how specific environmental variables influence the key ecological processes underpinning population dynamics (Shertzer *et al.* 2002; Bakker *et al.* 2009). Due to uncertainty in the structure and functioning of communities, environmental change often causes unexpected fluctuations in population abundances and demographic rates (Paine *et al.* 1998; Wood & Thomas 1999; Christensen *et al.* 2006; Fox & Barreto 2006; Doak *et al.* 2008; Darling & Côté 2008). There is a large theoretical literature investigating the likely impacts of environmental change on species dynamics (Savage *et al.* 2004; Brown *et al.* 2004; Woodward *et al.* 2010; Petchey *et al.* 2010; Binzer *et al.* 2012; Reuman *et al.* 2014). However the predictions of conceptual models are rarely tested and theoretical models are seldom applied to make inferences from empirical datasets (For examples of combining theoretical models and data see: Desharnais 2005; King *et al.* 2008; Hiltunen *et al.* 2014).

As multiple environmental changes often occur concurrently, it can also be difficult to reliably identify the major environmental drivers of variation in ecological rates (Teller *et al.* 2016). In wild populations, this inference is made more difficult because data is usually observation, rather than experimental, and often restricted to annual samples over a fairly small number of years (Grosbois *et al.* 2008; Frederiksen *et al.* 2014). Furthermore, only a limited set of environmental variables that may influence demographic rates are measured, despite a large number of unmeasured factors also having influences. To allow reliable quantification of the drivers of variation in ecological rates, data-driven analytical tools need to be developed and validated (for examples of this see Lebreton *et al.* 2012; Gimenez *et al.* 2012).

Purpose of this thesis

In this thesis, we examine the impacts of multi-faceted abiotic and biotic environmental change on species traits, vital rates and population dynamics. The main objectives of this work are to: A) examine how environmental changes influence species dynamics and their interaction with other species, and B) determine how reliable inferences of climatic impacts on vital rates can be made in complex environments.

We develop data driven modelling approaches to confront ecological and evolutionary theory concerning the environmental impacts on populations, with empirical data from the field and laboratory. Throughout the thesis we use models to formalise rival hypotheses of how climate influences populations and their vital rates. We then use a range of statistical tools to determine the likelihood of these hypotheses and to gain quantitative insights into the environmental impacts on species abundances and vital rates.

In Chapter 2, we present a study of the experimentally observed invasion dynamics of competing species under each combination of three environmental manipulations. We assess whether the impacts of environmental change upon species dynamics can be assumed to be independent of the other environmental conditions. We also develop a novel method to partition the relative importance of a) direct effects of environmental change on physiological processes from b) the indirect effects mediated by changes in species interactions.

It is increasingly appreciated that evolution and trait plasticity have important roles in driving species dynamics by temporally modifying the biotic environment. For example, inducible defence of prey can effectively reduce the availability of resources for a predator (Tollrian &

Harvell 1999; Yoshida *et al.* 2007). In Chapter 3 we describe a novel methodology for quantifying the impacts of predator exposure on the key ecological rates of prey population growth and predator defence against consumption. Theory predicts that under sufficiently strong predation, prey should increase their resource allocation to defence at the cost of growth (Coley, Bryant & Chapin 1985; Tollrian & Harvell 1999). However, if the predation pressure is removed, we would expect an inducible defence to be lost and the positive selection for a costly defence trait to be relaxed, causing prey to revert to the undefended and fast growing state. To test this prediction, we examine the differences in population growth and defence between microbial populations that are either: 1) naive to predation, 2) exposed to predation, or 3) have historically been exposed to predation, but not for several generations.

Our findings from Chapter 3 lead us to question which individual morphological or behavioural traits were linked to the dynamics of species abundance and how changes in species densities feedback to influence trait change. Therefore, in Chapter 4 we simultaneously study the dynamics of species' traits and abundances in a predator-prey-resource system. We develop a novel approach to connect trait and abundance dynamics, allowing identification of ecologically important traits. This allows the quantification of the trait dependence of vital rates, such as predation. We then identify species whose density influences the direction and magnitude of trait change. The analysis of these two processes allows unique insights into the feedbacks between trait and ecological change and permits a better understanding of the resource allocation trade-offs influencing the community dynamics.

In Chapter 5, we examine how environmental warming influenced the trait dependent community dynamics and the nature of interspecific interactions, using the predator-prey-resource system established in Chapter 4. We investigate the traits that influence species vital rates at a range of temperatures along an environmental gradient, assessing whether the key traits vary in response to environmental change. We then investigate how warming effects the way that species interactions depend on species' traits.

Finally, in Chapter 6 we evaluate the methods used to identify the environmental variables driving species demographic rates. We focus specifically on the reliability of statistical tools used to identify the climate dependence of survival when analysing individual mark and recapture data from long term field studies. We identify statistical issues that complicate the identification of the main drivers when there are many potential hypotheses. We then review the recent literature to assess if and how these statistical challenges are overcome. We use

simulations to test the performance of potential frameworks at identifying and quantifying climate effects. This allows us to provide guidance about the modelling approaches that should be undertaken in future to determine the effects of environmental change on the demographic rates.

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Chapter 2: Multiple environmental changes interact to modify species dynamics and invasion rates

Jason I. Griffiths, Philip H. Warren & Dylan Z. Childs (manuscript appears as published in the peer-reviewed journal OIKOS (2015) 124.4,458-468.)

Abstract

Multiple aspects of the environment often change at the same time, influencing populations directly by modifying their physiology, but also indirectly by influencing other interacting species. The impacts of each environmental change upon population dynamics are usually assumed be independent of the state of other aspects of the environment, despite evidence at the individual level indicating that the combined impacts are often non-additive. The importance of indirect effects mediated through community interactions also has high uncertainty. We used experimental microcosms to determine whether environmental factors interact to affect species dynamics and the relative importance of direct and indirect effects on species dynamics.

We factorially manipulated three aspects of the environment (temperature, food availability and salinity) and examined reciprocal invasions of competing protist species under each environment. Experimental observations were used to parameterize a dynamic model of the system. Using this model and a novel variance decomposition method, we examined the mechanisms by which environmental changes altered species invasion rates.

The three environmental factors interacted when modifying species growth rates, intra- and interspecific competition, causing the impact of each environmental change on species dynamics to depend crucially on the state of other aspects of the environment. Indirect changes in the abundance of the resident competitor and its interspecific competitive ability were the main cause of environmental driven variation in invasion rates, whilst direct effects on species intrinsic growth rates were relatively unimportant. This indicates that, to understand and ultimately predict species and community responses to multiple environmental changes, we should consider their joint impacts and the mechanisms by which they interact to modify key ecological processes such as competition.

Key words: Environmental change; Protist microcosms; Population dynamics; Invasion; Maximum likelihood estimation; ANOVA decomposition

Introduction

Environmental change is a pervasive feature of natural systems (Halpern *et al*. 2008), influencing species physiology, behaviours and phenology (Tylianakis *et al*. 2008; Gilman *et al.* 2010). These changes impact upon species abundances and interactions (Jiang & Morin 2007; Tylianakis *et al*. 2008; Lafferty 2009; Beveridge *et al*. 2010a; Reuman *et al*. 2013). Consequently they can modify ecological processes such as the invasion rates of non-native species and alter the structure and functioning of natural communities (Walther *et al.* 2010; Didham *et al.* 2007; Villalpando *et al.* 2009; Sorribas *et al.* 2012; Cockrell & Sorte 2013). These environmental impacts have primarily been studied by considering the effects of specific environmental drivers or large scale climatic patterns (Sala *et al*. 2000; Stenseth *et al.* 2002; Ruete *et al.* 2012; Jenouvrier *et al.* 2013). However, because environmental changes rarely occur in isolation, it is increasingly appreciated that there may be interactions between them (Brook *et al.* 2008; Laurance & Useche 2009; Paine *et al.* 1998; Didham *et al.* 2007; Wernberg *et al*. 2012; Kroeker *et al.* 2013).

Evaluating whether the effects of particular environmental factors are dependent upon the state of others has important consequences for understanding and, ultimately, predicting species responses to environmental change (Evans *et al.* 2012; Parmesan *et al.* 2013). If multiple environmental drivers interact to modify species growth rates or intra- and interspecific density dependence, community level responses may occur which would not be predictable from knowledge of the effects of the same drivers individually (Bellwood *et al*. 2004; Moe *et al*. 2013). Several ecological catastrophes are now thought to have resulted from the combined effects of co-occurring environmental drivers. For example, the unforeseen crash of the Peruvian anchovy populations is proposed to have resulted from the interaction between El Niño driven warming and reduced productivity, in combination with overfishing (Jackson *et al*. 2001; Cushing 1995). Likewise, in Costa Rica, the rapid extinction of 67% of endemic frog species has been attributed to the combined changes in temperature and humidity and subsequent effects of trophic interactions (Pounds *et al*. 2006). More generally, meta-analyses of individual level studies have indicated that the cumulative effects of multiple environmental changes upon birth, survival and death rates are frequently non-additive, each change influencing the effects of others (Craine *et al.* 2008; Darling & Côté 2008).

As well as identifying when environmental changes interact to modify species abundances, it is important to appreciate the key biological processes that are affected in order to understand the mechanisms that produce the resulting dynamics. Environmental changes may have direct

effects on a focal species' physiology, vital rates and intraspecific density dependence (Reading 2007; Tylianakis *et al*. 2008), but may also have indirect effects through changes in the abundance or interaction strength of other species with which the focal species interacts (Emmerson *et al.* 2004, 2005; Suttle *et al*. 2007). Understanding the relative importance of direct and indirect mechanisms is essential for developing a predictive framework. If indirect effects are relatively unimportant, physiological based predictive methods can be used to forecast species responses and range shifts (e.g. Bradley *et al*. 2010). Alternatively if, as Gilman *et al.* (2010) suggest, species interactions frequently influence how environmental changes affect individual fitness, and the dynamics and structure of communities, then predictions from such methods will be inadequate. However, there are very few empirical tests examining the relative contribution of direct and indirect impacts of environmental change (Allison *et al*. 2007; Adler *et al*. 2009; Barton *et al*. 2009).

Assessing how multiple environmental changes interact to modify natural communities is very difficult, requiring long-term data sets and knowledge of the state of the system when singular and combined changes in the environment occur. Here we utilize an experimental protist microcosm system to obtain such data; observing and quantifying the effects of combinations of environmental change on the invasion dynamics of competing species. We manipulated three aspects of the environment: food availability, salinity and temperature, in a factorial design and fitted a statistical model to the observed invasion dynamics in order to quantify the extent to which environmental changes interact when modifying species intrinsic growth rates, intra- and interspecific competition. We then present a novel variance decomposition method, which uses the parameterized model to calculate the changes in species invasion rates that result from environmental impacts on the species intrinsic growth rates and inta- and interspecific competition. This gives a quantitative understanding of the mechanisms that cause variation in species invasion rates under different environmental conditions, allowing us to evaluate how direct and indirect effects contributed to this variation.

Methods

EMPIRICAL SYSTEM

STUDY ORGANISMS AND CULTURING

We examined the impacts of combined environmental changes by examining the reciprocal invasion dynamics of two species of bacterivorous protist: *Blepharisma japonicum* (Suzuki) and *Paramecium caudatum* (Ehrenberg). Microcosms consisted of 240ml glass jars containing 100ml Chalkley's solution (Thompson *et al*. 1988), $0.55gL^{-1}$ of crushed protozoan pellets (Carolina Biological Supply, USA) and 2 wheat seeds. Jars were capped with aluminium foil and kept in controlled temperature chambers at 20^0C. All microcosms, and media, were autoclaved before use. Two days prior to the initiation of the experiment, the medium was inoculated with a known bacterial species (*Serratia marcescens*). This allowed the development of a resource base for establishment of the two competing protists*.*

ENVIRONMENTAL MANIPULATION TREATMENTS

Three components of the environment were manipulated, each relative to the above stock culture conditions (Control). The selected factors represent distinctly different type of environmental influence and are conditions that often covary in aquatic systems (Crain *et al.* 2008). These manipulations were: a) reduced food availability (F⇓), b) increased salinity (S⇑) and c) increased temperature (T⇑). Following a three-way fully factorial design (Fig.2.1.a), we manipulated each of the three environmental factors individually (F⇓; T⇑; S⇑), in paired combinations (F⇓+ T⇑; F⇓+S⇑; T⇑+ S⇑) and all together (F⇓+ T⇑+ S⇑). This resulted in eight environmental treatment groups, representing each possible combination of manipulation of the three factors.

Low food treatments (F⇓) contained less protozoan pellet mass in the medium ($0.25gL^{-1}$) and just one wheat seed. In increased salinity treatments (S⇑), the osmolarity was increased from 0.0001 osM (standard salt concentration in Chalkley's medium) to 0.0006 osM, whilst maintaining the same ratios of salts. Finally, microcosms from increased temperature treatments (T⇑) were kept in controlled temperature chambers set to 25^0C (5^0C warmer than controls). Manipulation levels were selected that caused moderate but detectable shifts in carrying capacities or population growth rates, based on preliminary tests of the individual

species along gradients of each factor. The bench positions of microcosms were randomized with respect to the treatments, with the exception of the temperature treatments for which all microcosms from a specific temperature were kept in the same controlled environment chamber. Cultures were replenished weekly by replacing the small amount of evaporative loss with sterile distilled water.
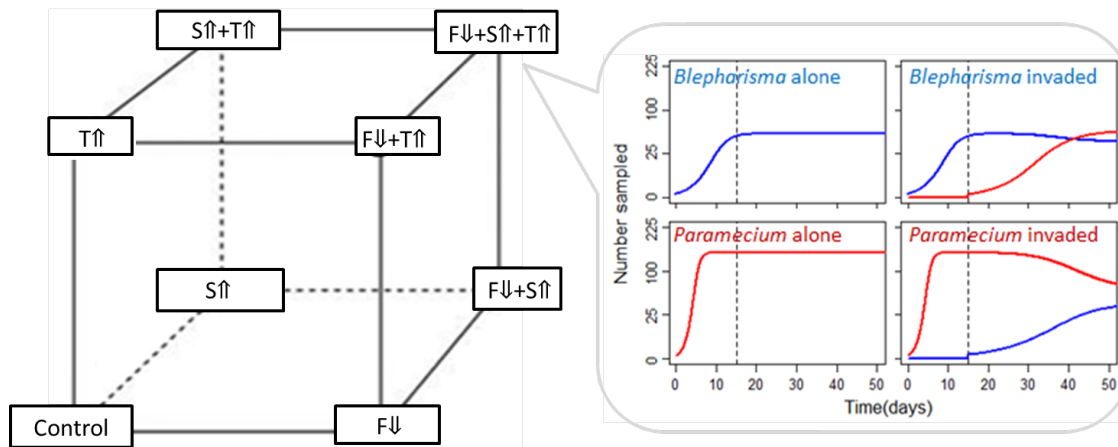
Figure 2.1) Schematic of experimental design showing: A) the three way factorial environmental manipulation of food availability (F⇓), temperature (T⇑) and salinity (S⇑), relative to control stock conditions, and B) the controlled and reciprocal invasion experiments nested within each environmental treatment. Under each environmental condition, *Blepharisma* and *Paramecium* are cultured individually and subject to invasion by its competitor at day 15 (dashed lines).

INVASION EXPERIMENT UNDER COMBINED ENVIRONMENTAL STRESSES

Under each environmental manipulation treatment, reciprocal invasion experiments were undertaken between the two species (Fig.2.1.b). Our invasion experiments consisted of replicate populations in four invasion treatments: a) *Blepharisma* uninvaded, b) *Blepharisma* invaded by *Paramecium*, c) *Paramecium* uninvaded, and d) *Paramecium* invaded by *Blepharisma*.

Under each environmental treatment, five single-species replicates were initiated for each species. Protists were obtained from 2 week old stock cultures, standardizing any influences of individual quality and thus growth rates (Price & Morin 2004; Olito & Fukami 2009). Populations were left to grow for 15 days, by which time species abundances had stopped increasing exponentially. After this growth phase, three replicate microcosms of each resident species were invaded by its competitor. Inoculation abundances, at both the initiation of the experiment and the invasion time, were of 120 individuals if *Blepharisma* or 80 individuals if

Paramecium (to allow for the latter's faster growth rate). At the invasion time, the remaining two control replicates received an equivalent volume of stock medium that had been sieved to remove protists. We used this controlled competition experimental design, with un-invaded replicates and differential replication, to give high statistical power, for inference of key biological processes such as the intensity of competition.

SAMPLING

For all replicates, we sampled species densities twice a day over the first 4 days and then daily over the growth phase, until the invasion point (day 15). After this, sampling was done 3 times a week for the duration of the experiment. Prior to sampling, the medium was agitated and 0.2ml of medium was then transferred (with replacement) to a sterile petri dish and protists were counted under a stereoscopic microscope (surveyed at 7.5 - 30 x magnification). Microcosms were sampled 40 times.

MODEL INFERENCE: DYNAMIC MODEL PARAMETERIZATION

QUANTIFICATION OF THE EFFECTS OF COMBINED ENVIRONMENTAL CHANGES ON DYNAMICS

We used maximum likelihood based inference to fit dynamic models to the observed species abundances, in order to describe how ecological processes drive changes in species abundances under each experimental condition (Bolker 2005). For each environmental treatment level, sample data from all invasion replicates and the uninvaded single species replicates were simultaneously used to parameterize a dynamic model with a common structure across all environments. This approach explicitly linked hypotheses with data, accounted for non-independence of repeat samples and allowed long term trend in abundances, which occur at varying rates between environmental treatments, to be accounted for. Such trends are common in microcosm dynamics, resulting from gradual changes in conditions due to bacterial decomposition of abiotic nutrient stores or environmental degradation (e.g. Fox 2007). Additionally, the parameterized model was further analyzed, to investigate how environmental changes influence specific ecological processes (i.e. intra- or interspecific competition) and how these changes individually contribute to changes in invasion rates. This novel insight could not have been exposed by analyzing differences in invasion rates between treatment groups, as the direct and indirect component of environmental change are entirely confounded.

Two-species competition models were formulated in continuous time, allowing for nonstationary parameters and non-Gaussian observational error distributions. The dynamics were assumed to be governed by a deterministic model that followed a simple Lotka-Volterra (L-V) competition model:

$$\frac{dN_i}{dt} = r_i * N_i * \left(1 - \alpha_{ii}(t) * N_i - \alpha_{ij}(t) * N_j\right) \qquad [1]$$

Here N_i is the state variable of the abundance of species i, r_i is the intrinsic growth rate, $\alpha_{ii}(t)$ is the intraspecific competition term and $\alpha_{ij}(t)$ is the interspecific competition coefficient which signifies the per capita effect of species j on the abundance of species i. This model choice reflects the asexual and largely unstructured life history of protists and follows from the observation that protists are a rare example where species interactions and dynamics are often well approximated by L-V models (Jiang & Morin 2004; Fox 2007), with few higher order interactions (Vandermeer 1969). As in Fox (2007), competition terms, which define density dependence, were allowed to vary over time (Appendix.1) to reflect the trends in abundance and interaction strength over the experiment. More complex model structures were fitted which incorporated: decreasing r_i with abundance (theta logistic), more complex patterns of change in completion with time or thresholds in parameter values after invasion. The alternative models did not sufficiently improve the model fit to justify their increased complexity.

We assumed a negative binomial sampling process, $(Y_t) \sim NB\left(X_t, \frac{\gamma}{\gamma + X_t}\right)$, to accommodate for overdispersion (γ) which results from aggregative behaviours of individuals and would also be further inflated by process variability. A simplifying assumption of the deterministic method of parameter estimation employed here is that process variability is absent; meaning that all error was assumed observational. In validation of this assumption, the overdispersion of the negative binomial was not greatly more than expected under a Poisson sampling process and a comparison with estimates of a more computationally expensive stochastic dynamic model revealed no significant difference in parameter estimates; indicating that process variation was small (Appendix.2A). Due to this simplifying assumption the likelihood function could be more rapidly optimized allowing parameter confidence intervals to be obtained from likelihood profiles.

COUNTERFACTUAL ANOVA DECOMPOSITION

This analysis decomposes how each species per capita invasion rate was modified by combinations of environmentally driven effects that influence: the intrinsic growth rate (r_{iE}), intraspecific competition (α_{jjE}) and interspecific competition (α_{ijE}); where the superscript E specifies the environment under which the model coefficient is evaluated. The invasion rate under a specific environmental condition (v_{iE}) is the rate that a rare colonizer of species i invades an equilibrium resident population of the competitor j. Formally, it is the per capita rate of change given $N_i \approx 1$ and $N_j = N_j^*$ and was calculated as:

$$v_{iE} = r_{iE} * \left(1 - \frac{\alpha_{ijE}}{\alpha_{jjE}} \right) \qquad [2]$$

The fitted model estimates of our factorial experimental design provided coefficients to calculate v_{iE} under all combinations of our environmental manipulation. Additionally, individual coefficients in Eq. 2 can be independently modified to reflect their value under alternative environmental treatments. This allows simulation of counterfactual environments, where only certain biological quantities are altered by environmental change. For example, by only allowing environmental effects on intrinsic growth rates, the direct effects of environmental change can be estimated. Conversely by allowing only competition coefficients of the resident to vary, the indirect effects of environmental change are identified. We calculated invasion rates for both species under all 512 possible counterfactual environments (i.e. all possible combinations of treatment effects modifying each parameter individually).

ANOVA decomposition was then undertaken to partition the pathways of effect of environmental factors on invasion rate (Rees *et al.* 2004). We defined the environmentally dependent invasion rate of a species (v_{iE}), as a linear function of its invasion rate in a control environment (v_{ic}), plus the effects on invasion rate of each environmental manipulation acting on: (i) the intrinsic growth rate ($\Delta_{F\Downarrow}r_i, \Delta_{T\Uparrow}r_i, \Delta_{S\Uparrow}r_i$), (ii) the residents intraspecific density dependence ($\Delta_{F\Downarrow}a_{jj}, \Delta_{T\Uparrow}a_{jj}, \Delta_{S\Uparrow}a_{jj}$), and (iii) the interspecific competition ($\Delta_{F\Downarrow}a_{ij}, \Delta_{T\Uparrow}a_{ij}, \Delta_{S\Uparrow}a_{ij}$) . Delta subscripts refer to the specific environmental factor and arrows signify its direction of change (e.g. $\Delta_{F\Downarrow}r_i$ =the effect of decreased food on invasion rate, caused by changes to the invaders intrinsic growth rate).

The resulting simulation experiment followed a nine-way fully factorial ANOVA without replication, with the following linear model structure:

$$\overbrace{\hspace{8cm}}^{\textit{main effects of environmental manipulations on biological coefficient}}$$

$$v_{iE} = v_{iC} + \left[\varDelta_{F\Downarrow}r_i + \varDelta_{T\Uparrow}r_i + \varDelta_{S\Uparrow}r_i + \varDelta_{F\Downarrow}a_{ij} + \varDelta_{T\Uparrow}a_{ij} + \varDelta_{S\Uparrow}a_{ij} + \varDelta_{F\Downarrow}a_{jj} + \varDelta_{T\Uparrow}a_{jj} + \varDelta_{S\Uparrow}a_{jj} \right]$$

$$+ \; Interactions \hspace{6cm} [3.a]$$

The sum of model predictors (*e.g.* $\varDelta_{F\Downarrow}r_i + \varDelta_{T\Uparrow}r_i$) gives the additive expectation of the combined independent effects of environmental changes on invasion rate, via those model coefficients.

By definition, this gives the expected effect size if one predictor does not moderate the effect of the other on invasion rate. Additionally, higher order interaction terms between model predictors were considered. For example, variation in invasion rate could be caused by the effects of the three factors acting on the intrinsic growth rate and the interactions between pairs of them. In this case, the appropriate linear model would include the main effects of the factors on r_i and the second order interactions between them. Here we use the following short-hand notation to describe this: $v_{iE} \sim v_{iC} + (\varDelta_{F\Downarrow}r_i + \varDelta_{T\Uparrow}r_i + \varDelta_{S\Uparrow}r_i)^2$. Alternatively, variation in invasion rate could be due to the effect of temperature acting on three different L-V coefficients and third order interactions of them. In this case, the linear model structure should be: $v_{iE} \sim v_{iC} + \left(\varDelta_{T\Uparrow}r_i + \varDelta_{T\Uparrow}\alpha_{jj} + \varDelta_{T\Uparrow}\alpha_{ij} \right)^3$.

Such rival hypotheses about the important causes of variation in invasion rate were formalized as competing linear models and were fitted to the counterfactual data set (Appendix.3). These differed in the predictors they use to explain environmentally driven changes in invasion rate and the highest order of interactions between predictors. Decomposition revealed the amount of variation in invasion rate accounted for by a specific level of model complexity, allowing identification of the major sources of differences from control invasion rates and an examination of the importance of higher order interactions. All analyses were carried out using the R statistical program (R Development Core Team 2006).

Results

All populations persisted for the duration of the experiment and the observed dynamics were largely consistent within replicates treatments (Fig.2.2).   Across treatments there were large differences in observed species abundances and variation in the direction of population trends. These differences reflected whether or not environments were warmed as these tended to deteriorate faster (Appendix.1).
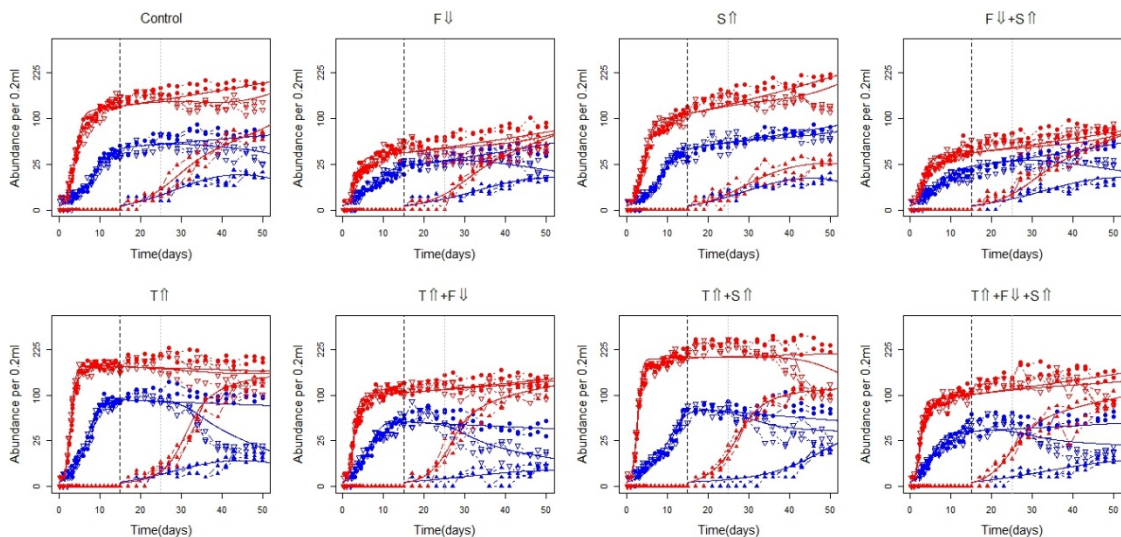
Figure 2.2) Observed invasion dynamics of protists under eight treatment combinations with fitted models predicted from equation 1. Sampled abundances of *Blepharisma* (Blue) and *Paramecium* (Red) are plotted when alone (filled circles; solid fitted line), as a resident (invaded at day 15) (open downward triangles; short-dashed fitted line) and as an invader; increasing into a near equilibrium population of the competitor (invade on day 15) (Filled upward triangles; long-dashed fitted line). Black dashed line is the invasion time and grey line is the midpoint of the experiment, when competition coefficients are considered.

MODEL INFERENCE: ENVIRONMENTAL EFFECTS ON BIOLOGICAL PROCESSES AND SPECIES DYNAMICS

The fitted model captured the differences in dynamics between environmental conditions; using a common deterministic structure across environments to allow direct comparisons (Fig.2.2). One model simultaneously describes species dynamics in isolation and their reciprocal invasion dynamics. This is a strong structural assumption, but nonetheless, the model generally fits the data well. There is a minor bias in the model prediction of *Paramecium* abundance in isolation, which is most evident towards the end of the experiment. This may reflect: i) a deviation in the carrying capacity of single and joint species (e.g. as a response to changing grazer pressure on their bacterial resource), or ii) a minor humpback trend in

abundance is apparent under some but not all environments, producing a small systematic deviation in the model fits over the final stages. We believe the additional model complexity required to capture these detailed inter-treatment differences would result in poorly identifiable parameter estimates. Moreover, the invasion dynamics do not show large deviations over the invasion phase and so parameter estimates will be robust to the structural constraints. The model correctly predicts the reciprocal success of invasion of both species, allowing coexistence under all environments. The rate of change in both species was also accurately captured. The parameters quantifying the dynamics of each treatment were well identified, as indicated by an analysis of the parameters likelihood profiles (Appendix.2B). Non-overlap of model coefficients confidence intervals represents a highly conservative test of treatment differences at the 95% level. Figure 2.3 reveals the substantial and statistically significant variation across treatments in the processes driving the dynamic behaviours.

Intrinsic growth rates of both species increased significantly with warming (Fig.2.3a+b). One apparent exception in Fig.2.3.a. is that of *Blepharisma* under increased temperature and salinity environments. However, examination of the time series, during the growth phase (Fig.2.2. T⇑+S⇑) indicates that this was a result of be a brief plateau during the growth phase which is not captured by the deterministic model. Therefore we do not consider this estimate to be good evidence of interactions between environmental effects. We do however find reliable evidence of interactions between environmental factors on other biological quantities. In *Paramecium*, under the same environmental treatment of increased salinity and temperature we did reveal evidence of interactions between environmental factors. Increased salinity caused an increase in the intrinsic growth rate under cool condition, but a reduction under warmed conditions.

Environmental factors also clearly interacted to influence intra- and interspecific competition (Fig.2.3. c-f). Competition was greatly intensified under low resource conditions in both species, however resource depletion caused larger increases in the competitive effects of *Paramecium* (α_{PP} & α_{BP}; Fig.2.3. d+e) compared to *Blepharisma* (α_{BB} & α_{PB}; Fig.2.3. c+f). Warming had the opposite effect, reducing interspecific competition and allowing higher abundances. Importantly, the intensification of competition produced in low resource environments under cooler conditions was greatly reduced in warm environments. This antagonistic interaction between warming and resource depletion acted on both intra-and interspecific competition. Finally, a subtle third order interaction between all three factors affected intraspecific competition (Fig.2.3.c+d). Here increased salinity interacted with low

resource conditions only at lower temperatures to produce a synergistic increase in α_{BB}(Fig.2.3.c) and an antagonistic reduction of α_{PP}(Fig.2.3.d). This translates into a reduction in *Blepharisma* abundance and an increase in *Paramecium* abundance with increased salinity which is only meaningfully apparent under low resource and low temperature conditions. These non-additive effects of combined environmental changes, which were usually common to both species, produced species dynamics that would be unexpected from the study of the effects of drivers in isolation.
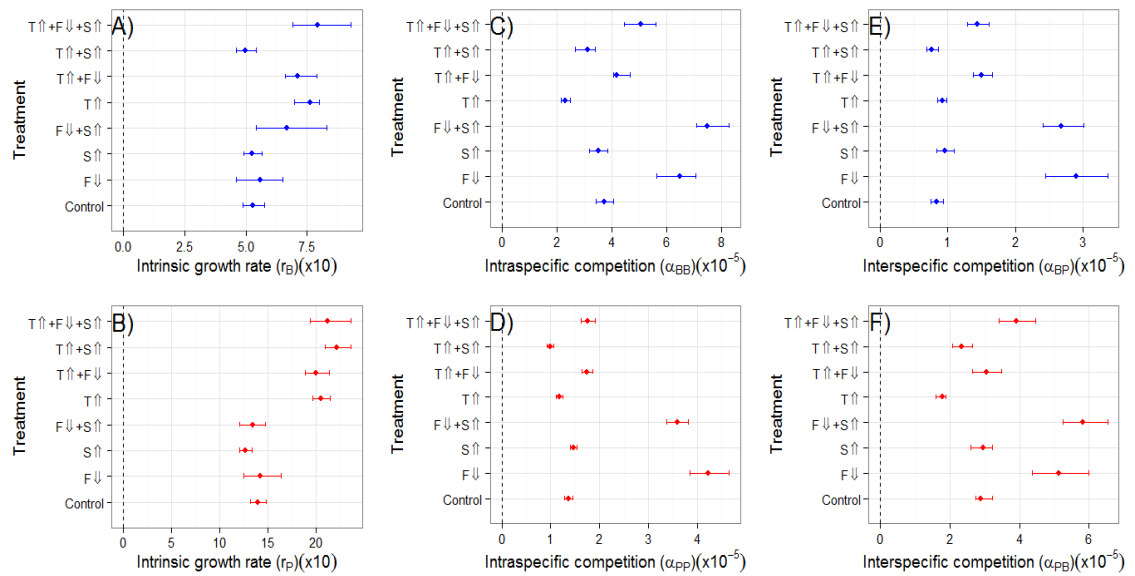
Figure 2.3) Model inference: maximum likelihood estimates of the intrinsic growth rate, intraspecific and interspecific compeitition coefficients of *Blepherisma* (blue) and *Paramecium* (red) under each environmental treatment. Bars represent 95% confidence intervals obtained from profile likelihoods.

COUNTERFACTUAL ANOVA DECOMPOSITION

A) IDENTIFYING THE BIOLOGICAL PROCESSES CAUSING VARIATION IN INVASION RATES

Applying our counterfactual ANOVA decomposition method to the fitted models, we examined the mechanisms by which combined environmental changes cause variation in species invasion rates. The simplest decomposition model with an R-squared value accounting for over 90% of the variance was chosen to infer the change in invasion rate associated with environmental driven changes in intrinsic growth rate, and competitor abundance or interaction strength. For both species, the same candidate model most simply and sufficiently explains this variation:

$$v_{iE} \sim v_{ic} + \left(\Delta_{F\Downarrow}\alpha_{jj} + \Delta_{T\Uparrow}\alpha_{jj} + \Delta_{S\Uparrow}\alpha_{jj} + \Delta_{F\Downarrow}\alpha_{ij} + \Delta_{T\Uparrow}\alpha_{ij} + \Delta_{S\Uparrow}\alpha_{ij} \right)^2 , \quad [3.b]$$

This model captures above 93% of the variation, using just 22/512 coefficients of the saturated model (Appendix.3). Notably, the model structure (eq 3.b) neglects the impacts of environmental change on the intrinsic growth rate and limits interactions of the remaining six predictors of invasion rate to the second order. These predictors reflect impacts of the three environmental factors upon intra- and interspecific competition. Thus, ANOVA decomposition revealed that the major pathways by which environmental changes modified invasion rates are two indirect competitive effects. Invasion rates were largely dependent upon the effects of environmental manipulations on: 1) the intensity of intraspecific competition in the resident population; which determines competitor abundance ($\Delta\alpha_{jj}$), and 2) interspecific competitive effect of each competitor upon the invader ($\Delta\alpha_{ij}$). Further, the environmental effects on both pathways were modified through interactions between pairs of environmental changes, whilst higher order interactions had small effects.

B) QUANTIFYING MECHANISMS BY WHICH ENVIRONMENTAL FACTORS INFLUENCE INVASION RATES

The decomposition model coefficients (Eq. 3b - predictors & 2nd order interactions) provided estimates of the invasion rates in the control environment. They also show the direction and magnitude of changes in invasion rate, associated with environmental effects via two distinct components of competition: 1) alterations to the interspecific competitive effect of the resident and 2) changes in the intensity of intraspecific competition and thus abundance of the resident population. Finally the interaction coefficients indicate the effect of interactions between pairs of environmental factors on the invasion rates; acting via both pathways. We present these quantitative results in Figure 2.4 and, with reference to it, describe below the inferred mechanisms by which environmental factors alter invasion rates. This analysis isolates how the environmental impacts upon each component of competition independently influence invasion rates. As a result, the total environmental impact upon invasion rates should not be re-estimated from the decomposition output by summing the effects via both pathways as the competition coefficient (α_{ij} and α_{jj}) in the invasion calculation (equ2) are non-additively related.

i) How individual environmental changes modify invasion rates

Consistent with our observations, the estimated invasion rate under control conditions (horizontal solid black line) was lower for *Blepharisma* than *Paramecium* ($v_{B}c$= 0.215 vs. $v_{P}c$= 0.360). The three environmental factors caused differing directional changes in invasion rates (coloured capped bars), acting via the two indirect pathways (left vs. right panel of Fig.2.4). Notably, the direction of change caused by an environmental factors effect on interspecific competition consistently opposed its main effect on invasion rates via impacts on intraspecific competition in the resident (compare the direction of light and dark shades). This occurred because the environmental effects on competition were in the same direction within and between species and because these pathways have opposing effects on invasion rates. Reducing intraspecific competition in the resident (α_{jj}) increases equilibrium competitor abundance (N_{j}^{*}); indirectly decreasing the invasion rate by raising the total population level competitive effect ($\alpha_{jj} * N_{j}^{*}$). Contrastingly, reducing interspecific competition (α_{ij}) decreases this total competitive effect, increasing the invasion rate.

The greatest magnitude of change in invasion rate was caused by decreasing food availability. Predominantly, low food availability reduced invasion rates, with the main mechanism causing this being an increase in the per capita interspecific competitive effect of the resident (Fig.2.4- light green bars; $\Delta_{F\Downarrow}a_{BP}$=-0.803; $\Delta_{F\Downarrow}a_{PB}$=-1.16). Low food availability also produced a strong but only partially opposing increase in invasion rates by increasing intraspecific competition in the resident population, which reduced the number of resident competitors and the population level competitive effect (Fig.2.4- dark green bar; $\Delta_{F\Downarrow}a_{BB}$=0.34; $\Delta_{F\Downarrow}a_{PP}$ =0.55). Temperature had the next largest main effects on invasion rates, also predominantly reducing them. This was, however, caused by a intraspecific process, where the invasion rate was primarily reduced due to an increase in the number of resident competitors, due to a reduction in the intensity of intraspecific competition (Fig.2.4-dark red bars; $\Delta_{T\Uparrow}a_{BB}$=-0.77; $\Delta_{T\Uparrow}a_{PP}$=-0.124). The reduction in the interspecific competitive effect of the resident in warmed environments allowed only a smaller counteracting increase in invasion rate (Fig.2.4- light red bars; $\Delta_{T\Uparrow}a_{PB}$=0.57; $\Delta_{T\Uparrow}a_{BP}$=0.023). Relative to the effects of other environmental factors, the magnitude of the temperature effects on invasion rate were much greater in *Paramecium* than *Blepharisma.* This was true for effects on both indirect pathways. Finally, increasing salinity alone had negligible effects on the invasion rate of both species; minimally affecting the intra- or interspecific competition pathways (Fig.2.4- blue bars $\Delta_{S\Uparrow}a_{BB}$=-0.12; $\Delta_{S\Uparrow}a_{PB}$=0.08; $\Delta_{S\Uparrow}a_{BP}$=0.025; $\Delta_{S\Uparrow}a_{PP}$=-0.005 =-0.12).

ii) Environmental factors interact to modify mechanisms influencing invasion rates

Pairs of environmental changes interacted, via both intra- and interspecific pathways, causing invasion rates to be modified in a way that would be unanticipated if the individual factors were assumed to act independently. These non-additive effects of environmental change on species invasion rates can be seen in Fig.2.4 as the vertical difference between the additive expectations if factors acted independently (white bars) and the calculated combined effects (thick grey bars).

Under combined warming and food reduction, the interspecific competitive effect of residents increased much less than expected if the effects of the factors were additive and independent. This reduced intensification of competition resulted in invasion rates being less reduced (Fig.2.4- light green + red vs. grey bars). This antagonistic interaction was common to both *Blepharisma* and *Paramecium* ($\Delta_{F\Downarrow}a_{BP} * \Delta_{T\Uparrow}a_{BP}$=0.507; $\Delta_{F\Downarrow}a_{PB} * \Delta_{T\Uparrow}a_{PB}$=0.482). Increased temperature and food reduction also interacted to affect the opposing intraspecific pathway, which modifies invasion rates by changing the abundance of residents (Fig.2.4- dark green + red vs. grey bars). Additively, this pathway would be expected to allow invasion rates to increase, due to the larger impact of reduced food availability compared to temperature, which intensifies competition between residents, reducing their abundance. Here the size of the non-additive effect differed between the species; with *Paramecium*'s invasion rate being further increased, whilst *Blepharisma*'s was relatively unaffected ($\Delta_{F\Downarrow}a_{BB} * \Delta_{T\Uparrow}a_{BB}$=0.203; $\Delta_{F\Downarrow}a_{PP} * \Delta_{T\Uparrow}a_{PP}$=-0.087).

High salinity and temperature also interacted to modify invasion rates, primarily by affecting the intensity of intraspecific competition and thus abundance of the resident competitor. The interaction between these factors had differing effects on the two species, reflecting their differential responses to salinity. In *Paramecium*, the reduction in invasion rate, expected due to the large negative main effect of temperature, was largely reduced by an interaction with salinity (Fig.2.4b- dark red + blue vs. grey bars). Notably, the magnitude of this antagonistic interaction is 5 times greater than the small main effect of increased salinity ($\Delta_{S\Uparrow}a_{BB} * \Delta_{T\Uparrow}a_{BB}$=0.416). This large effect reflects part of the subtle three-way interaction identified above. Contrastingly, in *Blepharisma,* this pair of factors interacted less strongly to modify intraspecific competition and invasion rates, as the resident (*Paramecium*) is less variable in its sensitivity to salinity across other environmental treatments ($\Delta_{S\Uparrow}a_{PP} * \Delta_{T\Uparrow}a_{PP}$=-0.0764). We also found that other pairs of environmental factors only interacted to modify the invasion rate of *Paramecium.* These remaining interactions are weaker in magnitude but all include the

effect of salinity and one other factor upon the invasion rate of *Paramecium* ($\Delta_{S\Uparrow}a_{BB} *$ $\Delta_{F\Downarrow}a_{BB}$=-0.145; $\Delta_{S\Uparrow}a_{PB} * \Delta_{T\Uparrow}a_{PB}$=-0.129; $\Delta_{S\Uparrow}a_{PB} * \Delta_{F\Downarrow}a_{PB}$=-0.195). Indirectly, these interactions further reflect the higher variability in the sensitivity of *Blepharisma* to salinity sensitivity; with its competitive ability being more variably affected by salinity between other environmental conditions.
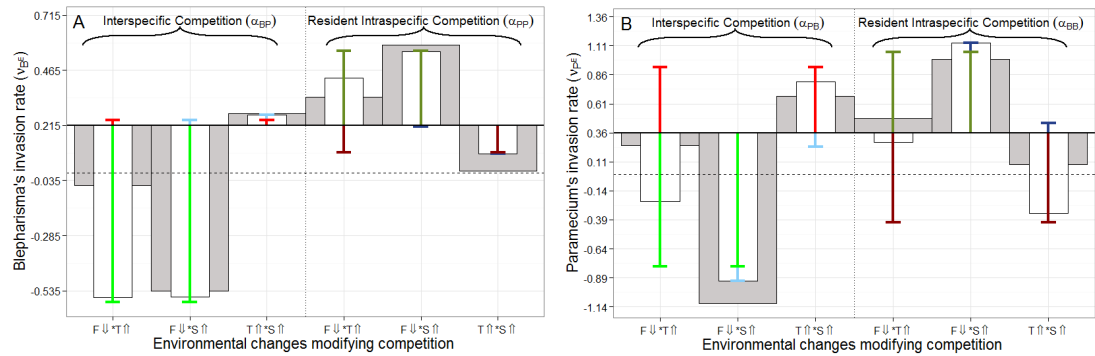
Figure 2.4) ANOVA decomposition- How estimated invasion rates of *Blepharisma* and *Paramecium* are modified by the impacts of environmental changes, acting upon competition with the resident species. The two subpanels of each plot relate to the components of the total competitive effect: (left panel) the intensity of the resident's per capita interspecific competitive effect on the invader, and (right panel) the intensity of intraspecific competition among residents (inversely proportional to the resident's carrying capacity). Environmental effects on invasion rate are plotted as the magnitude and direction of the changes they cause relative to the invasion rate under control treatments (solid horizontal black line). Within each subpanel, the three sets of bars (i.e. an open bar, a grey bar, and two green/blue/red capped bars) represent the effects of pairs of environmental changes on the invasion rate, via a component of competition (e.g. the left-hand group in the left panel of A represents the impacts on *Blepharisma's* invasion rate of reduced food availability (F⇓) and increased temperature (T⇑) caused by changes in the per capita interaction strength of the resident competitor, *Paramecium*). The narrow capped bars show the individual impacts of reduced food availability (F⇓, green bars), increased temperature (T⇑, red bars) and increased salinity (S⇑, blue bars). Regular white bars show the combined effects of the pairs of environmental changes, assuming that the factors act independently (i.e. the sum of the two individual effects shown by the coloured lines). In comparison, thick grey bars show the combined impact when the interaction between factors is included. The horizontal dashed line indicates the threshold for species invasions to be successful, to give a scale to the magnitude of these effects.

Discussion

In this study, we obtain a quantitative understanding of the combined effects of environmental changes on the dynamics of coexisting species, by fitting simple statistical models to empirical data. We show that there were strong non-additive effects of productivity, temperature and salinity, acting upon intra- and interspecific competition, modifying the invasion dynamics. Decomposition of the overall effect on species invasion rates into its component parts reveals the relative unimportance of direct effects on species intrinsic performance compared to that of indirect effects of competitor abundance and per capita interaction strength.

These results provide clear evidence that environmental changes are likely to interact when modifying population level processes, supporting the widely documented evidence of similar non-additive effects on key life history processes (e.g. Folt 1999). In a meta-analysis of 112 studies, Darling & Côté 2008 uncovered nonadditve effects of multiple drivers on mortality rates in 75% of relevant studies. Crain *et al*. (2008), expanded this meta-analysis approach, examining interactions between drivers on other individual level responses in marine communities and similarly found non-additive effects in 74% of cases. However, interactive effects at the individual level do not necessarily translate simply to the population and community level. By excluding experiments focusing on indirect effects via trophic interactions, this meta-analysis fails to account for the long term population and community effects of combined drivers. It may therefore provide a conservative estimate of the frequency of non-additive environmental effects. Our study addresses this problem, thus helping to fill this widely acknowledged (Paine *et al.* 1998; Breitburg *et al.* 1998; Crain *et al*. 2008; Moe *et al.* 2013) knowledge gap.

Considering the causes of coexistence in this system is an important prerequisite to understanding the dynamics and the combined impacts of environmental changes. Despite the apparent overlap of resource base between competitors in laboratory microcosms, coexistence frequently occurs (e.g. Vandermeer 1969). Although coexistence may be permitted through a combination of several mechanisms, Fox & Barrento (2006) have shown that even after experimentally ruling out interspecific bacterial diversity, differential habitat use and chemical interference, unexplained coexistence still occurs. The demonstration by Delong & Vasseur (2012) of protist coexistence based on size partitioning of their bacterial resource, indicates that intraspecific bacterial variation, over their life history or evolutionary divergence, is potentially a general mechanism mediating coexistence.

In addition to the role of bacterial variation in permitting coexistence, changes in the bacterial abundance have a crucial role in driving the protist responses to environmental change, potentially explaining the non-additive effects of combined changes. Bacterial responses to reduced resource levels have been shown to follow simple models of consumer resource theory (Abrams 1998; Tilman 1982). Under low resource conditions, bacterial resource density is reduced, subsequently driving the reduction in the abundance of bactiverous protists (Balčiūnas & Lawler 1995; Kaunzinger & Morin 1998). The increase in protist abundance under warmed conditions, which is consistent with previous microcosm studies (e.g. Fox & Morin 2001; Beveridge *et al*. 2010b), is also likely to be largely driven by increased bacterial resource

abundance (Jiang & Morin 2004). Under similar warming, increased bacterial productivity and specific growth is known to be accompanied by increased protist consumption and metabolism (White *et al.* 1991; Peters 1994). This is consistent with metabolic theory, which suggests that motility and consumption rates are initially expected to increase faster than increases in mortality and metabolism under moderate warming (Savage *et al*. 2004; Rip & McCann 2011). This mechanism speeds up growth and, by shifting energy up trophic levels, reduces the intensity of density dependence. At higher temperatures, increases in metabolic cost will begin to outweigh the benefits of increased prey assimilation rates, preventing further increases in protist abundances. However the species used here are well adapted to warm temperatures and we have observed that their abundances continue to increase until substantially warmer temperatures (personal unpublished observations). These mechanisms explain the similar responses of the competitors to changes in productivity and temperature.

Together, environmental changes clearly moderated the effect of one another. This generated non-additive effects on both species' intrinsic growth rates and intra- and interspecific competitive effects which translated into variation in the rate of invasion. Most prominently, we found that the intensification of intra- and interspecific competition, which is anticipated under resource depletion (Abrams 1998), was consistently offset at higher temperatures. The mechanism driving this may be a modification of ecosystem functioning at higher temperatures. Warming has been demonstrated to increase bacterial decomposition of wheat seeds and recycling of nutrients (Beveridge *et al*. 2010a). This modification would offset competition by unlocking a greater amount of potential resource from abiotic source. Our study was designed to quantify the interactive effects rather than to identify the underlying physiological mechanisms, however warmed treatments showed more rapid declines in abundance, suggesting more rapid exploitation of the wheat seed, which acts as a slow nutrient release. Future experiments aiming to identify these mechanisms should consider monitoring changes in the bacterial abundance, the biomass decomposition of the wheat seed and may also need to consider how demographic processes of the competing species are influenced. Changes in body size in response to warming may be important in altering individual resource requirements and driving these interactive effects on species abundance patterns (Reuman *et al.* 2013).

Our analysis also reveals subtle interactions of increased salinity when in combination with cool temperatures and low food levels. The impacts of salinity upon protist dynamics have not previously been reported. However, in short term experiments *Blepharisma* is known to have a

lower tolerance to increased salinity compared to *Paramecium*, which performs well even at much higher salinities (Finley 1930; Smurov & Fokin 2001). We found that under most combinations of conditions studied, the dynamics of both species were largely insensitive to differences in salinity, although *Paramecium* showed a small increase in abundance due to increased salinity, whilst *Blepharisma* showed a slight decline. There was however a subset of conditions causing the abundance of both species to be affected by salinity to a greater extent, through strengthened impacts on intraspecific competition. These changes are in the direction expected based on our limited knowledge of their salinity tolerances (i.e. *Paramecium* increased in abundance whilst *Blepharisma* decreased).

Although these interactions were smaller than the interactions of productivity and temperature, they were large relative to the lesser main effect of increased salinity under most conditions. This finding suggests that it may be difficult to identify some important drivers of change in species abundance because the main effect size is generally small, but becomes much more important under a narrow set of environmental conditions. This is in accordance with shorter term evidence of density dependence in Soay sheep populations: Coulson *et al*. (2008) revealed that harsh winter weather produced strong density dependence, through mortality, but this only occurs after previous periods of high resource conditions which produce a population boom. Interestingly, in this system, it is only when bacterial abundance is expected to be low (i.e. low resources and temperature) that salinity strongly influences protist dynamics and only via intraspecific competition. It appears that differences in species physiological performance between salinity levels are amplified under these low prey conditions. Identifying conditions, and the underlying mechanisms, that are likely to lead to these amplification effects is essential for a transition to a more predictive framework.

Unconsidered interactions between many environmental changes may prove problematic for the development of predictive frameworks mechanistically linking environmental changes to species dynamics. Metabolic theory, for example, has shown promise, in tightly controlled laboratory microcosms, as a method for scaling species feeding rates, interaction strengths and extinction risks with temperature (Rall *et al*. 2010, Petchey *et al.* 1999; O'Connor *et al.* 2009; 2013; Clements *et al.* 2014). However in natural systems, these scaling rates may vary continuously as other interacting facets of the environment change. Thus strong interactions, such as that seen here between temperature and productivity, will have important ramifications for the development of predictive frameworks for forecasting species responses under environmental change.

Fortunately, our results also indicate that by considering the impacts of environmental changes on species' physiological performances, their prey resource and ecosystem functioning, these interactions may be quite predictable. Given the large possible number of interactions that could have occurred between our treatments, we actually find a fairly small number of rather consistent effects. It appears likely that general bottom-up mechanisms produce the comparable responses of the competing species and causes the similar changes to intra and interspecific competition. This is likely the case in many natural systems. For example, nutrient enrichment is generally beneficial to the performance of all plant species in a community, although they will vary in their sensitivity. As a result the per capita intensity of intra- and interspecific competition can be reduced. Notably, when the resultant changes in abundance of competitors and its interaction strength are considered, the net effect of enrichment can still be negative (Suttle *et al.* 2007).

Our counterfactual decomposition analysis revealed that much of the dynamic changes in invasibility were mediated via such indirect changes in the density of the resident species and its competitive ability. This is surprising as increases in intrinsic growth rates caused by temperature were large; representing a switch from approximately two to three protist generations per day. Such increases in species intrinsic growth rates are often assumed to drive substantial increases in species' invasion rates (Sorribas *et al*. 2012; Sorte *et al*. 2012), but were unimportant here. Our results echo the theoretical conclusions of Case (1990) that in communities of strongly interacting species, the possibility of invasion is reduced. The invasion rate in this system is largely dependent upon the ratio of interspecific: intraspecific competition. Therefore in order to deduce if some combination of environmental changes will be good or bad for an invader, understanding whether the changes raises or lowers density of residents is highly informative (although our results suggest that density and interaction strength may often be negatively related) .

When indirect effects of environmental change predominate, the direct effects of environmental changes on a species alone will be very poor predictors of changes in invasion rates and the dynamics in general (Barton *et al*. 2009; Gilman *et al.* 2010). Direct effects may be more important in situations where the focal species has a significant intraspecific competitive effect ($\alpha_{ii} * N_i^*$). For example, the resilience of an established species or the immediate response to environmental trends in an equilibrium population may be more dependent on the direct response of the focal species. When questioning the relative importance of direct and indirect effects of environmental change, the answer will vary

depending on the biological process of interest. Our work indicates that forecasts of species responses to environmental changes, which do not incorporate species interactions (e.g. species distribution models), will have limited predictive capacity. It is therefore important to develop methods to integrate interspecific interactions into abundance and range forecasting model (e.g. species interaction distribution models; Kissling *et al.* 2012) in order to make accurate predictions of the consequences of environmental change.

Overall our results support two important points. Firstly, as combined environmental changes impacted species dynamics non-additively through effects on several biological processes, the response of species will depend crucially upon the states of numerous aspects of the environment experiencing change. This suggests that multiple features of the environment will need to be incorporated into population models in order to understand responses to environmental change. Furthermore, as environmental effects are frequently non-additive, the mechanisms driving the interactions between must be identified. Fortunately, the interactions between environmental drivers are not as complex as may be expected given the vast number of possible interactive effects. By considering both the direct impacts of climate on species physiology as well as the indirect effects on competitors and their resources, the underlying mechanisms causing these interactive effects appear quite interpretable. This suggests the possibility of anticipating and generalizing about these effects in other environments. This understanding will likely require detailed demographic data relating environmental conditions to individual birth, growth and survival rates rather than phenomenological time series approaches. Methods linking demographic and abundance data into a single model inference framework are now beginning to be utilized (Coulson *et al*. 2008), but the prediction of species responses to environmental change is still a nascent field (Jenouvrier *et al.* 2013). Secondly, invasion rates can be strongly influenced by environmental changes as a result of variation in the abundance and per capita interaction strength of competitors; not just by impacts on their intrinsic physiological performance. Therefore ecological models must embrace the interplay of species within a community by incorporating strongly interacting species into models. Our work demonstrates that even in very simple systems, different components of the environment interact to strongly impact on the many aspects of species biology and modify community level processes via the interactions with other species.

Acknowledgements

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Appendices

Appendix 1) Competition terms modelled as a function of time

It is common to find non-stationary dynamics in closed microcosm systems (e.g. Vandermeer 1969). Trends of increasing abundance, after the population growth phase, is often attributed to a net influx of resource from decomposition of wheat seeds, whilst declining abundances results from depletion of the protozoan pellet resource base. An inspection of our time series suggested that we needed to include temporal variation in the parameters of the model to reflect this. For all treatments, the functional form of temporal changes in parameters was examined and models were compared based on AIC scores (Table.2.S1).

Table 2.S1) Comparison of the relative performance of candidate models defining the temporal variation in the intrinsic growth rate, intra – and interspecific competition. AIC scores averaged across treatments are used to select the preferred model structure (shaded).

Form of temporal variation				
Intrinsic growth rate (r_i)	**Intraspecific competition (α_{ii}(t))**	**Interspecific competition (α_{ij}(t))**	*AIC*	*d.f.*
Exponential	Exponential	Linear	2960	13
Linear	Exponential	Linear	2963	13
Constant	Exponential	Exponential	2748	11
Constant	**Exponential**	**Linear**	**2746**	**11**
Constant	Linear	Exponential	2784	11
Constant	Linear	Linear	2785	11
Constant	Exponential	Constant	3068	9
Constant	Linear	Constant	3101	9
Exponential	Constant	Constant	3064	9
Linear	Constant	Constant	3132	9
Constant	Constant	Constant	3079	7

Model comparisons advocated that intraspecific density dependence (α_{ii}) changed as an exponential function of time, whilst interspecific density dependence (α_{ij}) changed linearly. Temporal variation in the intrinsic growth rate was also examined, however this did not significantly improve the model fits. A translation was applied to the parameters which temporally moderate the intensity of competition, so that we could assess the relative

importance of direct and indirect effects of environmental change at the midpoint of the experiment (day 25). Thus the functional form of the competition coefficients where:

$$\alpha_{ii}(t) = \alpha_{ii} * e^{\beta_{ii}(t-25)} \qquad \text{[1a]}$$

$$\alpha_{ij}(t) = \alpha_{ij} * \left(1 + \psi_{ij}(t-25)\right) \qquad \text{[1b]}$$

The intensity of intra- and interpecific competition varied over time, however the magnitude and direction of these changes varied between treatments and species. The maximum likelihood models estimates (Fig.2.S1), quantified the nonstationarity that this caused in the dynamics. In many replicates, especially those initiated with low nutrient levels, intraspecific competition lessened over the experimental period (Fig.2.S1.A+B; negative values of β_i). This produced the trends of increasing abundance of sinlge species systems in many treatments. It is likely that bacterial decomposition of the wheat seed nutrient source caused this decline of intra-specific competition through a net influx of energy into the system. In hot treatments, *Blepharisma* showed a noticably different behaviour. The positive estimates of β_i under warm conditions (Fig.2.S1.A), captures the intensification of intraspecific competition, causing their declining abundances. *Paramecium* also had higher values of β_i in hot environments, although stilll negative (Fig.2.S1.B). This commonality indicates that warming had a general effect of causing these microcosms to be degraded more rapidly. This is in line with expections of metabolic theory and other microcosm experiment that manipulate temperature. Warming induces metabolic rises, increasing decomposition of nutrients stored in the wheat grains and the rate that the nutrients within this closed system will be depleted (Beveridge *et al*. 2010a; Binzer *et al.* 2012).
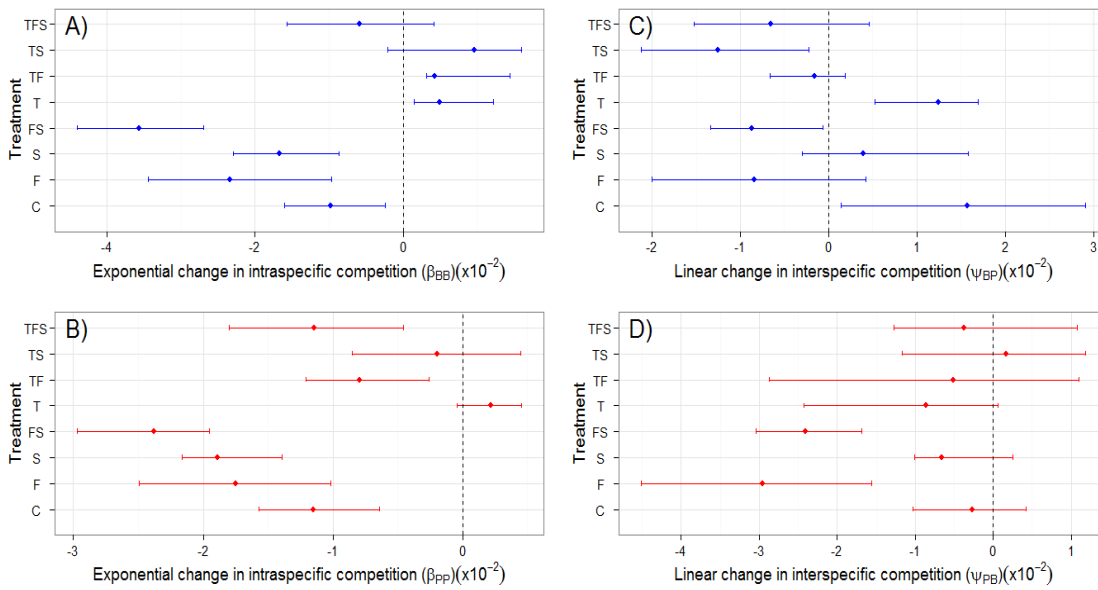
Figure 2.S1) Maximum likelihood estimates of the temporal changes in intra- and interspecific competition in *Blepherisma* (blue) and *Paramecium* (red) under each environmental treatment. Note that positive coefficients signify an intensification of competition over time. Bars represent 95% confidence intervals obtained from profile likelihoods.

Although temporal changes in interspecific competiton were more difficult to identify, the results suggest a slight decline in interspecific competition over time in most treatments (Fig.2.S1.C+D; negative ψ_{ij} values). Such an amealoration of interspecific competition would be the result of niche seperation, which could have occurred over this time scale as a result of character displacement. Additional and more detailed observations would be needed to test this hypothesis.

The temporal variation in competition coefficients indicated that despite the simplicity of microcosm systems,the abiotic conditions varied considerably over the experiemntal period and influenced the two species to differing extents.The frequency and magnitude with which competition varied suggests that temporal varaition in density dependence is likely to be a pervasive and important feature of natural systems.

Appendix 2) Reliability and accuracy of parameter estimation

<u>*2A) Reliability of estimates: The deterministic assumption is valid for this microcosm system.*</u>

Stochastic environmental and demographic effects produce process variability, which influences the future trajectories of population dynamics. This is distinctly different from observation (measurement) error, which is not inherent to the system and therefore does not influence future dynamics (Turchin 1995; Hilborn& Mangel 1997). Both sources of variation can be large in ecological data sets, and improper attribution can result biased parameter estimates (Ellner *et al*. 1998; Bjørnstad et al. 2001, Dennis et al. 2006). As we employed a deterministic model to estimate biological quantities from our experimental data, we assume implicitly that the process variability is absent. This assumption made feasible the comparison of rival model structures and the estimation of parameter confidence intervals. Here we present two analyses that strongly verify our modelling approach for these controlled laboratory experiments. Firstly, we examined whether there was evidence of excessive variability in the abundances in samples, which would signify a large stochastic source. Secondly, we constructed a stochastic model and compared the estimate of the two model types.

i) Inspection of overdispersion in the samples of species abundance, which could be attributed to a stochastic source.

In our deterministic model, all variability in the data is attributed to the observation process. Resultantly, additional process variation would inflate the amount of sample variability above that expected by a random sampling process alone. By formalizing the observation process as a negative binomial, we could estimate the amount of excessive variation in the data. This was quantified by a stochastic parameter (γ). This captures overdispersion of variance in the likelihood function relative to a poisson (i.e. random samples in a well mixed environment).

Overdispersion was consistently small across treatments. It increased the variation in count (averaged across treatments) by 0.45 individuals above the mean over the entire duration, which is 46.92 individuals per 0.2ml sample. This lack of overdispersion indicates that, process variability was minimal and also that spatial clumping was unimportant.

ii) Comparison of deterministic and stochastic model estimates.

Computational advances and the development of statistical algorithms are making it increasingly possible to tailor statistical models to describe the biological scenario of interest

whilst concurrently accounting for both process and observational uncertainty (Clark & Bjornstad 2004; de Valpine 2002, de Valpine and Hastings 2002, Calder et al. 2003). We used one recently developed package, POMP (King et al 2009), which aids the construction of semi-mechanistic partially-observed Markov process models. This supports likelihood based parameter inference in nonlinear stochastic dynamic systems that contain both process and observation error (Ionides et al 2006; King et al. 2008).

Partially-observed Markov process (POMP) models comprise of the unobserved Markovian state process, X_t, and an observation process generating the observed data, Y_t. Given a time series of n observations, $Y_{1:n}$, taken at discrete times $t_{1:n}$, the model is fully specified by:

1) The process model defining the conditional transition density of the hidden state, $f(X_t|X_{t-1}, \theta)$, dependent on a vector of (unknown) parameters.

2) The observation model, defining the measurement process and its probability density function, expressed by the conditional distribution of the observation process: $f(Y_t|Y_{1:(t-1)}, X_{1:t}, \theta) = f(Y_t|X_t, \theta)$.

3) The initial densities of the system: $f(X_0|\theta)$.

The state of the system at $t_{1:N}$ then contribute to the likelihood of the data: $f(Y_{1:t}|\theta) = \prod_{t=1}^{T} f(Y_t|Y_{1:(t-1)}, \theta)$.

Utilizing this state space framework, two species stochastic competition models were formulated in continuous time, allowing for nonstationary parameters, non-Gaussian error distributions, and hidden and discrete state variables. This stochastic differential equation model, with a time step (δt) of 0.1, mirrored the deterministic Lotka-Volterra (L-V) competition model in the main paper (Equ1.). However the stochasticity parameters (σ_i where introduced, changing the stochastic process x_t at each δt by a normally distributed amount with expectation $\mu(x_t, t)$ and variance $\sigma(x_t, t)^2$. These parameters determined the amount of demographic noise and the extent of the diffusion process. The resulting stochastic model structure was:

$$dN_i = r_i * N_i * \left(1 - \alpha_{ii}(t) * N_i - \alpha_{ij}(t) * N_j\right) dt + \sigma x_t dW \qquad [1.a]$$

Measurements were assumed to follow a poisson process, $p(Yt) \sim Poisson(Xt)$, given that microcosms were well mixed prior to a random sampling of a small proportion of discrete individuals.

To verify if the deterministic model provides a reliable simplification, we used simple linear models, comparing the estimates of the two methods. We examined whether the deterministic estimates differ from those of the complex stochastic model.   When the parameter estimates of the two techniques are compared, we expect that the values to lie along the 1:1 line, if the estimates are equivalent. Thus we test whether the gradient of the linear model is significantly different from one; taking differences in the gradient and intercept of different parameters into account.
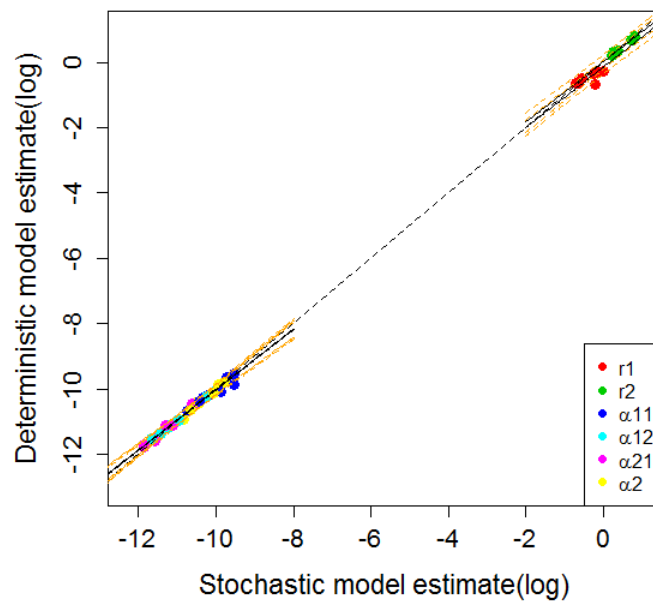
Figure 2.S2) Comparison of the deterministic and stochastic parameter estimates for all treatments. All parameters have a gradient that is estimated not significantly differ from one. Furthermore, although there are minor differences in the intercept of competition coefficients versus intrinsic growth rates, intercepts are not significantly different to one. The confidence intervals reveal that stochastic and determinstc models are likely to follow a 1:1 relationship; verifying that the methods give very similar results.

 The comparison of the stochastic and deterministic model estimates revealed a strong agreement of the two parameter estimation methodologies (Fig.2.S2 ; F=19740, df=39, p < 0.001). There is no significant evidence to reject the hypothesis that parameter estimates of these model types follow a 1:1 relationship (t=-1.84, p=0.08). Therefore, we conclude that our deterministic methodology provided estimates which are not significantly different to a more complex stochastic model. This result strongly justifies the use of our practical approach in further analysis of the data set.

The result that process variation appears not to be dynamically important in this system is likely a reflection of the controlled nature of our experimental system. This precludes many of the natural sources of natural variation, diminishing environmental stochasticity. Additionally,

the high abundances of individuals in our study system means that effect of demographic stochasticity are likely to be small (Lande *et al.* 2003). This suggests that in this system, where coexistence is stable and densities of both species remain high, issues of demographic stochasticity can be neglected. However, in systems with fluctuating, non-equilibrium dynamics or where species abundances fall to low levels, process variability is far more likely to produce phase shifts or stochastic extinction events. Process error cannot therefore be ignored in these cases (Ellner *et al*. 1998). For this reason we endorse the continued development of state space models to separate these sources of variation in the data.

2B) Accuracy of estimates

So that we could identify significant treatment effects and interactions between environmental drivers, it was vital that we could estimate or uncertainty of parameter values. To do this we constructed likelihood profile curves for all parameters. This technique requires estimation of how the likelihood of observing the dataset changes as the value of a focal parameter varies around its maximum likelihood point. In this process all other parameters are optimized at each value of the focal parameter. The functional form of the likelihood curve (the number of degrees of freedom) was determined by penalized regression splines and generalized cross validation (Wood 2001). Confidence intervals were then computed from this curve using the likelihood ratio test (Bolker 2005). The likelihood profiling methodology was necessary as not all parameters could be closely estimated using a quadratic approximation, due to a log transformation enforced in the parameter estimation process.
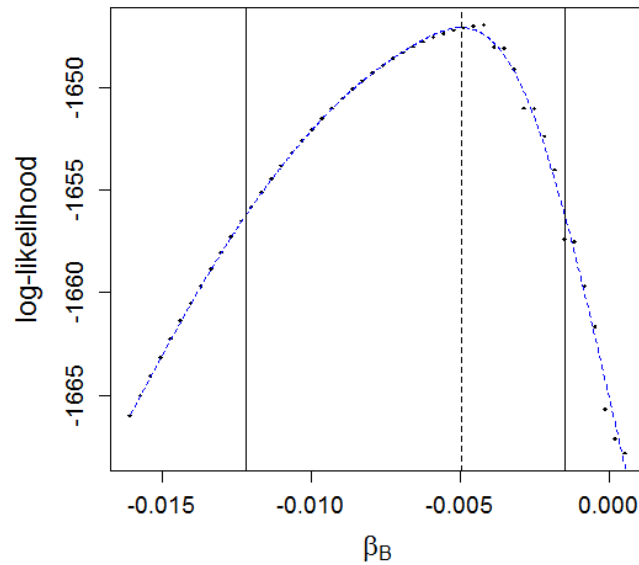
Figure 2.S3) Example likelihood profile curve for one parameter under a specific environment . This captures how the likelihood of observing the data changes as the value of parameter β_B is varied. This process allows the likelihood function to be optimized under each fixed value of the parameter by allowing other biological quantities tovary in the optimization process. General Additive Models used to obtain fitted curve and Likelihood ratio test used to obtain 95% confidence intervals (solid line) around the MLE (dashed line).

The smoothness of the likelihood profile curves and the small residuals of individual likelihood calculation points from their smoothing spline (Fig.2.S3), indicate that the likelihood function was consistently optimized. The smooth decline in the likelihood away from the maximum likelihood estimate and uni-modal shape of curves in the 95% confidence interval indicates that parameters were well identified and without evidence of multiple local maxima.

Appendix 3 Formalizing rival linear model hypotheses of the important causes of variation in invasion rate

In order to assess relatively how much the direct and indirect effects of environmental change impacted invasion rates (i.e. effects on the focal species physiology vs. effects of variation in the abundance and interaction strength of resident competitors), we formalized a suite of linear models. These models (Table 2.S2+2.S3), defining rival hypotheses of the important sources of variation in invasion rates, were fitted to the counterfactual data set. Our counterfactual decomposition method then allowed us to reveal the amount of variation in invasion rate accounted for by a specific level of model complexity, allowing identification of the major sources of variance from control invasion and an examination of the importance of higher order interactions.

We found that simple linear models, which did not account for the effects of on both competition coefficients (e.g Table.2.S2+ 2.S3: mod.1+2) and interactions between environmental drivers on these coefficients, captured a small proportion of the variance (e.g Table.2.S2+2.S3: mod.3). Models incorporating the effects of changes in the intrinsic growth rate did not greatly improve the amount of variance accounted for. In both species environmental effects on the intrinsic growth rate poorly explained the variation in invasion rates. Models neglecting environmental effects on intrinsic growth rates but instead accounting for variation due to interactions of environmental drivers upon competition coefficients had greater explanatory power (Table.2.S2.mod 1 vs. mod 7). This meant that much simpler models could account for most of the variance in invasion rate, without incorporating effects of environmental drivers on intrinsic growth rate (Table.2.S2: mod.6).

The complex models, with higher order interactions, did not improve the amount of variation explained, with the addition 134 more parameters being required to explain an additional 5.6% of the variance (Table.2.S2: mod6 vs. mod.14). This suggests that many interactions between drivers acted on different biological rates in our experimental system, but the effect size of many were small. Yet there were clearly several strong interactions that required inclusion in order to account for a great deal of the variation.

The selected decomposition model (highlighted in Table.2.S2+2.S3), which was the same for both species, is actually rather simple. It has only 22 out of a possible 512 parameters. Beyond this level of complexity we found that little extra explanatory power is gained by adding extra term. Thus higher order interactions appear relatively unimportant in this study. Notably the decomposition model does not include environmental impacts on intrinsic growth rates as a predictor, revealing that effects on this biological quantity are unimportant in modifying invasion rates. Variation in species invasion rates is largely dependent environmental effects upon the ratio between intra- and interspecific competition; as modified by interactions between pairs of environmental drivers. These interactions encompass the effect of: *a)* combined environmental changes modifying one coefficient; *b)* two environmental drivers effecting different coefficients; and *c)* a single driver mediating both the intra- and interspecific parameter pathways.

Table 2.S2) Comparison of proportion of variance in *Blepharisma's* invasion rate explained by rival linear models decomposing the counterfactual data set: as signified by the adjusted R^2 value. The predictors in the formula brackets define interacting main effects, with the maximum order of interactions limited to the integer superscript. The chosen model is highlighted in grey.

Model	Blepharisma model structure: (response~ control intercept + (predictors)$^{max\ order\ of\ interactions}$)	No. of coefficients	Adj.R^2 value
1	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}r_B + \Delta_{T\Uparrow}r_B + \Delta_{S\Uparrow}r_B)^3 + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP})^3$	15	0.297
2	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}r_B + \Delta_{T\Uparrow}r_B + \Delta_{S\Uparrow}r_B)^3 + (\Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^3$	15	0.552
3	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}r_B + \Delta_{T\Uparrow}r_B + \Delta_{S\Uparrow}r_B) + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP}) + (\Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})$	10	0.768
4	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}r_B + \Delta_{T\Uparrow}r_B + \Delta_{S\Uparrow}r_B)^2 + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP})^2 + (\Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^2$	19	0.868
5	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP})^2 + (\Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^2$	13	0.869
6	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}r_B + \Delta_{T\Uparrow}r_B + \Delta_{S\Uparrow}r_B)^3 + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP})^3 + (\Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^3$	22	0.875
7	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP})^3 + (\Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^3$	15	0.876
8	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP} + \Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^2$	**22**	0.942
9	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}r_B + \Delta_{T\Uparrow}r_B + \Delta_{S\Uparrow}r_B + \Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP} + \Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^2$	46	0.954
10	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP} + \Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^3$	42	0.965
11	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP} + \Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^4$	57	0.966
12	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP} + \Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^5$	63	0.966
13	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}r_B + \Delta_{T\Uparrow}r_B + \Delta_{S\Uparrow}r_B + \Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP} + \Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^3$	130	0.991
14	$\nu_{B^E} \sim \nu_{B^c} + (\Delta_{F\Downarrow}r_B + \Delta_{T\Uparrow}r_B + \Delta_{S\Uparrow}r_B + \Delta_{F\Downarrow}\alpha_{PP} + \Delta_{T\Uparrow}\alpha_{PP} + \Delta_{S\Uparrow}\alpha_{PP} + \Delta_{F\Downarrow}\alpha_{BP} + \Delta_{T\Uparrow}\alpha_{BP} + \Delta_{S\Uparrow}\alpha_{BP})^4$	256	0.998

We concentrate on interactions of type *a)*: examining the main effects on invasion rates of pairs of environmental changes and their interactive effects, acting via upon a single bioological quantity. Several interactions were found belonging to the other categories; however they do not reflect interactive effects by any single ecological process. Because the coefficients are multiplicative terms in the invasion rate calculation (Eq.2), an exact interpretation of these coefficients is difficult.

Table 2.S3) Comparison of proportion of variance in *Paramecium's* invasion rate explained by rival linear models decomposing the counterfactual data set: as signified by the adjusted R^2 value. The predictors in the formula brackets define interacting main effects, with the maximum order of interactions limited to the integer superscript. The chosen model is highlighted in grey.

Model	Paramecium model structure: $(response\sim control\ intercept + (predictors)^{max\ order\ of\ interactions})$	No. of coefficients	Adj.R^2 value
1	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow} r_P + \Delta_{T\Uparrow} r_P + \Delta_{S\Uparrow} r_P)^3 + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB})^3$	15	0.420
2	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow} r_P + \Delta_{T\Uparrow} r_P + \Delta_{S\Uparrow} r_P)^3 + (\Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^3$	15	0.447
3	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow} r_P + \Delta_{T\Uparrow} r_P + \Delta_{S\Uparrow} r_P) + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB}) + (\Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})$	10	0.837
4	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB})^2 + (\Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^2$	13	0.882
5	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow} r_P + \Delta_{T\Uparrow} r_P + \Delta_{S\Uparrow} r_P)^2 + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB})^2 + (\Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^2$	19	0.884
6	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB})^3 + (\Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^3$	15	0.889
7	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow} r_P + \Delta_{T\Uparrow} r_P + \Delta_{S\Uparrow} r_P)^3 + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB})^3 + (\Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^3$	22	0.891
8	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB} + \Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^2$	22	0.933
9	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB} + \Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^5$	63	0.945
10	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB} + \Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^4$	57	0.946
11	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB} + \Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^3$	42	0.946
12	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow} r_P + \Delta_{T\Uparrow} r_P + \Delta_{S\Uparrow} r_P + \Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB} + \Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^2$	46	0.976
13	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow} r_P + \Delta_{T\Uparrow} r_P + \Delta_{S\Uparrow} r_P + \Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB} + \Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^3$	130	0.998
14	$\nu_{P^E} \sim \nu_{P^C} + (\Delta_{F\Downarrow} r_P + \Delta_{T\Uparrow} r_P + \Delta_{S\Uparrow} r_P + \Delta_{F\Downarrow}\alpha_{BB} + \Delta_{T\Uparrow}\alpha_{BB} + \Delta_{S\Uparrow}\alpha_{BB} + \Delta_{F\Downarrow}\alpha_{PB} + \Delta_{T\Uparrow}\alpha_{PB} + \Delta_{S\Uparrow}\alpha_{PB})^4$	256	0.999

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Chapter 3: Quantifying the ecological costs and benefits of inducible defence.

Jason I. Griffiths, Philip H. Warren & Dylan Z. Childs

Abstract

Ecologists often examine the individual level impacts of producing defences to reduce predation. However, the population level costs and benefits of developing defence are rarely investigated. In this chapter, we developed a novel methodology for quantifying the impacts of predator exposure on the key ecological rates of prey population growth and defence against consumption. The method combines data collected in prey growth trials and predator feeding assays, to allow changes in species abundances driven by population growth to be clearly distinguished from changes driven by consumption by a predator.

Theory predicts under sufficiently strong predation, prey should increase their resource allocation to defence at the cost of growth. If however the predation pressure is removed, we would expect an inducible defence to be lost. To test this prediction, we used our novel methodology to examine the population growth and defence of microbial populations that were recently either: 1) naive to predation, 2) exposed to predation or 3) historically exposed to predation, but not recently.

Application of this analysis revealed clear evidence of such a trade-off between growth and defence. Prey exposed to predation developed a lower vulnerability to predation (reduced attack rate and increased handling time). However, this came with a cost of reduced resource allocation into population growth. When the predator was removed, the defence was lost and growth rates increased to the levels observed in naive populations. This temporal variation in species growth and consumption rates is expected if prey resource allocation is driven by a growth-defence trade-off. Such variation in vital rates is theoretically predicted to permit complex dynamical behaviours to occur.

Key words: Inducible defences; Growth-defence trade-offs; Functional responses; Predator-prey interactions; Protist microcosms; State space model

Introduction

A wide variety of species develop defensive traits, in response to increasing densities of their predators (Schmitz, Beckerman & Brien 1997; Tollrian & Harvell 1999; Agrawal 2001; Peacor & Werner 2001; Karban & Baldwin 2007; Bourdeau & Johansson 2012; Padilla & Savedo 2013; Shaffery & Relyea 2015). Defence may result from an evolutionary response in a variably defended population (Yoshida *et al.* 2004; Kasada *et al.* 2014) or from changes in individual resource allocation permitted through trait development plasticity (Agrawal 2001; Fordyce 2006). A key factor driving temporal variation in defence is that, under different predator abundances, different levels of defence gives the highest individual fitness and population rate of increase (Abrams *et al.* 1993). When predators are abundant and prey are relatively rare, it is advantageous for prey to invest resources into defence, rather than growth, to reduced vulnerability to mortality through predation. However, when predators are rare and conspecifics are abundant, the limited resources available would be better allocated to growth instead (Tollrian & Harvell 1999; Andersson & Hughes 2010). This trade-off prevents the predators defended state from always being beneficial, driving temporal variation in growth and consumption (Coley *et al.* 1985; Abrams *et al.* 1993).

Defence driven variation in ecological rates, allows populations to exhibit a rich array of dynamical behaviours that are not expected from purely ecological theories based on species abundances (Abrams *et al.* 1993; Tien & Ellner 2012; Kasada *et al.* 2014; Koch *et al.* 2014). For example, a well-known consequence of defence trait dependent predation is the modification of predator-prey cycles from classical quarter-period lagged oscillations to anti-phase cycles (Abrams & Matsuda 1997; Cortez 2011; Mougi 2012; Cortez & Weitz 2014). Such signatures of fluctuating defence have recently been identified in many previously published predator-prey experimental systems (Hiltunen *et al.* 2014), although the mechanisms generating the dynamics were not usually explored.

To understand how predation induced defences influence population level processes, we need to determine how defence impacts on: A) the growth rate of the prey population, B) the rate at which predators search for and attack prey (the attack rate) and C) the time taken by predators to process a captured prey item (the handling time). Contrastingly, many studies of predation driven defence focus on individual level comparisons of the traits known to convey defence against predation in predated and un-predated populations, but do not provide quantitative estimates of population level ecological rates (Lass & Spaak 2003; Van Donk *et al.*

2011). Conversely, when population level rates of consumption are estimated, concurrent prey population growth, during the study period, is not usually accounted for.

Here we develop a novel methodology to quantify the impacts of predator exposure on a prey's population growth and its vulnerability to consumption by predators. We use short term experiments to observe prey population growth and density dependent consumption of prey by the predator. We then construct a novel functional response analysis that combines these two sources of data, to disentangle changes in prey population abundances caused by consumption by a predator from the balancing increases in abundance cause by prey reproduction during the exposure to predation. Accounting for this prey population growth is particularly important when analysing data on species with short generation times compared to the duration of study (Juliano & Williams 1987). Otherwise, estimates of predation rates may be systematically biased. Using the highly flexible dynamic state space framework, we were able to account for differential sampling effort, which depended on population density. We also accounted for the chance demographic variation inherent in the change in population abundances.

We apply this methodology to replicate microbial prey populations that have been previously either exposed or naive to predation for many generations. We estimate and compare rates of growth and consumption in these two groups. This allowed us to evaluate the presence of a theoretically predicted trade-off between growth and defence which is expected to drive temporal variation in species growth and consumption rates and permit complex dynamical behaviours to occur.

Methods

EMPIRICAL MICROCOSM SYSTEM

STUDY ORGANISMS: CULTURING & ESTABLISHING THE PREDATOR-PREY SYSTEM

We studied a predator-prey system, consisting of a bacteria resource, *Serratia marcescens*, a bactiverous ciliate prey *Colpidium striatum* (Stokes 1886), and a predator, *Stentor coeruleus* (Ehrenburg 1830). Laboratory microcosm cultures consisted of petri dishes containing 50ml Chalkley's solution (Thompson *et al*. 1988), $0.7gL^{-1}$ of crushed protozoan pellets (Carolina Biological Supply, USA) and 3 wheat seeds. All equipment was autoclaved

before use. Culture medium was first inoculated with bacteria, allowing the establishment of a resource base. Two days later, 100 individuals of the *Colpidium* prey were added to each microcosm. These were maintained at 20^0C with a 16: 8 light-dark photoperiod. The positions of microcosms within controlled temperature environments was randomized and frequently permuted. Cultures were replenished three times a week by renewing 1ml of medium and replacing any evaporative loss with distilled water.

TREATMENTS

Microcosms were initially divided into two treatment groups. Microcosms assigned to the first treatment (Naive to predation: 10 replicates) were not exposed to predation by *Stentor*. These microcosms were cultured in isolation from the predator for a 175 day period. The microcosms assigned to the second treatment (Predator exposed: 23 replicates) were invaded with 25 individuals of the predator *Stentor* on day 12 and also received a second equal invasion 2 days later, reducing the impacts of demographic stochasticity on the initial population trajectories and preventing chance predator extinctions. The replicates in the "naive to predation" treatment received additions of an equivalent volume of *Stentor* culture medium, but sieved to remove predators.

In 10 replicates of the predator exposed treatment, the predator went extinct prior to day 175. As replicate were monitored every two days during the exposure period, the extinctions were known to have occurred during a window of time in the middle/second half of the experiment, after day 90 and before day 140. These populations were classified into a third treatment, of being previously exposed to predation (n=10). They provided information about the response of the population once the predation pressure was removed. The remaining replicates were exposed to predation for duration of exposure period (n=13). Time series analyses of these predator-prey dynamics showed evidence of a transition from classical ¼ lagged predator-prey cycles to non-classical antiphase cycles indicative of systems experiencing inducible prey defence (Appendix.1; Hiltunen *et al.* 2014).

GROWTH RATE & PREDATION ASSAYS

After the exposure period (day 175), prey-containing medium was extracted from each replicate population, ensuring that any predators were removed. Extracted medium was divided into 8 subsamples and diluted with protist-free medium (bacterially-inoculated), using dilution factors of: 1, 1.2, 1.5, 2, 3, 6, 12, or 30. Each dilution subsample was used to initiate a pair of subpopulations. This produced eight pairs of prey populations along a gradient of prey densities. Each population was 3ml in volume and was located in a separate compartment of a 5ml well plate. One of each pair, along the gradient of prey densities, was invaded with 8 predators. The other served as a predation free control treatment, allowing changes in abundance due to predation to be un-confounded from effects of prey reproduction.

SAMPLING

Replicate exposure microcosms were each agitated and then sampled three times a week for the duration of the exposure period. To count predator density, 5ml of medium was transferred (with replacement) to a sterile petri dish and scanned under a stereoscopic microscope (surveyed at 7.5–30 x magnification). Prey density was estimated by visually scanning a 0.1ml subsample. This sampling effort was increased when *Colpidium* was rare and the proportion of habitat recorded. For all populations in the growth and predation assays, prey abundance was sampled immediately after initiation and then after 4 and 24 hours later. This produced observations of the changes in prey abundances under a range of initial population densities in the presence and absence of predation and enabled growth and consumption rates to be estimated.

INFERRING THE GROWTH-DEFENCE TRADE-OFF: A DYNAMIC FUNCTIONAL RESPONSE ANALYSIS

Changes in prey abundances, observed during growth and predator feeding trials, were used to estimate prey growth and predator consumption rates. By combining both sources of data, population increases, caused by prey population growth in the predation trials, was accounted for when assessing the reduction of prey abundance caused by predator consumption. Comparison of the rates of growth and predation between prey that were exposed and naive

to predation allowed evaluation of the presence of inducible prey defence and the theoretically predicted growth defence trade-off .

Model formalization and parameterization

The change in abundance of prey (N) during the growth and predation trials, was described using a Rosenzweig-MacArthur stochastic differential equation model, where the abundance of predators is fixed (predation trials P=8; growth trials P=0) (Rosenzweig & MacArthur 1963):

$$dN = \left(rN \left(1 - \frac{N}{K} \right) - \frac{aPN}{1 + ahN} \right) dt + \sigma x_t dW \qquad \text{1a)}$$

Here the prey's intrinsic growth rate is r and population growth is prey density dependent, towards a carrying capacity (K). Predation depends on prey density, following a satiating type II functional response (Hollings 1959). The attack rate (a) denotes the rate that a predator searches for prey when it is not currently consuming a prey. The predators handling time (h) is the average proportion of time a predator uses to consume a prey. At high prey densities predators become limited by their handling time h and so the maximum prey consumption rate is $1/h$. Stochastic dynamics were simulated using a Euler approximation. A stochasticity parameter, introduces normally distributed demographic noise (dW) into the changes in prey abundance at each Euler time step (δt =0.025 hrs) with expectation $\mu(N_t, t)$ and noise intensity $\sigma(N_t, t)^2$.

The parameters of the dynamic model were inferred by constructing a partially-observed Markov process (POMP) model within the POMP package (Appendix.2) (King, Nguyen & Ionides 2015). POMP models comprise of the unobserved state transition process and an experimental observation process generating the data. Thus, they explicitly account for the separate sources of process and sampling variation. Measurements were assumed to follow a Poisson process, given that microcosms were well mixed prior to a random sampling of a proportion of individuals. The Sequential Monte Carlo (SMC) algorithm (Liu and West 2001) was utilized to perform parameter estimation, with uninformative uniformly distributed priors covering a broad range of biologically reasonable values. This allowed estimation of population growth and predation rates for each of the replicate populations.

Identifying differences between treatments

Multiple regression was used to model the differences in population growth, handling time and attack rate between replicate populations that were a) naive to predation, b) exposed to predation or c) previously exposed to predation, but the predator previously went extinct. A model was constructed formalizing the hypothesis that all three predation treatments (treats: naive, exposed, previously exposed) influence the response variables (vars: attack rate, handling time and population growth rate): $Y_{n \times vars} = X_{n \times treats} \ B_{treats \times vars} + \varepsilon_{n \times treats}$. Here the sample size (n=33) is the total number of microcosms. This full model was then compared with the nested models in which certain treatment effects were removed (i.e. some microcosms with differing histories of predation had the same ecological rates). The appropriate model structure was identified using likelihood ratio testing. Treatments that caused significantly different responses (vars) could then be identified. For treatments group that differed significantly, the probable distribution of the three response variables was characterized by performing discriminant analysis using Gaussian finite mixture modelling.

Results

PARAMETER ESTIMATION

The dynamical state space functional response analysis allowed changes in prey abundances driven by population growth to be clearly distinguished from changes driven by consumption by the predator (Fig.3.1). This is evident from the weak correlation in the values of the posterior samples between model parameters. Our approach also provided precise estimates of the rate of growth and consumption. For example, in the analysis of the first replicate in the naive to predation treatment group, the high probability density interval for the growth rate parameter indicates that the generation time of *Colpidium* could be identified to be between 12.7 and 14 hours. With similar precision, the attack rate of a predator that is not satiated by prey, could be identified to be between 16.7 and 19% of the prey per day.
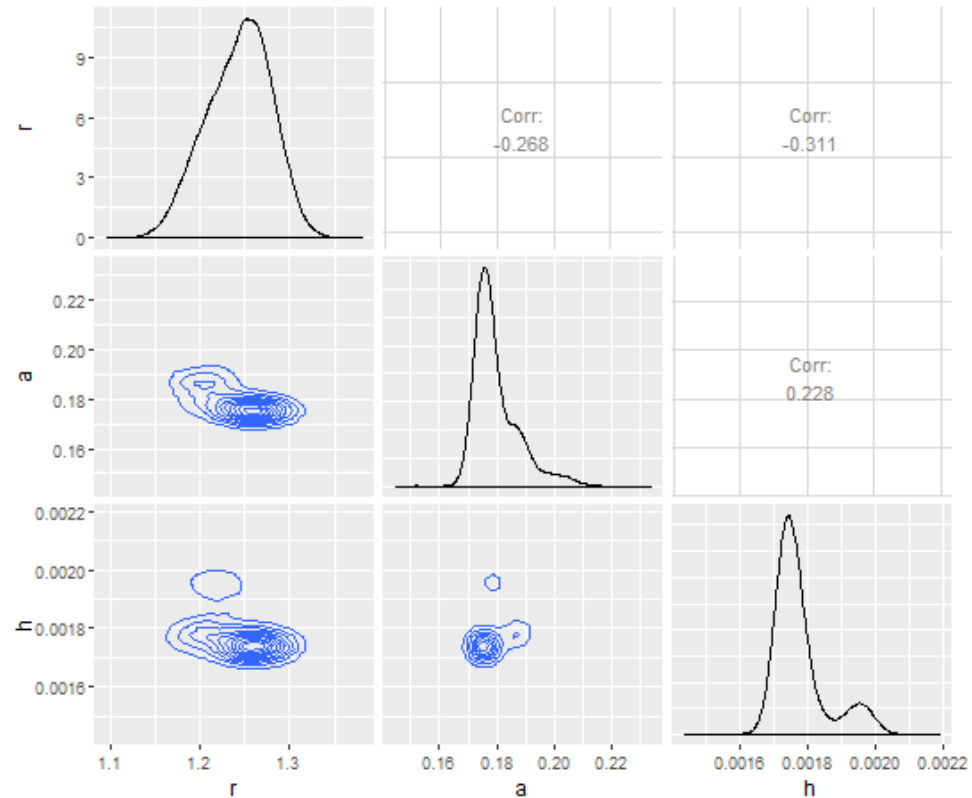
Figure 3.1) Model inferences of the dynamical state-space functional response analysis. Results obtained when the analysis is applied to the growth rate and predation assay data set for the first replicate in the naive to predation treatment group. Bayesian posterior distributions of the growth rate (r) of *Colpidium*, and the attack rate (a) and handling time (h) of its predator *Stentor*. The marginal probability density of each parameter estimate are shown on the diagonal panels. The lower off-diagonal panels use contour plots to display the bivariate probability density of pairs of parameters to show the weakness of the correlation between estimates (correlation values are shown in the upper off-diagonals).

INFERRING THE GROWTH-DEFENCE TRADE-OFF

We evaluated whether extended exposure to predation caused changes in prey growth and consumption by predators. These rates were compared between populations exposed to predation and those naive to predation. Populations that had been exposed to predation exhibited significantly different ecological rates compared to populations that were naive to predation (Fig.3.2). Vulnerability to predation was reduced, through a reduction in attack rate (*t=4.0, d.f.=30, p<0.001*) and increase in per capita handling time (*t=2.8, d.f.=30, p<0.001*). As theoretically predicted, this defence was associated with a reduction in population growth rate (*t=-4.7, d.f.=30, p<0.001*). Populations that experienced predation in the past but had not been exposed recently, due to predator extinction, no longer showed this defence. They had attack rates and handling times that were not significantly different from naive prey (attack

rate: *t=-1.6, d.f.=30, p=0.27;* handling time: *t=0.28, d.f.=30, p=0.78*). When defence was lost, the growth rate returned to the higher level found in the naive populations; causing no significant difference in growth between these groups (*t=-0.4, d.f.=30, p=0.96*).
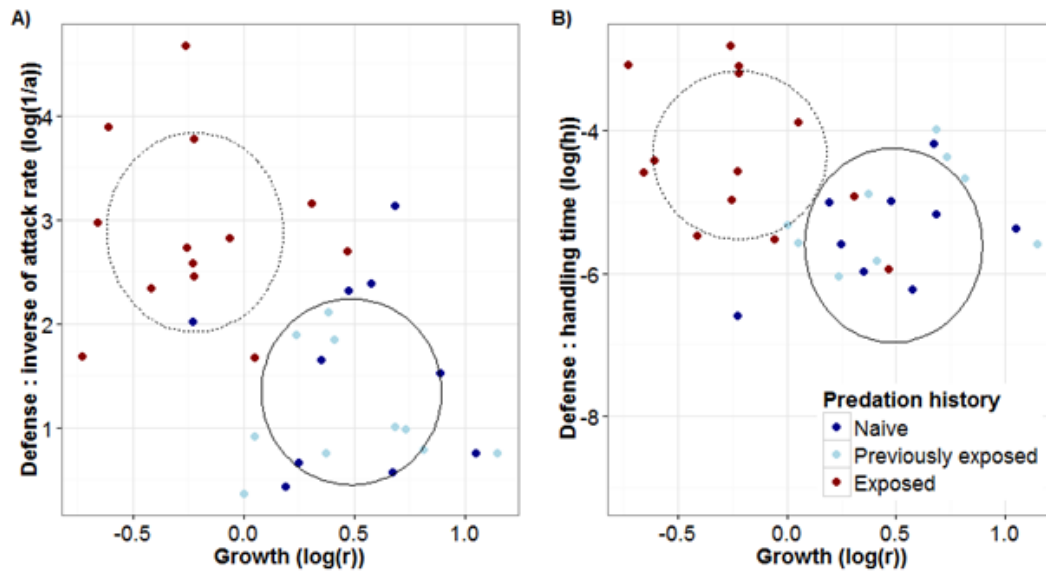
Figure 3.2) The growth defence trade-off identified by examining the growth and predation rates of populations with differing histories of predation. Growth rate was related to the level of prey defence in population naive to predation, exposed to predation or previously exposed to predation, but with a recent history in isolation. Defence was quantified based on: A) the estimated inverse of the attack rate, which measures the time required for a successful prey capture and B) the estimated handling time required for a predator to process a captured prey individual. The difference of the ecological rates in the exposed populations was identified using multivariate regression. The ellipses show the regions in the growth-defence space in which the exposed populations occur (dotted line) and the region where the naïve and previously exposed groups are found (solid line). The regions were identified using discriminant analysis.

Discussion

We developed a novel method for quantifying the impacts of exposure to predation on the key ecological rates of prey population growth and predator consumption. This method allowed data from prey growth trials and predator feeding trials to be combined. By incorporating greater biological detail into functional response analyses, our method allowed changes in species abundances caused by population growth to be distinguished from decreases caused by predation. The technique was used to analyse prey growth and predation feeding trial data from experiments conducted using replicate microbial prey populations. Populations came from three treatment groups which beforehand were either: 1) naive to predation, 2) exposed

to predation or c) previously exposed to predation. By comparing the rates of growth and predation between populations in these groups, we revealed a trade-off between growth and defence. Prey exposed to predation displayed a reduced vulnerability to predation (lower attack rate and higher handling time), at the cost of reduced resource allocation to population growth. This costly defence was lost when the predator was absent once more, with growth and defence returning back to the levels found in naive populations.

Evaluation of the inferred functional response curves, reveals that over the observed range of prey densities, changes in both attack rate and handling time contributed to the difference in predation between defended and undefended populations (Fig.3.3). Similar changes in defence have been identified between populations that are naive and exposed to predation, in other protist predator-prey systems (Altwegg *et al.* 2006; Hammill *et al.* 2010). In agreement with our results, both studies identify changes in the attack rate and handling time. This indicates that multiple defence mechanisms may be operating, as modified attack rates are driven by changes in the catchability of prey whereas modified handling time suggests altered digestibility. There is evidence in the literature of predation driven changes in morphology, behaviours and chemical composition in similar protist species (Kusch 1993; Hammill *et al.* 2010; Roberts *et al.* 2011). Generally, further research is needed to elucidate these mechanisms. Previous studies investigating inducible protist defence have not assessed the concurrent changes in prey growth rates and therefore do not allow evaluation of trade-offs between growth and defence.
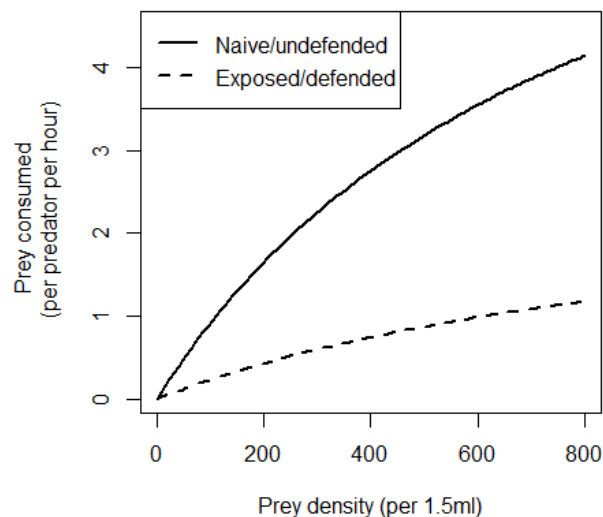
Figure 3.3) Evaluation of the estimated density dependent rate of prey consumption by the predator when prey are previously naive or exposed to predation. Functional responses curves predicted using the average point estimate of attack rate and handling time for each treatment. Prey density (x-axis) spans the range of densities observed.

Combining experimental assays and statistical analyses to understand the ecological costs and benefits of defence is a key tool for identifying whether dynamics are likely to be influenced by changes in defence. The use of our innovative dynamic state space functional response analysis has several advantages over a conventional functional response analysis. These benefits largely result from the improved biological realism that is incorporated into the state space approach and the fact that the models explicitly describe changes in abundance through time, rather than simply fitting nonlinear curves to observed changes in abundances. An important benefit, resulting from the dynamic modelling, is that estimates are not biased by the depletion of available prey to very low levels. Using the typical static functional response approach of fitting a type of parametric functional response curve to changes in prey abundance does not allow for prey depletion (Vonesh & Bolker 2005). This makes the method only suitable for modelling predation studies where prey are replenished or are too numerous to overexploit (Bolker 2005). Using dynamic models instead is known to be a suitable approach to account for resource depletion, because the approach towards prey extinction can be modelled directly. For example, the Rogers random-predator equation is a dynamic equivalent to fitting a type 2 functional response but accounts for prey depletion (Rogers 1972; Juliano & Williams 1987).

We extend the dynamics modelling approach in several ways. Firstly, by modelling the growth of the prey concurrently with its consumption by predators we were able to combine information from replicate trials and multiple sources of information. This approach was permitted by using the state space framework along with Bayesian estimation procedures. This meant that we were able to use stochastic dynamic models to account for chance demographic changes in population abundance, caused by random variation in births and deaths. Closed form solutions to the transition likelihood function of such models are not usually known, causing it to be very difficult to parameterize them without using estimation of the likelihood through simulation (Clark & Bjørnstad 2004; King 2012). Furthermore, the state space approach separately models the biological process and the sampling procedure, allowing variable and complex sampling efforts to be easily accounted for. As a result, rare individuals could be searched for more thoroughly for and this effort incorporated into the model. Non-Gaussian error distributions and discrete individual abundances are also accounted for (Newman *et al.* 2006).

Due to the flexibility of the state space approach, extra biological realism can be included in the analysis, by modifying the part of the model simulating the biological process or the

sampling procedure. Given that the system can be simulated, the likelihood of a parameter set can be estimated. Therefore, the approach is generalizable to systems with predator interference, multiple predator, or a few competing prey species.

The methodology developed in this study allowed us to identify predator induced defences in prey populations and the presence of a theoretically predicted trade-off between growth and defence. This trade-off is expected to drive temporal variation in species growth and consumption rates and permit complex dynamical behaviours to occur (Abrams *et al.* 1993; Tien & Ellner 2012; Kasada *et al.* 2014; Koch *et al.* 2014). Investigation of the trait dependence of species interactions would allow greater understanding of the growth defence trade-off and the long-term population dynamics of this system. Our findings indicate that, ecological interpretations population dynamics may be challenging, without considering the impacts of defensive traits on the ecological rates of population growth and consumption. Future work should concurrently study the dynamics of species traits as well as abundance in order to permit an understanding of the feedbacks between these components and their linked dynamics.

Acknowledgements

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Appendices

Appendix.1) Time series analysis of predator-prey abundances reveals evidence of trait dependent predation and community dynamics

"Ecological predictability" refers to the idea that changes in species' abundances can be predicted from knowledge of densities of other species. This is the basis of much classical ecological theory and underpins many contemporary efforts to develop predictive theories of ecology. However, recent theoretical and empirical work have emphasized that both rapid evolution and trait change driven by phenotypic plasticity will reduce ecological predictability because variation in species' abundance and traits determine ecological dynamics (Thompson 1998; Agrawal 2001; Yoshida *et al.* 2003; Yoshida *et al.* 2007a; Fussmann *et al.* 2003; Cortez 2011). It is now widely accepted that trait change, driven by evolution or plasticity, is common and often occurs over similar time-scales to ecological changes in species abundance (Hairston *et al*. 2005; Pelletier *et al*. 2007; Berg & Ellers 2010; Schoener 2011; Ellner *et al.* 2011; Travis *et al*. 2014).

Trait changes influencing behaviour and morphology alter individual performance and cause key ecological rates such as consumption, growth, birth, and death to vary over time (Bolker *et al*. 2003; Preisser *et al*. 2005; Pelletier *et al*. 2007; Harmon *et al*. 2009; Bassar *et al*. 2010; terHorst *et al.* 2010; Turcotte *et al.* 2011). When the strength or direction of trait change is in turn modified by the resulting change in ecological conditions, a feedback loop between trait dependent demography and density dependent trait evolution is produced (Yoshida *et al.* 2003; Yoshida *et al.* 2007; Pelletier *et al.* 2007; Becks *et al.* 2010, 2012; Schoener 2011;

Agrawal *et al*. 2013). The feedback between ecological and trait dynamics permits a wide range of community dynamics that would not be expected from purely ecological theories, based on species abundances (Abrams & Matsuda 1997; Jones & Ellner 2007; Palkovacs *et al*. 2009; Pelletier *et al.* 2009; Jones *et al.* 2009; Berg & Ellers 2010; Kishida *et al.* 2010; Ellner & Becks 2010; Mougi 2012a; b; Cortez & Weitz 2014; Hiltunen *et al.* 2014a).

A well-known consequence of trait dependent predation, driven specifically by evolution, is the modification of predator-prey cycles from classical ¼-period lagged oscillations to anti-phase cycles (Abrams & Matsuda 1997; Cortez 2011; Mougi 2012a; Cortez & Weitz 2014; Hiltunen *et al*. 2014b). Dynamic behaviours such as this, where trajectories can differ over time, despite having equal states of abundance, are not possible in unstructured ecological models (but see de Roos & Persson 2003 for an alternate mechanism based on predator maturation delays). Such "eco-evolutionary" signatures have recently been identified in many previously published experimental systems (Hiltunen *et al*. 2014b), although the mechanisms generating them were not usually explored at the time (Ellner & Becks 2010; Hiltunen *et al.* 2014a).

In such anti- phase cycles, the maximum predator abundance coincides with minimum prey abundance. This shift from ¼-period lagged to antiphase cycles can be identified when plotting predator vs. prey abundance over the course of the time series. In this phase plane view, the expected eco-evolutionary signature is a transition from an elliptical phase plane trajectory (produced by standard predator–prey models without prey evolution) to a pattern approaching a line with negative slope (indicating that prey abundance is inversely related to predator number). By analysing changes in the elliptical-ness of this phase plane over the experiment, statistical method can be used to probe for a transition from classic to eco-evolutionary cycles. Here we apply this statistical time series analysis, developed by Hiltunen *et al*. (2014a), to probe predator-prey datasets for evidence of a transition from classical ¼-period lag consumer-resource dynamics to anti-phase "eco-evolutionary" dynamics. The presence of such a signature would suggest that population trajectories are not governed by purely density dependent processes that are constant over time.

Materials and methods

EMPIRICAL MICROCOSM SYSTEM

STUDY ORGANISMS, CULTURING & ESTABLISHING THE PREDATOR-PREY SYSTEM

We examined the population dynamics of an experimental predator-prey system containing two protist species; the predator *Stentor coeruleus* (Ehrenburg 1830) and its prey *Colpidium striatum* (Stokes 1886). Experimental microcosms consisted of aluminium foil capped glass dishes containing 50ml Chalkley's solution (Thompson *et al*. 1988) and $0.7gL^{-1}$ of crushed protozoan pellets (Carolina Biological Supply, USA). All microcosms, and media, were autoclaved before use and kept in controlled temperature chambers at 20^0C. Two days prior to the initiation of the experiment, the medium was inoculated with a known bacterial species (*Serratia marcescens*). This allowed the establishment of a resource base for *Colpidium*, the bactiverous prey. On day zero, five replicates were initiated with 100 *Colpidium*, which were all obtained from 2 week old stock cultures, standardizing any influences of individual quality and thus growth rates (Price & Morin 2004; Olito & Fukami 2009). The microcosms were first invaded with 25 individuals of the predator *Stentor* on day 12. They also received a second equal invasion 2 days later, reducing the impacts of demographic stochasticity on the initial population trajectories and preventing chance predator extinctions.

SAMPLING

For all replicates, we sampled species densities daily during the prey growth phase, until the predator invasion (day 12). After this, sampling was done every third day for the duration of the experiment. In order to obtain reliable estimates of species densities, sampling effort was modified between species and over time, reflecting changing densities (approx. 3-5ml for predators and 0.1ml for prey). Prior to sampling, the medium was agitated. Medium was then transferred (with replacement) to a sterile petri dish and protists were counted under a stereoscopic microscope (surveyed at 7.5 - 30 x magnification). All microcosms were sampled 62 times over 175 days.

ANALYSIS - INFERENCE TECHNIQUES

QUANTIFYING PHASE-SHIFTS AWAY FROM CLASSICAL P-P CYCLES

Predator-prey replicates were analysed to detect signatures of eco-evolutionary dynamics. A transition from classical ¼-period lagged predator–prey cycles to out of phase eco-evolutionary dynamics over the course of our experimental time series can be identified in phase-plane plots of predator versus prey abundance. In this phase-plane view, the expected eco-

evolutionary signature is a transition from an elliptical phase-plane trajectory (produced by standard predator–prey models without prey evolution) to a pattern approaching a line with a negative slope (indicating that prey abundance is inversely related to predator number).

Following the methodology of Hiltunen *et al.* (2014a), changes in the predator-prey phase lag over the time series can be quantified by defining an Evolutionary Dynamics Index (EDI) which evaluates the elliptical-ness of the phase trajectory over successive windows of the data. The EDI is calculated by measuring the orbits inverse eccentricity (the ratio between the orthogonal short and long axes of an ellipse) over moving windows of the data. The inverse eccentricity is a measure of the circularity of an ellipse, with bounds of 0 and 1 signifying parabolic and circular orbits respectively. Reductions in inverse eccentricity measures over successive windows of the time series generates a negative EDI, indicating a transition to a more linear phase-plane and therefore anti-phase dynamics.

When calculating the EDI, phase-plane trajectories are first estimated by applying a spline smoother to log-transformed abundance data for each population (smoothing parameter chosen by ordinary cross-validation). Smoothed phase-plane trajectories were then constructed by evaluating the fitted spline at 100 equally spaced time points and were rescaled to have standard deviation 1.The inverse eccentricity of smoothed trajectories were then analysed for moving windows, each containing 50 interpolated points (more than one complete predator prey oscillation), using a principal components analysis. The change in inverse eccentricity across windows was characterized by fitting a suite of nonlinear regression models (Linear, exponential, hyperbolic and translated hyperbolic) and using model comparison based upon AIC scores to select a best fitting model. Finally, the EDI values is calculated by multiplying the estimated initial slope of the fitted nonlinear model by the difference in the first and last inverse eccentricity estimates.

The statistical significance of phase transitions from clasical to antiphase cycles (negative EDI) were estimated using a residual bootstrap approach. To implement this, bias-corrected residuals were resampled and added to the fitted values. The EDI calculation procedure (described above) was then applied to the resulting bootstrap data set. For each replicate, this was repeated 5000 times. Due to the clear nonlinearity of trends, significance was additionally tested based on non-parametric correlation (Kendall's tau) between window number and inverse eccentricity. For each method, the EDI of the observed data set was then judged to be significant at the 5% level if 95% or more of the bootstrap EDI values or resampled tau values

were less than zero. The qualitative results of the tests were similar although the parametric approach consistently gave more conservative probability estimates.

Results & Discussion

QUANTIFYING PHASE-SHIFTS AWAY FROM CLASSICAL P-P CYCLES

Analysis of predator-prey time series revealed a consistent transition from classic predator–prey cycles to anti-phase cycles, characteristic of eco-evolutionary dynamics (Fig.3.S1). A transition to anti-phase dynamics can be seen as a transition from an elliptical phase plane trajectory (produced by standard predator–prey cycles without prey evolution) to a pattern approaching a line with negative slope. The transition from an elliptical phase trajectory to a linear phase trajectory (indicative of eco-evolutionary dynamics) was quantified using the eccentricity analysis (see materials and methods & Fig.3.S1).
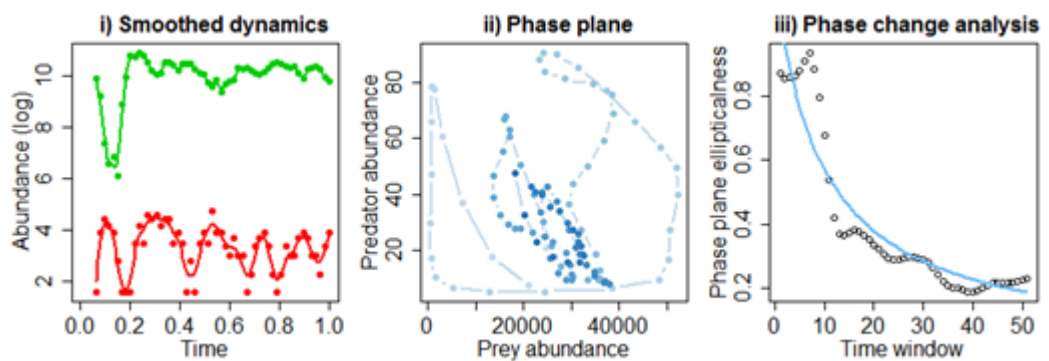
Figure 3.S1) Transition from classic ¼ lagged predator–prey cycles to anti-phase cycles characteristic of eco-evolutionary dynamics. Subpanels show the main steps in quantifying the transition to anti-phase cycles: (i) Raw experimental predator-prey population abundances were smoothed using a piecewise polynomial model. Here we show a representative replicate showing abundances of predators (red points) and prey (green points). Smoothed predator and prey dynamics are displayed using coloured lines. Time is rescaled so that the time range of the experiment is between zero and one. (ii) At equidistant times the abundance of predators is plotted against the number of prey (darkening colour indicates movement forwards in time). In this phase plane, a pattern of points approximating a line with negative slope shows antiphase dynamics. (iii) The phase of the dynamics is measured for windows of the time series. The lower the elliptical-ness of the trajectories the more anti-phase the dynamics. This, is measured based on the ratio of the trajectories eigenvalues (individual points). The change in the elliptical-ness (phase of dynamics) over time is captured using an exponential decay model (solid line). The rate of this decline is the evolutionary dynamic index (EDI). A statistically significant negative slope indicates a transition to antiphase dynamics.

Over successive windows of time, all five predator-prey time series showed a significant transition in the phase of predator and prey oscillations (Supplement.1). All replicates had a negative evolutionary dynamic index (EDI) indicating a transition from limit cycles towards anti-phase dynamics (Table.3.S1; combing replicates, p<0.001). This pattern is thought to be diagnostic of the appearance of prey traits that confer reduced vulnerability to predation (Abrams & Matsuda 1997; Becks *et al.* 2010).

Our findings suggest that the population dynamics of this predator-prey systems dynamics are not purely driven by density dependent processes. The nature of the observed changes in phase are consistent with the predictions of theoretical models in which prey evolve costly defences in response to high predator abundance (Abrams & Matsuda 1997; Mougi 2012a). One mechanism generating antiphase consumer–resource cycling is rapid contemporary evolution of either prey defence or competitive ability as predator densities oscillate (Abrams & Matsuda 1997; Yoshida *et al.* 2003; Jones & Ellner 2007; Becks *et al.* 2012). Under this eco-evolutionary model, there is selection for prey defence, when predators are numerous. The resulting increase in the frequency of defended individuals then causes a decrease in predator abundance, even though defended prey are abundant. However, the costliness of defence produces a competitive advantage for undefended genotypes when prey are numerous and predators are rare. This then drives selection for less defended prey, allowing predators to increase once more.

Table 3.S1) Quantification of the transition to anti-phase dynamics from an inverse eccentricity analysis (Hiltunen *et al.*2014a). For each replicate, the changes in predator-prey phase lag over time was defined as the initial rate of change in inverse eccentricity (trajectories eigenvalue ratio) over time and gave an Evolutionary Dynamics Index (EDI; see Hiltunen *et al.*2014a), based on the slope of a non-linear regression model. A significant negative EDI (slope) indicates a transition towards anti-phase dynamics. Significance of EDI was assessed parametrically using a residual bootstrap significance test and non-parametrically using a Kendall's tau correlation test.

Replicate	Evolutionary Dynamics Index	Residual Bootstrap significance test	Kendall's tau significance test
1	-604	0.0001	**0.0001**
2	-381	0.0734	**0.0322**
3	-273	0.0058	**0.0014**
4	-361	0.0438	**0.0232**
5	-218	0.0145	**0.0212**

Our results contribute to the increasing evidence suggesting that temporal changes in traits of individuals in the population drive changes in demographic rate over time. Contemporary work indicates that the trait dependencies of demographic rates has important impacts on population dynamics. The "ecological predictability" of many population dynamics may be greatly improved by combining observations of abundance with trait and genetic information when making inferences. It has long been realized that individuals within a population differ. They can have multiple life history stages and sexes (Coulson *et al.* 2001; Cameron & Benton 2004; de Roos *et al.* 2008), exhibit inducible phenotypic variation to environmental conditions (Tollrian & Harvell 1999) and evolve in response to selection pressures (Grant & Grant 2002). Future work should be focused on understanding the role of trait change in population and community dynamics, by unifying ecological and evolutionary theories.

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Supplement 1: Phase transition analysis results for each replicate

Summary plots of the eccentricity analysis applied to each replicate individually are shown below (Fig.3.S2). There is consistency across replicates. Between replicates, smoothed predator-prey datasets look qualitatively similar and phase plane dynamics show similar patterns. The form and rate of change in phase is also consistent between replicates. In all cases, the phase shifts rapidly towards antiphase dynamics and the rate of change then slows as populations remain in this out of phase state.
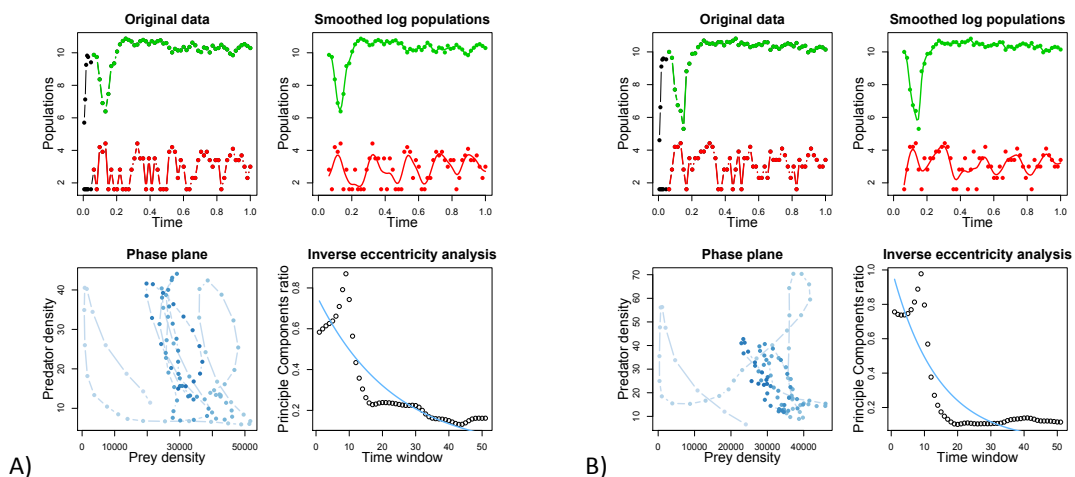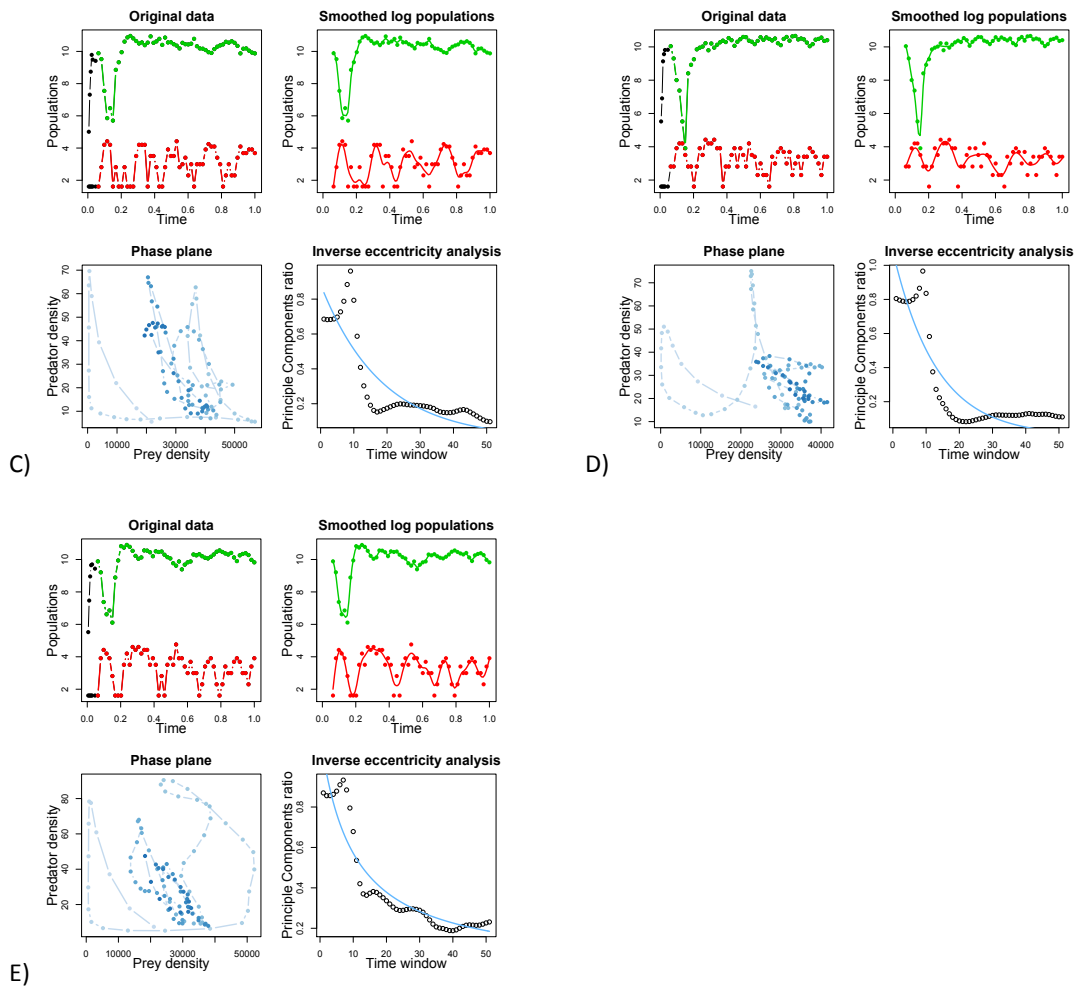
Figure 3.S2) Identifying a transition from classic predator–prey cycles to anti-phase cycles characteristic of eco-evolutionary dynamics in the five predator-prey replicates. For each of the five replicate predator-prey populations, an inverse eccentricity analysis was performed (see Hiltunen et al. 2014). The four main steps of the leading to the inverse eccentricity analysis are summarised for each replicate: a) Original time series data of predator and prey density are log transformed and plotted against standardized time (black part omitted from analysis), b) Time series are smoothed using penalized splines, c) Smoothed predator-prey densities are plotted in phase plane with darkening of colour indicating movement forwards in time. A transition to out of phase dynamics can be seen as a transition from an elliptical phase plane trajectory (produced by standard predator–prey cycles without prey evolution) to a pattern approaching a line with negative slope. d) This change in phase plane trajectory is quantified as the decrease in inverse eccentricity (the ratio between the orthogonal long and short axes of an ellipse). This is measured over a moving window of the smoothed data as the eigenvalue ratios (circles) of a principal components analysis. The solid blue curves show the fitted nonlinear regression of eigenvalue ratio over the windows of data. Linear, exponential, hyperbolic and translated hyperbolic model were fitted and model comparison was made based on AIC scores.

Appendix.2) Constructing the stochastic dynamic functional response model

Utilizing the state space framework, continuous time Rosenzweig-MacArthur stochastic predator-prey models were formulated and parameterized. During the simulation of the stochastic differential equations, the Euler approximation was used. At each Euler time step (dt), the population rate of change was calculated and used to forecast the expected change in abundance by the next time step. Process variation was then added to the one step ahead prediction of abundance, by allowing the simulated abundance at t+dt to be sampled from a normal distribution with a mean equal to the expected value and a standard deviation determined by a demographic stochasticity parameter.

Given a time series of observations, taken at discrete times, the state space model is fully specified by:

1) X_0: The initial abundances in the system:

2) $f(X(t)|X(t-1);\theta)$: The process model, simulating stochastic realizations of the hidden predator-prey interaction, dependent on a vector of unknown parameters (θ).This defines the conditional transition density: $f\left(Y_t|Y_{1:(t-1)},X_{1:t},\theta\right)=f(Y_t|X_t,\theta)$.

3) $f(Y(t)|X(t);\theta)$:The measurement model, defining the observation process and its probability density function ($P(Y(t)|X(t);\theta)$). Measurements were assumed to follow a Poisson process, given that microcosms were well mixed prior to a random sampling of a known proportion of habitat.

The state of the system at t$_{1:N}$ then contribute to the likelihood of the data: $f(Y_{1:t}|\theta)=\prod_{t=1}^{T}f\left(Y_t|Y_{1:(t-1)},\theta\right)$. Within this state space framework, we accounted for, non-Gaussian error distributions, discrete abundances, variation in sampling effort and the confounding sources of process and observation variation (Clark & Bjornstad 2004, de Valpine & Hastings 2002, Newman *et al.* 2008). The Bayesian sequential Monte Carlo BSMC algorithm of Liu & West (2001) was utilized to perform parameter estimation, using uninformative priors covering a broad range of biologically reasonable values.

Chapter 4: Linking trait-abundance dynamics to quantify feedbacks driving trait dependent species interactions

Jason I. Griffiths, Owen Petchey, Frank Pennekamp, Philip H. Warren & Dylan Z. Childs

Abstract

Traits change can cause significant temporal variation in key ecological rates. In turn, the modification of ecological interactions can simultaneously feedback to alter the strength and direction of trait change. The resulting trait dependent vital rates and ecologically dependent trait change permits a wide range of community dynamics that would not be expected from ecological theories purely based on species abundances. It has been recommended that trait dynamics should be directly studied in parallel with the dynamics of community abundances, however so far these two dynamic components remain largely disconnected.

Here we investigated the role of trait mediated demography in determining community dynamics and also examined how ecological interactions influence trait change. Using an experimental microbial predator-prey-resource system, we concurrently monitored the dynamics of community abundances and various traits at the individual level. We formulated an empirically derived trait dependent community model, to identify key ecologically relevant traits and to link their dynamics with those of species abundances. By modelling the trait dependence of population growth and species interactions, we obtained an understanding of the growth-defence trade-offs that underpin the temporal variation in ecological rates.

Our results provide clear evidence of a feedback between trait change and species dynamics. This suggests that an understanding of trait or community dynamics will require unification of theories of trait dependent ecological interactions and ecologically dependent trait change.

Key words: Community dynamics; Trait dependent interaction; Density dependent trait change; Trait-abundance feedbacks; Growth-defence trade-offs; Predator-prey experiment; Generalized additive models

Introduction

Trait variation within species is increasingly recognized as having impacts on the population dynamics of natural communities (Berg & Ellers 2010; Schoener 2011). It can be driven by evolutionary selection pressures, favouring certain heritable traits in a genetically variable population (Thompson 1998; Yoshida *et al.* 2004; Kasada *et al.* 2014) or alternatively, trait variation can be caused by phenotypic plasticity, when a single genotype produces different phenotypes under differing environments (Tollrian & Harvell 1999; Agrawal 2001; Fordyce 2006; Cortez 2011). For example the timing of life history events or the allocation of resource to growth and defence may depend on the density of predators and resources or the environmental conditions (Finlay 1977; Lampert 1994; Travis *et al.* 2014; Riessen 2015). Trait change can mediate significant temporal variation in vital ecological rates such as resource consumption, growth, birth, and death (Bolker *et al.* 2003; Preisser, Bolnick & Bernard 2005; Pelletier *et al.* 2007; Harmon *et al.* 2009; Bassar *et al.* 2010; terHorst, Miller & Levitan 2010; Turcotte, Reznick & Hare 2011). When the modification of ecological interactions alters the strength or direction of trait change, a feedback loop is produced between ecological abundance and evolutionary/plasticity-driven trait dynamics (Yoshida *et al*. 2003; Yoshida *et al.* 2007; Pelletier *et al.* 2007; Becks *et al.* 2010, 2012; Schoener 2011; Agrawal *et al.* 2013).

Trait dependent vital rates permit a broad array of community dynamics that are not expected from purely ecological theories based on species abundances (Abrams & Matsuda 1997; Jones & Ellner 2007; Palkovacs *et al.* 2009; Pelletier, Garant & Hendry 2009; Jones *et al.* 2009; Berg & Ellers 2010; Kishida *et al.* 2010a; Ellner & Becks 2010; Mougi 2012a; b; Cortez & Weitz 2014; Hiltunen *et al.* 2014a). For example, in intra-guild predation systems, where a predator consumes a prey and simultaneously competes for the resource of the prey, purely ecological theory predicts that peaks of resource abundance should be followed by a peak in the prey and then in the predator ("turn-taking") (Holt & Polis 1997; Hipfner *et al.* 2013). The inclusion of trait mediated variation of interaction strengths makes possible a variety of novel dynamical behaviours, by allowing the community to have differing trajectories, at different times, despite having equal states of abundance (Ellner & Becks 2010; Hiltunen *et al.* 2014a). Such crossing of trajectories is not possible in unstructured ecological models (but see de Roos & Persson 2003 for an alternate mechanism based on predator maturation delays). The dynamics can become increasingly complex as the number of species, interactions and trait dependencies increases (Jones *et al.* 2009; Ellner & Becks 2010; Strauss 2014; Hiltunen *et al.* 2014a).

For trait change to substantially influence community dynamics, it must be sufficiently rapid to influence demographic processes on an ecological timescale (Thompson 1998; Hairston *et al.* 2005; Ellner *et al.* 2011). Observations of rapid trait change, in response to predation or other selection processes, are increasingly being reported (Kuhlmann & Heckmann 1985; Kusch 1993; Relyea & Auld 2004; Losos *et al*. 2004; Boulanger *et al.* 2013). When rapid trait change substantially impacts ecological rates, purely ecological theories will not allow a full understanding of a community's dynamics and may give unreliable predictions of future abundances (Shertzer *et al.* 2002; Schreiber *et al.* 2011; Ellner & Becks 2011; Strauss 2014). It has been proposed that trait dynamics should be directly studied in parallel with the dynamics of species abundances (Hiltunen *et al.* 2014b). The difficulty of using empirical datasets to statistically model the linked dynamics of trait dependent communities and ecological dependent trait change has resulted in these two dynamic components remaining largely disconnected (Kishida *et al.* 2010b), despite the body of theoretical work suggesting the importance and interrelatedness of these processes (Abrams & Matsuda 1997; Ellner & Becks 2010; Cortez & Weitz 2014).

Here we investigate the role of trait mediated demography in determining community dynamics and also examine how ecological interactions in turn influence trait change. We examine whether the feedback between these processes can be understood by modelling the trait dependence of population growth and species interaction and the ecological dependence of trait change. Using an experimental microbial predator-prey system, we concurrently monitored the dynamics of community abundances and a range of traits. We identified key ecologically relevant traits and linked their dynamics with those of species abundances, by formulating an empirically-derived trait-dependent community model. Finally, the divergence of individual traits between predator exposed and un-exposed populations was evaluated and we examined the selection pressure, acting on these traits during a brief period of predation.

Methods

EMPIRICAL MICROCOSM SYSTEM

STUDY ORGANISMS: CULTURING & ESTABLISHING THE PREDATOR-PREY SYSTEM

We studied the community dynamics of a tri-trophic predator-prey-resource system, consisting of a bacteria resource, *Serratia marcescens*, an intermediate bactiverous ciliate *Colpidium striatum* (Stokes 1886; referred to as the prey), and a top predator, *Stentor coeruleus* (Ehrenburg 1830). *Stentor* populations consume bacteria during filter feeding, but do not persist on a purely bacterial diet, generating weak intra-guild predation (Slabodnick & Marshall 2014). The microcosm experiments consisted of petri dishes containing 50ml Chalkley's solution (Thompson *et al.* 1988), $0.7gL^{-1}$ of crushed protozoan pellets (Carolina Biological Supply, USA) and 3 wheat seeds. All microcosms and media were autoclaved before use. Two days prior to the initiation of the experiments, the medium was inoculated with bacteria and kept at 37^0C, allowing the establishment of a resource base. Protist microcosms were subsequently maintained at 20^0C with a 16: 8 light-dark photoperiod. The positions of microcosms within controlled temperature environments was randomized and frequently permuted. Cultures were replenished three times a week by renewing 1ml of medium and replacing any evaporative loss with distilled water.

TREATMENTS

Microcosms were either assigned to a predator-prey-resource treatment (exposed to predation: replication =10) or a prey-resource treatment (naive to predation: replication=4). Higher replication was used to study the treatment including predation to provide more information to infer vital rates in this more complex system. On day zero, replicate microcosms were initiated with 100 *Colpidium*. During the first 12 days, all treatments contained just the prey and resource, and were treated identically. On day 12, the microcosms exposed to predation were each invaded with 25 individuals of *Stentor*. They also received a second equal invasion 2 days later, reducing the impacts of demographic stochasticity on the initial population trajectories and preventing chance predator extinctions. The replicates in which prey were naive to predation received additions of equivalent volumes of *Stentor* culture medium, but sieved to remove predators. All populations persisted for the 82-day duration of the study.

SAMPLING

Replicates were agitated and then sampled three times a week for the duration of the experiment. To count predator density, 5ml of medium was transferred (with replacement) to a sterile petri dish and scanned under a stereomicroscope (Leica M205 C: surveyed at 7.8x magnification). Prey density was measured by transferring 0.044ml of medium into a Sedgewick Rafter cell (S52, SPI supplies, Westchester, PA), and taking a 5 second video (25 fps) using the stereomicroscope with a 259 magnification mounted digital CMOS camera (Hamamatsu Orca C11440, Hamamatsu Photonics, Japan). The automated digital video processing R package, bemovi (Pennekamp *et al.* 2015) was used to remove static background debris, located and measure individual prey and link their trajectories. This allowed measurement of a range of individual level behavioural and morphological traits including: body size and shape, movement speed, net displacement rate, step lengths per video frame and turning angles. Bacteria density was estimated using flow cytometry, based on a 20µl sample of medium. Analysis of the flow cytometry measurements of scatter and absorbance, associated with each observation, allows the groups with similar qualities to be distinguished. As a characteristic background noise pattern was identified across samples, a Gaussian mixture model (GMM) was constructed to filter noise observations from the signal of bacterial observations (Fraley *et al.* 2006; Appendix.1.A). To identify different components in the bacterial resource, the signal observations were grouped into clusters of observations with differing characteristics. Model-based clustering was undertaken to achieve this and to determine the number of bacterial classes in the data (Appendix.1.B). During this process a set of GMM's were fitted, each hypothesizing a different number of clusters in the data. Model comparison was then applied, based on Bayesian Information Criterion (BIC) scores, to identify the most parsimonious model. Observations were then classified into bacterial categories. Two main distinct bacterial classes were identified and the proportion in each class calculated for every sample.

PREDATION & GROWTH TRIALS

At the end of the experiment, prey-containing medium was extracted from each replicate population, ensuring that any predators were removed. Extracted medium was divided into 8 subsamples and diluted with protist-free medium (bacterially-inoculated), using dilution factors of: 1, 1.2, 1.5, 2, 3, 6, 12, or 30. Each dilution subsample was used to initiate a pair of

subpopulations. This produced eight pairs of prey populations along a gradient of prey densities. Each population was 3ml in volume and was located in a separate compartment of a 5ml well plate. One of each pair, along the gradient of prey densities, was invaded with 8 predators. The other served as a predation free control treatment, allowing changes in size due to predation to be separated from effects of prey population density. For all populations, prey populations were sampled immediately after initiation and then after 4 and 24 hours later. This produced observations of the changes in prey's individual trait distributions following the long-term exposure to predation and then over the course of a short feeding and growth trials.

MODEL INFERENCE

PARAMETERIZATION OF AN EMPIRICALLY DERIVED TRAIT DEPENDENT PREDATION MODEL

Deriving a community model of traits and abundance

By combining the theoretical frameworks for modelling trait dependent species interactions (Holt & Polis 1997) and fitness dependent trait selection (Abrams *et al.* 1993), we formulated a community model to describe the linked dynamics of species traits and abundances. From this, theoretical continuous time dynamic model, we derived non-parametric regression model structures. These describe changes in community abundances and trait values between observations as smooth functions of species densities and mean trait values (Appendix.2). This allowed trait dependent community interactions and ecological impacts on trait dynamics to be described flexibly, without strong constraints on the functional forms of the model equations. The community dynamics were discretized into the following system of difference equations:

$$E(\log\left(\frac{R^{t+1}}{R^t}\right)) = g_{RR}(R, Z_i) - f_{RN}(R, Z_i)\,\frac{N}{R} - f_{RP}(R)\frac{P}{R} \qquad \text{1a)}$$

$$E(\log\left(\frac{N^{t+1}}{N^t}\right)) = \alpha_{RN}f_{RN}(R, Z_i) - f_{NP}(N, Z_i)\,\frac{P}{N} - d_N \qquad \text{1b)}$$

$$E(\log\left(\frac{P^{t+1}}{P^t}\right)) = \alpha_{NP}f_{NP}(N, Z_i) + \alpha_{RP}f_{RP}(R, P) - d_P \qquad \text{1c)}$$

$$E(\log\left(\frac{Z^{t+1}}{Z^t}\right)) = s_Z(R, N, P, Z_i) \qquad \text{1d)}$$

Here we model the expected (E) log difference in species abundances and trait values. The densities of the resource, prey and predator are, respectively, R, N, and P. Ecologically relevant traits influencing dynamics are denoted by Z_i. The term $g_{RR}(R, Z_i)$ is a smooth function describing the intra-specific density dependence of growth in the resource. The quantities f_{ab} (X, Y) are smooth functional response terms describing the dependence of the consumption rate, of species *a* by species *b,* on the state variables X and Y at the prior time step. For example, $f_{RP}(R, P, Z_i)$ describes the trait and density dependent intra-guild predation interaction between the resource and predator. The per capita mortality term of species *a* (d_a) and the conversion efficiency of biomass between species *i* and *j* (α_{ij}) are constants to be estimated. Finally, $s_Z(R, N, P, Z_i)$ is the density dependent rate of trait change and is proportional to the ecological selection pressure acting on the trait.

Model parameterization

Prior to model fitting, individual trait measurements were square root transformed to reduce skew in the data. Mean trait measurements were then calculated for each replicate at each sample point. Population abundance and trait dynamics were interpolated, using cubic hermite splines, to obtain data with equidistant time intervals and then standardized to have a standard deviation of 1. The finite rate of change of each species population abundance (X_s) was calculated, based on non-standardized measurements and log transformed to provide a measure of the observed linearized per capita rate of population growth: $\log\left(\frac{X_s(t+1)}{X_s(t)}\right)$. Similarly, the first log difference of dynamics of each trait (Z_i) was also calculated: $\log\left(\frac{Z_i(t+1)}{Z_i(t)}\right)$.

We used the "mgcv" package in R to construct generalized additive models (GAM) describing the linked community dynamics (equ.1) (Wood 2006). To account for heavy tailed response variables, we used a scaled-t distribution model. To avoid over-fitting, the model degrees of freedom in the gcv criterion was inflated by a factor of 1.2, following recommendations of Gu (2013) and Hiltunen *et al.* (2014a). Numerical optimization, using a box constrained variable metric algorithm (Limited-memory BFGS quasi-Newton method), was applied to identify the remaining constants (d_a and α_{ij}).

Formulation and comparison of candidate hypotheses

Competing hypotheses about the trait dependence of demography and community dynamics were formalized, by constructing a set of regression models. In these candidate models population growth and consumption rates depended upon different behavioural and morphological traits as well as species abundances. Similarly, hypotheses about the impacts of species abundances on trait dynamics were also formalized. Here regression models were constructed in which the rate of trait change depended upon species. There was a high degree of collinearity between the measurements of several traits. Those showing strong correlation, such as body length and width, were reduced to a single variable. The resulting candidate predictor variables describing prey traits were: body size (Z_{size}; area μm^2), swimming speed (Z_{speed}; $\mu m \, sec^{-1}$), displacement rate (Z_{disp}; describing the linear distance of habitat explored; $\mu m \, sec^{-1}$) and turning movement variability (Z_{turn}; describing the variability of movement direction; $radians \, turned \, sec^{-1}$). Finally, the composition of the resource population was also used as a putative predictor of changes in species abundances (Z_{comp}), by using the proportion of the total resource that was categorized into the initially rare bacterial class as an additional candidate model covariate. We constructed models including (or excluding) each prey trait individually, and including (or excluding) the resource composition information.

To test whether the inclusion of trait information improved our ability to explain community dynamics, the trait-dependent community models were compared with the null model based on abundance only. Cross validation was used to compare the predictive ability of candidate models. All but one replicate was used to parameterize the model and the remaining replicate was used to estimate the model's predictive performance. Predicted population changes between each observation were then compared against the observed data and the root mean square error (RMSE) was calculated to quantify model prediction error. This process was repeated, sequentially leaving out each replicate and measuring RMSE.

EXAMINING TRAIT SELECTION THROUGH PREDATION

The shift of individual-level trait values in response to exposure to predation was quantified following the long-term study of community and trait dynamics. Trait measurements were taken from individuals in replicate populations during the subsequent short-term growth and predation trials. We examined how the log-normally transformed distribution of individual

trait values (z_i) varied between populations depending upon: a) the initial density of the populations in feeding trials (x_{init_den}), b) the amount of time that the trials had been running (x_{time}), c) the history of predation (x_{pred_hist}: i.e. the presence/absence of predators in the microcosm from which the initial individuals in the trials where obtained) and d) the presence/absence of predators during the short term trials (x_{pred_curr}). A linear mixed model was formulated to incorporate random effects (γ) of replicate and incubator. Interactions between the four fixed effects (β) were proposed leading to the following full model:

$$\log(z_i) = x_{init\_den}\ \beta_{init\_density} \times x_{time}\ \beta_{time} \times x_{pred\_hist}\ \beta_{pred\_hist}$$
$$\times\ x_{pred\_curr}\ \beta_{pred\_curr} + u_{replicate}\gamma_{replicate} + u_{incubator}\gamma_{incubator}$$

A model reduction approach of model comparison was then applied using likelihood ratio tests to compare nested models and to obtain a parsimonious description of the factors influencing individual trait distributions.

Results

EVALUATION OF THE TRAIT DEPENDENT COMMUNITY MODEL

We assessed the predictive ability of community models that included different information about prey traits and the resource composition. The inclusion of information about the dynamics of prey body size and the resource composition, produced the largest reduction in the prediction error of the empirically derived community model (Fig.4.1). The inclusion of these factors individually was not sufficient to greatly reduce prediction error, but together they gave a 16.3% reduction in prediction error. The inclusion of other prey traits in combination with the resource composition information did not improve the predictive performance of the empirically derived models.

Inclusion of the body size and resource composition information into the model improved the agreement between predicted and observed changes in the abundance of the resource and prey, but did not greatly improve the prediction of predator change in abundance (Fig.4.2; time series of observed species and trait dynamics are presented in Appendix.3).
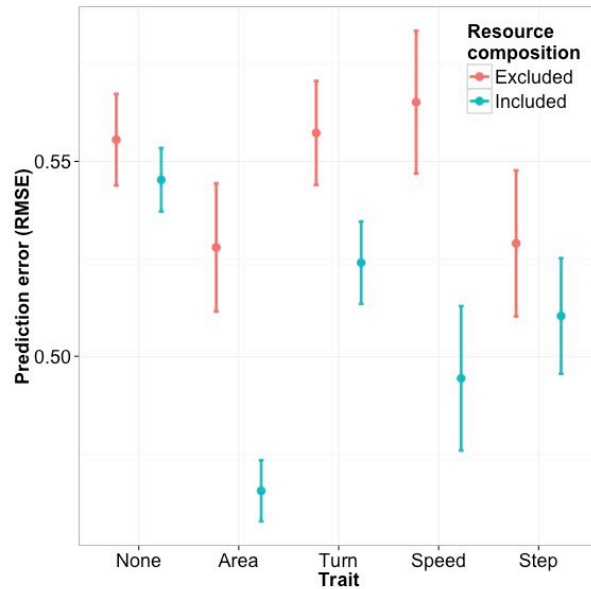
Figure 4.1) Predictability of changes in species abundances in population models containing different combinations of information about prey traits (x-axis) and the resource composition (bar colour). The predictability is quantified by measuring the prediction error between the observed rates of change of species abundances and model predictions, based on the RMSE of predictions during cross validation (lower RMSE is better). Error bars show the confidence interval of the prediction error observed between rounds of the cross validation, the midpoint being the mean prediction error.
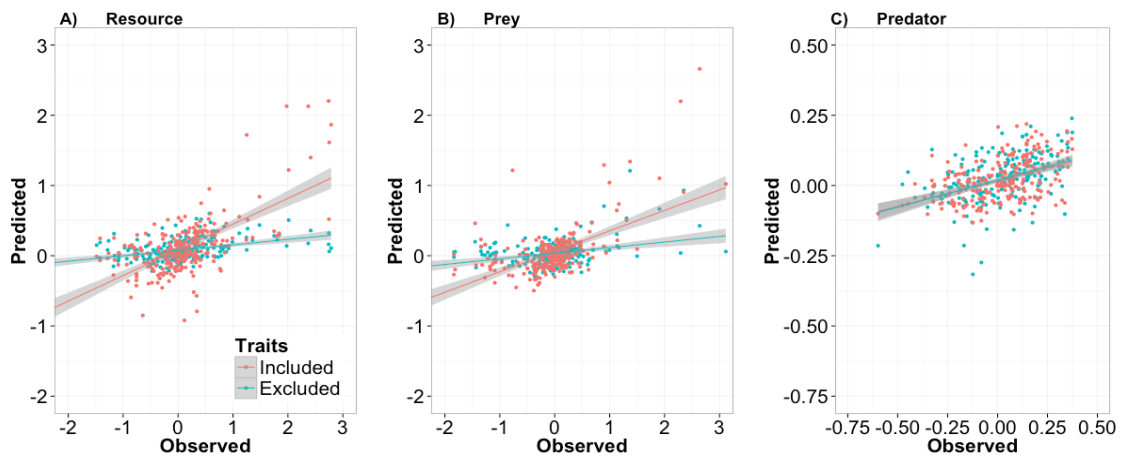
Figure 4.2) Comparison of the correspondence between predicted and observed changes in species abundances in models with and without trait information. Predicted and observed changes in A) resource, B) prey and C) predator abundance are shown for models with the trait information of prey size and resource composition either included (red) or excluded (blue). Linear regression is used to summarize the relationship and shaded regions show the standard errors of the regression.

Evaluation of the empirically derived trait dependent community model revealed density dependent processes and species interactions that were consistent with theoretical expectations (Fig.4.3). The resources growth rate showed negative density dependence, as would be expected when a finite nutrient supply limits population growth (Fig.4.3A). The functional form of consumer interaction between the prey and the predator (Fig.4.3C) is similar to a type one functional response when prey are small, and similar to a type two functional response when prey are large (Holling 1965). Consumption increases with victim density, but then saturates as the density gets higher. The consumer interaction between the resource and the prey also showed a pattern of increased consumption with increased victim density (Fig.4.3B), with a relationship similar to a type one functional response, i.e., consumption a linearly increasing function of resource density.

Inclusion of resource composition information into the community model revealed that the resource growth rate depended upon its composition. Resource growth decreased as the composition of the resource became increasingly comprised of the initially rare resource class (Fig.4.3A; line transparency related to resource composition). The consumption rate of resources by the prey was also influenced by the resource composition (Fig.4.3B). The preys' resource consumption was reduced as the composition of the resource became dominated by the originally rare bacterial class (line transparency). This effect was dependent on the prey size (line colour), which had the largest influence on the consumption rate. As prey size decreased over the course of the experiment, the rate of resource consumption declined. When prey were of a reduced size (red vs blue lines), the influence of resource composition was lessened, probably because consumption rates were already rather low.

The consumption rate of prey by the predator was also dependent on the size of prey, with larger prey being more rapidly consumed (Fig.4.3C). Over time, prey individuals became smaller and were both consumed by predators less rapidly and also ingested resources less quickly. The size of the prey changed considerably over the course of the experiment (Appendix.3). Figure 4.3D indicates that the reduction in size was related to higher density of predators (line colour) and prey themselves (x-axis). The negative effect of prey abundance may reflect a delayed feedback from resource availability, as current resource abundance did not help explain body size change. The negative effect of increased predator abundance on body size indicates that predation pressure is either directly selecting for smaller individuals or indirectly causing altered resource allocation to growth through plasticity.
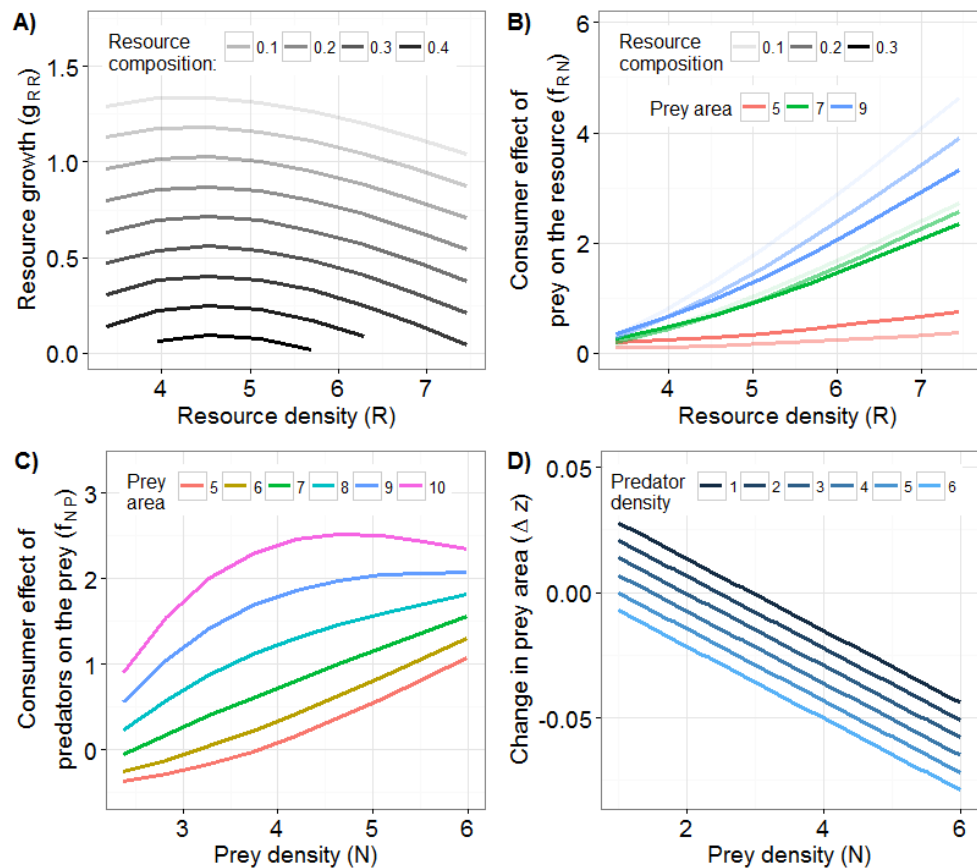
Figure 4.3) Evaluation of the trait and density dependence of community processes and trait change, identified in the empirically derived community model. The model showed: A) density dependent resource growth, which varied with the resource composition (fraction of resource composed of initially rare resource class; line transparency), B) Consumption of resources by prey, which varied with prey size (line wavelength colour) and resource composition, C) consumption of prey by predators, which varied with prey size, and D) change in prey size trait, which was dependent on predator and prey density (brightness of blue).

TRAIT SELECTION THROUGH PREDATION

The shift of individual trait distributions in response to exposure to predation was quantified at the end of the experiment. During the long-term community dynamic experiment there was a significant divergence in body size between individuals from populations that were previously exposed or naive to predation (n=535, $d.f.$=6, $\chi^2 = 387.08$, p<0.001). The size of prey exposed to predation, declined substantially compared to populations that were naive to predation but were otherwise kept in identical environmental conditions (Fig.4.4; transparent blue versus transparent red distributions).

During the repeated observation in the predation feeding trials, the mean body size in populations exposed to predation approached the level of naive populations (Fig.4.4 red

distributions of decreasing transparency, *n*=535, *d.f.*=8, $\chi^2 = 105.39$, *p*<0.0001). This transition back towards the state of naive individuals is likely because the predator density in the feeding trials was relatively low compared to that experienced in the microcosm experiment. An increased allocation to growth rather than defence may have been favoured, causing the increase in prey body size and a reduction in the trait divergence. The previously naive populations, which were exposed to a small number of predators, showed a stable body size distribution (Fig.4.4 blue distributions). This further indicates that the predation pressure was not strong enough to elicit a reduction in the size of prey over the short period of exposure.
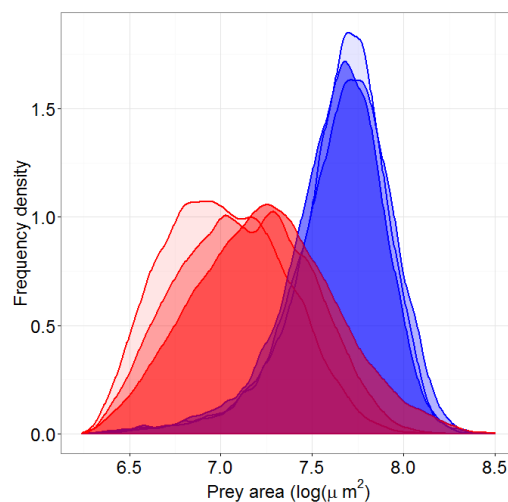
Figure 4.4) Shifting individual body size distribution of prey following a microcosm experiment in which some populations were exposed to predation (red) and others left naive (blue). During feeding trials populations of prey previously exposed and naive to predation were measured on three occasions (transparency levels). First, the individuals in feeding and growth trials were measured immediately after the microcosm experiment (most transparent), second after 4 hours (medium transparency) and finally 24 hours later (least transparent).

Discussion

In this study, we use a novel approach to connect abundance dynamics with the dynamics of individual traits. We identified traits that were modified by the ecological conditions and simultaneously influenced demographic rates and community dynamics. This allowed us to quantify the trait dependence of species growth and consumption rates. It also allowed us to understand how changes in species density feeds back to drive temporal trait change. Our results revealed a rich network of relationships among traits and resource, prey, and predator abundances, and demonstrated how inclusion of trait-abundance feedbacks increase the ability to predict ecological dynamics (Fig.4.5).
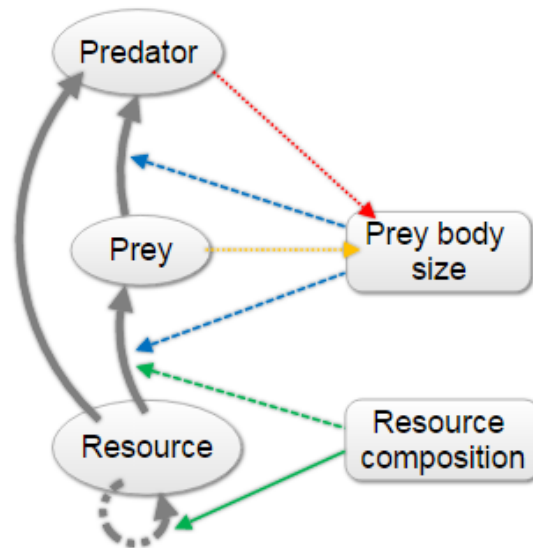
Figure 4.5) A conceptual diagram illustrating key relationships identified in the predator-prey-resource microbial system. Ecological interactions between species are depicted by thick grey arrows (dot dashed thick grey line signifies resource density dependent growth). Trait change (blue) and resource composition (green) influenced species consumptive interactions (dashed lines) and density dependent population growth (solid line). Predator and prey density influenced trait change (red and yellow dotted lines), creating a feedback between the dynamics of species traits and abundance.

BODY SIZE DEPENDENT PREY INTERACTIONS & THE FEEDBACK WITH TRAIT CHANGE

Body size has an important role in mediating the strength of predator-prey interactions in many ecological systems (Brooks & Dodson 1965; Zaret & Kerfoot 1975; Yoshida *et al.* 2004; Tucker *et al.* 2015). The substantial two-third reduction of prey body size observed over the course of our experiment was found to be coupled with two important community feedback processes: A) the top down effect of predation and B) the bottom up effect of resource consumption. We identified that temporal changes in prey body size modified its vulnerability to predation by *Stentor* and its resource consumption. As body size declined, partially driven by reduced resource availability, the vulnerability of prey (*Colpidium*) to predation decreased, but they also consumed resources at a lower rate. These ecological changes may result from changes in either the life history or resource allocation of the prey. We then linked the temporal changes in body size back to the abundance of predators and conspecifics. This implies a feedback between the ecological impacts of the trait change and the future selection pressure on the body size trait in the prey.

 The reduced vulnerability to predation at small sizes could have been attained through some combination of: a) reduced frequency of predator encounter, b) investment in physical

defence, c) improved escape/avoidance behaviour when encountering predators or d) predator saturation through increased consumption time. If high predator density caused prey to reduce their movement and foraging in order to reduce predator contacts, we would have expected the inclusion of movement rate information into our community model to have improved our ability to explain changes in species abundances. This was not the case. There was no obvious evidence of physical defence in *Colpidium* and chemical defences are usually associated with other species that have pigmented granules (Miyake *et al*. 2001). An improved ability to escape following predator encounters may have occurred if *Colpidium* utilize chemical cues or developed an aversion to the vortex of *Stentors* filtering. Such mechanisms have been proposed to be common in ciliates (Kusch 1993; Roberts *et al.* 2011). However, we have no direct measurements of such kairamones, nor behavioural changes. Finally, the handling time of captured prey may have increased if predators required longer to capture, subdue, and digest prey. Such changes has previously been reported in similar protist systems and may be related to an altered cellular composition (Hammill *et al.* 2010). The lower resource consumption in smaller prey is likely an acclimatory response to low energy inflow by switching to a life history strategy that uses minimal resources. Several previous studies have identified that *Colpidium striatum* responds to decreased productivity or lower effective prey availability by decreasing cell size (Balciunas & Lawler 1995; Jiang & Morin 2005).

Predator driven changes in body size, similar to those that we observed, appear to be common in similar microbial systems (Wiackowski & Starońska 1999; Kishida *et al.* 2010b; Kratina *et al.* 2010). For example, Fyda et al. (2005) observed that *Colpidium* exposed to predation by *Euplotes* or *Stylonychia*, became shorter and wider. Predator induced changes in growth rates and defence have also been observed (Fyda 1998; terHorst *et al.* 2010), however the links between individual traits, demographic rates and community dynamics have not previously been made (Kishida *et al.* 2010b). In systems where predators hunt for prey more active, such as the Trinidadian guppy system (Travis *et al.* 2014) or fish-zooplankton communities (Brooks & Dodson 1965), predation is often size selective. Predators preferentially attack larger individuals in order to obtain greater energetic gains per attack. Although *Stentor* is known to exhibit preferential feeding on certain prey species, prey consumption is thought to be unrelated to prey size (Tartar 1961; Rapport *et al.* 1972)**.** It is therefore unlikely that the predation directly induces smaller prey size through size selective predation. Instead, it is likely an indirect outcome of a reduced investment of resources into somatic growth due to an energetic cost of defence (Riessen & Sprules 1990; Schmitz *et al.* 1997; Bolker *et al.* 2003) or a modified life history strategy to allow earlier reproduction (Finlay 1977; Travis *et al.* 2014).

RESOURCE GROWTH DEFENCE TRADE-OFF

Protist grazing on a bacterial resource has been shown to lead to rapid changes in bacterial morphology, providing defence against predation and incurring an energetic cost to the bacteria (Jürgens & Güde 1994; Pernthaler *et al.* 1997; Hahn & Höfle 1999; Corno & Jürgens 2006). As the prey, *Colpidium,* is known to show selective feeding behaviour (Thurman *et al.* 2010), it is likely that the temporal change in the resource composition that we identified was driven by the strong consumptive selection pressure and represents a transition towards a population dominated by more defended and slow growing bacteria. The initially rare resource class became abundant and this was associated with a reduction in prey growth and also consumption. Increased defence in the resource population would produce a lower effective productivity for *Colpidium*, negatively affecting its population growth. We were able to separate the effects of changing resource abundance and availability/nutritional quality. Our results suggest that changes in resource quality or availability may have been as important as changes in the overall resource density in determining changes in consumer abundances. The rapid emergence of defence appears to be common in the basal trophic level of experimental aquatic food chains (Lampert 1994; Yoshida *et al.* 2003, 2004). It is important to examine whether this also occurs at higher trophic level, in established field systems, and in more complex communities, where conflicting energetic trade-offs potentially occur.

Overall, our results provide clear evidence of a feedback between trait change and species dynamics. This feedback appears to be underpinned by changes in the defence or life history of the species being consumed. Our findings indicate that theoretical frameworks for understanding trait or community dynamics will perform poorly in isolation. Theories of trait dependent ecological interactions and ecological dependent trait change are now well developed, however there is a clear empirical gap. Data driven modelling is needed to link theoretical and empirical insights into community and trait dynamics in a way that incorporate the feedbacks between these processes. A more mechanistic understanding of the processes driving the temporal variation in defence is required. This can be obtained by investigating the roles of evolution and plasticity in permitting trait change. This will allow an improved understanding of the costs and benefits of defences, the process driving trait dependence of ecological rates and the rates of trait change.

Acknowledgements

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Appendices

Appendix.1) Resource enumeration and classification into bacterial categories using Gaussian mixture modelling.

A) Removal of the noise background

Estimates of bacterial densities, sampled from experimental microcosms, were obtained by performing DNA marking of samples and undertaking flow cytometry (FCM). As sterile protist pellet medium was found to have a characteristic auto-flourescent signal, the raw data had to be filtered. This allowed the removal of the background noise, which would otherwise have biased estimates of bacterial densities. Using a validation data set comprised of samples from medium with a known composition, we characterised the regions of the FCM parameter space where observations would be expected to be noise. By removing observations that fall into this region from the overall FCM counts we obtained a more reliable estimate of bacterial density. FCM counts were found to be consistent with manual counts based on bacterial plating and also highly consistent between replicates.

Validation data set

A validation data set was produced in order to characterise the distribution of noise and bacteria events within the FCM parameter space. Different types of medium were sampled, including: highly purified water, sterilized standard tap water, sterilized protist pellet medium (PPM) and bacteria inoculated PPM. We assayed samples of PPM of differing concentrations (0.5, 1, 2 gL^{-1}) and ages (approx. 0.5,1,2 months). We also sampled cultures where the PPM had been filtered to remove large debris. Six replicate samples of each culture were made and FCM was used to analyse the resulting 84 samples

Data processing

FCM data comprises of a set of observations, each with a set of corresponding measurements about the scattering and colour intensity recorded for that observation. This provides a multidimensional characterisation of the individual observations, which allows different types of particles to be easily distinguished. The response measurements were log transformed and

principle components analysis (PCA) was used to transform/rotate the data and allow dimension reduction to a smaller set of uncorrelated response variables.

Noise characterisation

A Gaussian mixture model (GMM) was used to compare the observations from samples containing bacteria with those without bacteria. By comparing samples of known composition we were able to characterize the location of noise events in this FCM space. We first extracted a subset of the validation data set corresponding to data from samples containing bacteria (i.e. signal + noise). We parameterized the GMM to identify clusters within this data and produced an overall model of the FCM parameter space. The "mclust" package in R was used to construct the GMM using the expectation–maximization algorithm (Fraley & Raftery 2007). We then used the subset of the validation data set corresponding to data from samples containing no bacteria (i.e. noise only) to identify regions of this space that can be characterized as noise. (Fig.4.S1A). Clusters into which a high proportion of noise samples fell were identified and removed from the counts of bacteria density. Using this model and the inference about the characteristics of noise observations, novel observations could then be predicted to be noise or signal (Fig.4.S1B). We subsequently used the trained GMM to predict whether each observation from our experimental samples had characteristics of a bacterial signal or that of the noise background. Finally, signal observations were enumerated and their characteristics stored.
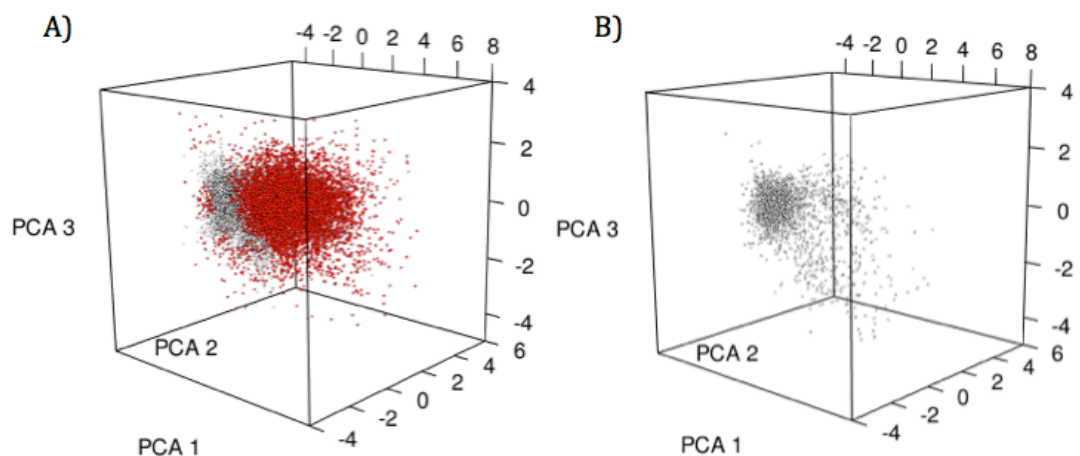
Figure 4.S1) Distribution of noise and signal observations in the FCM parameter space. In Subpanel (A) shows the distribution of observations from samples containing both bacteria and background noise. Observations are classified as background (grey) and signal (red). Subpanel (B) shows the distribution of observations from samples known to contain no bacteria (background only: grey). This characterize

characterises the region of the noise background. The bacterial signal is clearly visible in subpanel A as a large cluster that is separate from the noise component. FCM observation measurements are transformed using PCA of logged data and the three dominant axes of variation are plotted.

B) Classification of bacterial categories

To identify groups of differing bacterial classes, we further examined the bacterial signal remaining after filtering of the background noise component. A second GMM was parameterized using just the signal observations. Model-based hierarchical clustering was applied to identify the number of bacterial categories present in the data. To do this we fitted a set of models in which the number of clusters ranged from one to eight. Bayesian Information Criterion (BIC) was used to perform model comparison between models in the candidate set and identify the most parsimonious clustering structure. Observations were then classified into different bacterial categories and the proportion in each calculated.

The model-based hierarchical clustering revealed two major bacterial clusters in the data (Fig.4.S2). Two other clusters were identified but the abundances of observations predicted to belong to these categories was low.
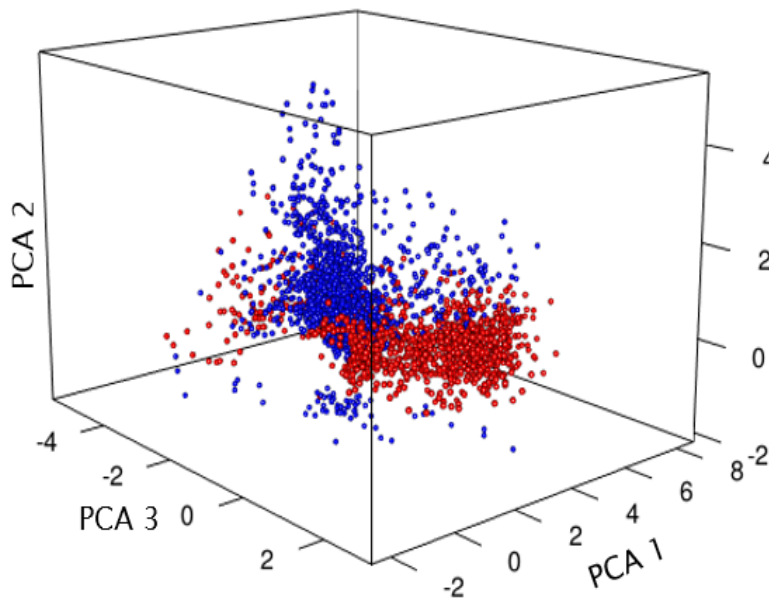
Figure 4.S2) Classification of the observations from the bacterial signal into bacterial categories using model-based hierarchical clustering. The FCM observations are classified into two major categories (blue vs red). FCM observation measurements are transformed using PCA of logged data and the three dominant axes of variation are plotted.

Appendix.2) Deriving the trait dependent community model structures

By combining the theoretical frameworks of Holt & Polis (1997) and Abrams *et al.* (1993) we formulated a community model with trait dependent species interactions and fitness dependent trait selection. An intra-guild predation food-web can then be characterized with the following coupled differential equations:

$$\frac{dR}{dt} = g_{RR}(R, Z_i)\, R - f_{RN}(R, Z_i)\, N - f_{RP}(R)\, P \qquad\qquad 1.a)$$

$$\frac{dN}{dt} = \alpha_{RN} f_{RN}(R, Z_i)\, N - f_{NP}(N, Z_i)\, P - m_N \qquad\qquad 1.b)$$

$$\frac{dP}{dt} = \alpha_{NP} f_{NP}(N, Z_i)\, P + \alpha_{RP} f_{RP}(R, P)\, P - m_P \qquad\qquad 1.c)$$

$$\frac{dZ_i}{dt} = \widetilde{G_N}\, \frac{\delta W_N}{\delta \hat{Z}_i}\,|\hat{Z}_i = Z_i \qquad\qquad 1.d.i)$$

$$W_N = \frac{1}{N}\frac{dN}{dt} \qquad\qquad 1.d.ii)$$

The densities of the resource, prey, and predator are, respectively, R, N, and P. Ecologically relevant traits influencing predation are denoted by Z_i. The term $g_{RR}(R)$ is a smooth function describing the intra-specific density dependence of growth in the resource. The quantities f_{ab} (X, Y) are functional response terms describing the dependence of the consumption rate, of species *a* by species *b,* on the state variables X and Y which can be either abundance or trait variables. For example, $f_{RP}(R, P, Z_i)$ describes the trait and density dependent intra-guild predation of the resource by the predator. The conversion efficiency during the consumption of species *a* by species *b* is denoted α_{ab}. Mortality of species *a* is m_a. Trait change dynamics are modelled using a quantitative trait evolution model where $\widetilde{G_N}$ represents the additive genetic variance divided by the prey generation time and characterizes the speed of evolutionary adaptation or acclimation. Prey fitness (W_N) is defined as the per capita rate of population growth conditional on the mean trait value *of* Z_i. The change in fitness with respect to trait change signifies the steepness of the selection gradient.

Appendix.3) Species and trait dynamics

All populations persisted for the duration of the experiment and the observed dynamics were largely consistent between replicates (Fig. 4.S3). We observed substantial fluctuations and trends in the abundances of species, prey body size and the resource composition over time.

Resource density showed a declining trend, but also spikes of increased abundance. Prey density initially increased, plateaued and began to decline after day 40. Similarly, predator density initially increased, then appeared to fluctuate before subsequently declining around the same time. The community models allowed a more detailed understanding of the relationships between changes in species densities. Prey body size decreased substantially over the course of the experiment (66% reduction). Some systematic fluctuations were also clearly identifiable across replicates. Finally, the resource composition also showed marked fluctuations in the relative frequency of different bacterial types, however no clear directional trend was apparent.
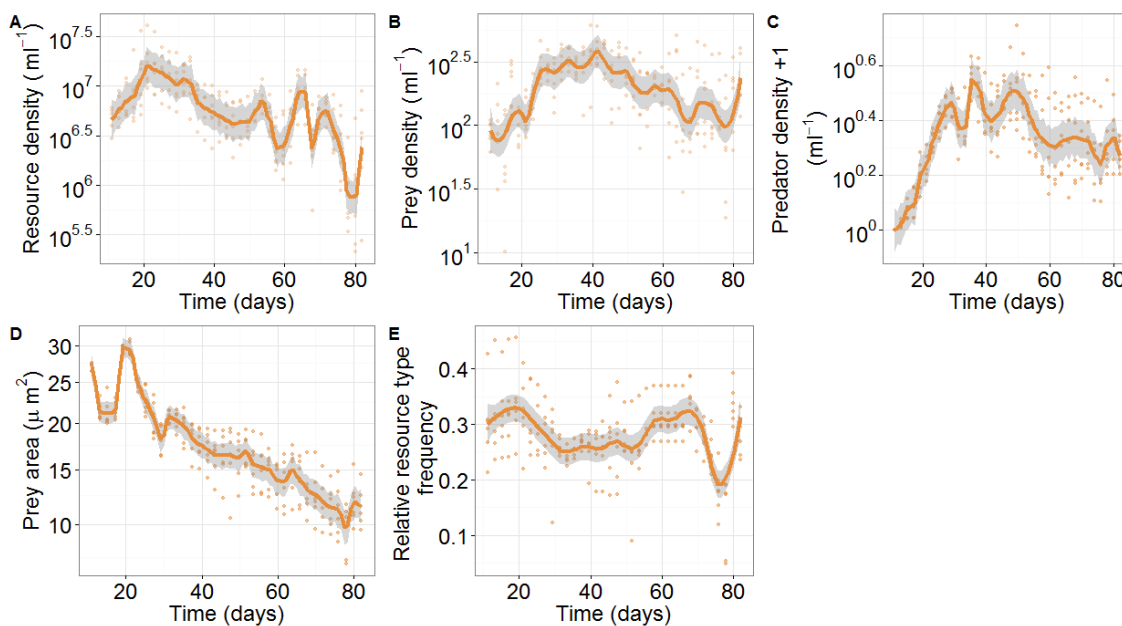
Figure 4.S3) The observed dynamics of A) resources, B) prey, C) predators, D) prey body size and E) the composition over the experiment (points). Predator additions were made on day 12. Population trends are indicated by smoothed population trajectories (coloured lines. These are local polynomial regressions and confidence bands).

Chapter 5: The impacts of warming on trait dependent community dynamics.

Jason I. Griffiths, Owen Petchey, Frank Pennekamp, Philip H. Warren & Dylan Z. Childs

Abstract

Warming can influence species' physiological performance and also drive changes in interspecific interactions, by modifying metabolic demands for consumption. Changes in species densities can drive temporal trait change, also driving variation in species interaction. Trait dependent interactions can permit complex community dynamics that are not expected by ecological theories based on species abundances. As warming can concurrently influence many ecological processes, including metabolism and development, it may modify the impacts of trait change on species consumption rates, driving unanticipated community dynamics.

Here we use an experimental predator-prey-resource system, to concurrently monitor the dynamics of species abundance and individual level traits at a range of temperatures. We formulated empirically derived trait dependent community models, to identify whether warming modifies the key traits associated with variation in consumption. We then quantify how the trait dependence of species consumption rates varies along a temperature gradient.

Across temperatures, body size was consistently associated with temporal variation in species interactions. Body size declines were observed at all temperatures, but were greatest in warm environments. Interestingly, in cool conditions, body size reductions were associated with reduced consumption by predators and consumption of resources. Conversely, at higher temperatures, reduced size was associated with increased intake of resources and consumption by predators.

Changes in prey body size may have indirectly resulted from a modified life history strategy or altered resources allocation. The findings indicates that warming reversed the life history strategy of prey to an investment of resources into consumption and growth rather than defence. This is likely a response to the increased metabolic demand of inhabiting a warm environment.

Introduction

Climatic warming has the potential to profoundly influence species abundances, through its impacts on a range of biological processes. Warming influences biochemical and physiological processes, modifying metabolic requirements and resource usage (Finlay 1977; Brown *et al.* 2004; Clarke & Fraser 2004; Diamond *et al.* 2012; Ohlberger 2013). This can drive behavioural or morphological change, as well as changes to life history schedules and resource allocation strategies (e.g. timing of reproduction or investment to growth and defence) (Post *et al.* 1999; Musolin 2007; Barros *et al.* 2010; Gardner *et al.* 2011). As a result, warming influences key ecological processes such as growth, birth, and death rates (Tylianakis *et al.* 2008a; Régnière *et al.* 2012; Schaper *et al.* 2012). Furthermore, warming induced changes in traits, metabolic demands or population abundance are likely to modify the strength of species interactions (Rall *et al.* 2010; Vucic-Pestic *et al.* 2011; Binzer *et al.* 2012; Lang *et al.* 2012; Ohlberger 2013; Öhlund 2015). For example, warming has been associated with intensification of pathogen effects on amphibians, greater predation rates in rocky intertidal ecosystems and disrupted plant pollinator interactions (Sanford 1999; Pounds *et al.* 2006; Memmott *et al.* 2007). By modifying interactions between species, warming can substantially modify a community's dynamics, structure and functioning (Harvell *et al.* 2002; Emmerson *et al.* 2004; Post *et al.* 2008; Gilman *et al.* 2010; Kordas *et al.* 2011; O'Connor *et al.* 2009; O'Connor *et al* 2011; O'Connor *et al.* 2012; Griffiths *et al.* 2014). Understanding how warming influences the strength of species interactions is therefore a vital component in the development of a predictive understanding of the ecological consequences of climate change (Hughes 2000; Tylianakis *et al.* 2008b).

Changes in species densities can also influence species traits (Berg & Ellers 2010; Schoener 2011). Trait change can be driven by density-dependent evolutionary selection pressures, favouring certain heritable traits in a genetically variable population (Thompson 1998; Yoshida *et al.* 2004; Kasada *et al.* 2014). Alternatively, trait change can result from phenotypic plasticity, where a single genotype produces different phenotypes under differing biotic environments (Tollrian & Harvell 1999; Agrawal 2001; Fordyce 2006; Cortez 2011). For example, the life history schedule or the resource allocation to growth and defence may depend on the density of predators and resources (Finlay 1977; Lampert 1994; Travis *et al.* 2014; Riessen 2015). Density mediated trait change can cause temporal variation in vital rates, such as resource consumption and population growth, modifying interspecific interactions. (Bolker *et al.* 2003; Preisser *et al.* 2005; Pelletier *et al.* 2007; Harmon *et al.* 2009; Bassar *et al.*

2010; terHorst *et al.* 2010; Turcotte *et al.* 2011). When the modification of interspecific interactions, such as the intensity of predation, alters the strength or direction of trait change, a feedback loop is produced between abundance and trait dynamics (Yoshida *et al*. 2003; Yoshida *et al.* 2007; Pelletier *et al.* 2007; Becks *et al.* 2010, 2012; Schoener 2011; Agrawal *et al.* 2013). This coupling between trait dependent vital rates and density dependent trait change allows a diversity of community dynamics to occur that would be unexpected from purely ecological theories, based on species abundances (Abrams & Matsuda 1997; Jones *et al.* 2009; Berg & Ellers 2010; Kishida *et al.* 2010; Ellner & Becks 2010; Cortez 2011; Mougi 2012a; b; Walsh *et al.* 2012; Cortez & Weitz 2014; Hiltunen *et al.* 2014).

Ecologically relevant traits, that can modify species interactions, may be influenced by changes in both species density and climate conditions. For example, changes in body size have been observed in many taxa, either in response to warming (Atkinson 1994; Brakefield & Kesbeke 1997; Bradshaw & Holzapfel 2006; Gardner *et al.* 2011; Sheridan & Bickford 2011) or due to changes in resource or predator abundances (Blumenshine *et al.* 2000; Yoshida *et al.* 2004; Travis *et al.* 2014; Reznick 2016). Such changes in body size can substantially modify predation rates (Brooks & Dodson 1965; Vonesh & Bolker 2005). Crucially, because warming concurrently alters a range of metabolic and physiological processes, it may alter the impact of trait change on the strength of species interactions.

Theory and empirical evidence indicates that warming generally intensifies consumptive interactions, by accelerating the metabolism of predators, increasing their growth, activity, and digestion rates (Sanford 1999; Brown *et al.* 2004; Jiang & Morin 2004; Vasseur & McCann 2005; O'Connor *et al.* 2009; Beveridge, Humphries & Petchey 2010; Yvon-Durocher *et al.* 2010; Hoekman 2010; Rall *et al.* 2010; Öhlund 2015). However, the impacts of climate change on trait dependent consumption rates are largely unknown. Despite this, recent theory indicates that such dependencies may be highly influential in determining community dynamics (Moya-Larano *et al.* 2012; Northfield & Ives 2013; Koch *et al.* 2014).

The relative influence of different morphological and behavioural traits in determining consumption rates may change with warming (Dijk *et al.* 2015). For example, in lizards, the importance of behavioural defence mechanisms depends on the climate and the individuals body size (Barros *et al.* 2010). Furthermore, the strength of the trait dependence of vital rates may be modified by climate change. For example, populations may shift from strongly trait dependent dynamics to predominantly density dependent dynamics, or vice versa. Furthermore, the effect of trait change on vital rates may vary over a climatic gradient. That is,

large trait values may increase vital rates under some climate conditions, but reduce them in others.

Here we develop a novel approach to examine the consequences of climate warming on the trait dependence of vital rates. We utilize an experimental microbial community to concurrently study species' abundance and trait dynamics at a range of environments along a temperature gradient. At each temperature, we identified key ecologically relevant traits and linked their dynamics with those of species abundances, by fitting community models to the observed dynamics at each environmental condition. Using this approach we determined the temperature and trait dependence of community dynamics along a temperature gradient and quantify the influences of warming on the trait dependence of species interactions. This allowed us to examine how warming impacts upon the strength of intraspecific interactions and how they varied with temporal changes in species' traits and densities.

Methods

EMPIRICAL MICROCOSM SYSTEM

STUDY ORGANISMS: CULTURING & ESTABLISHING THE PREDATOR-PREY SYSTEM

We studied the community dynamics of a tri-trophic predator-prey-resource system along a temperature gradient. The community consisted of a bacteria resource, *Serratia marcescens*, an intermediate bactiverous ciliate *Colpidium striatum* (Stokes 1886; referred to as the prey), and a top predator, *Stentor coeruleus* (Ehrenburg 1830). *Stentor* populations consume bacteria during filter feeding, but do not persist on a purely bacterial diet, generating intra-guild predation (Slabodnick & Marshall 2014). Microcosm experiments were conducted in petri dishes containing 50ml Chalkley's solution (Thompson *et al.* 1988), $0.7gL^{-1}$ of crushed protozoan pellets (Carolina Biological Supply, USA) and 3 wheat seeds. Microcosms and media were autoclaved before use. Two days prior to the experiments initiation, the medium was inoculated with bacteria and kept at 37^0C, allowing the establishment of a resource base.

TREATMENTS

Temperature and species manipulations

During the experiment, 14 microcosm populations were maintained at each of 6 temperature treatment levels: 14, 16, 18, 20, 22 and 24°C. These microcosms were equally distributed between two controlled temperature incubators and exposed to a 16: 8 light-dark photoperiod. The positions of microcosms within controlled temperature environments was randomized and frequently permuted. Cultures were replenished three times a week by renewing 1ml of medium and replacing any evaporative loss with distilled water.

Of the 14 microcosms, at each temperature level, 10 replicates were assigned to a predator-prey-resource treatment (exposed to predation: 5 reps per incubator). The remaining 4 replicates were assigned to a prey-resource treatment (naive to predation: 2 reps per incubator). On day zero, all microcosms were initiated with 100 *Colpidium* and populations of prey were allowed to grow by feeding on the bacterial resource. On day 12, each microcosms in the exposed to predation treatment was invaded with 25 *Stentor* predators. Two days later 25 more were added, to prevent chance predator extinctions. The replicates in the naive to predation treatment received an equivalent volume of *Stentor* culture medium, but sieved to remove predators. Populations were maintained for an 82 day study duration.

SAMPLING

Replicates were agitated and then sampled three times a week. Predator density was estimated by transferring (with replacement) 5ml of medium to a sterile petri dish and scanning the medium under a stereomicroscope (Leica M205 C: surveyed at 7.8x magnification). To measure prey density and trait values, 0.044ml of medium was transferred into a Sedgewick Rafter cell (S52, SPI supplies, Westchester, PA). A 5 second video (25 fps) of the medium was taken using a digital CMOS camera (Orca C11440, Hamamatsu Photonics, Japan) mounted on a stereomicroscope with a 259 magnification. Individual prey were located, measured and had their trajectories mapped using the automated digital video processing R package, bemovi (Pennekamp *et al.* 2015). For each sample, individual level behavioural and morphological trait measurements were produced summarising body size and shape as well as movement speed and pattern.

Bacteria density was estimated by passing a 20μl sample of medium through a flow cytometer. A characteristic background noise pattern was identified across samples, generated by auto-florescence of the sterile protest pellet medium. To filter noise observations from the signal of bacterial observations, a Gaussian mixture model (GMM) was constructed using the "mclust" R package (Fraley *et al.* 2006) and parameterized using subsidiary training data (Chapter.4 see Appendix.4.1.A). Model-based clustering was then undertaken to determine the number of distinct bacterial types in the data set and to classify signal observations into appropriate bacterial type categories. Two main distinct bacterial types were identified and the proportion in each class calculated for every sample.

MODEL INFERENCE

PARAMETERIZATION OF AN EMPIRICALLY DERIVED TRAIT DEPENDENT PREDATION MODEL

Deriving a community model of traits and abundance

A community model describing the linked dynamics of species traits and abundances was formulated, by combining theoretical frameworks for modelling trait dependent species interactions (Holt & Polis 1997) and fitness dependent trait selection (Abrams *et al.* 1993). We then derived a discretized non-parametric regression model structure, describing changes in community abundances and trait values between observations (Chapter.4 Appendix.4.2). This allowed trait dependent community interactions and ecological impacts on trait dynamics to be described flexibly as smooth functions of species densities and mean trait values. It also avoids strong assumptions about the functional forms of species interaction terms. The community dynamics were described by the following system of difference equations:

$$E(\log\left(\frac{R^{t+1}}{R^t}\right)) = g_{RR}(R, Z_i, T) - f_{RN}(R, Z_i, T)\frac{N}{R} - f_{RP}(R, T)\frac{P}{R} \qquad \text{1a)}$$

$$E(\log\left(\frac{N^{t+1}}{N^t}\right)) = \alpha_{RN}f_{RN}(R, Z_i, T) - f_{NP}(N, Z_i, T)\frac{P}{N} - d_N \qquad \text{1b)}$$

$$E(\log\left(\frac{P^{t+1}}{P^t}\right)) = \alpha_{NP}f_{NP}(N, Z_i, T) + \alpha_{RP}f_{RP}(R, P, T) - d_P \qquad \text{1c)}$$

$$E(\log\left(\frac{Z^{t+1}}{Z^t}\right)) = s_Z(R, N, P, Z_i, T) \qquad \text{1d)}$$

Here we model the expected (E) log difference in species abundances and trait values. The densities of the resource, prey, and predator are, respectively, R, N, and P. Ecologically relevant traits influencing dynamics are denoted by Z_i. And the temperature of the environment is denoted T. The term $g_{RR}(R, Z_i, T)$ is a smooth function describing the intra-specific density dependence of growth in the resource. The quantities f_{ab} (X, Y) are smooth functional response terms describing the dependence of the consumption rate, of species *a* by species *b,* on the state variables X and Y at the prior time step. For example, $f_{NP}(R, P, Z_i, T)$ describes the temperature, trait and density dependent consumption of prey by predators. The per capita mortality term of species *a* (d_a) and the conversion efficiency of biomass between species *i* and *j* (α_{ij}) are constants to be estimated. Finally, $s_Z(R, N, P, Z_i, T)$ is the temperature and density dependent rate of trait change and is proportional to the ecological selection pressure acting on the trait.

Model parameterization

Prior to model fitting, individual trait measurements were square root transformed to reduce skew in the data. Mean trait measurements were then calculated for each replicate at each sample point. Population abundance and trait dynamics were interpolated, using cubic hermite splines, to obtain data with equidistant time intervals and then standardized to have a standard deviation of 1. The finite rate of change of each species population abundance (X_s) was calculated based on non-standardized measurements and log transformed to provide a measure of the observed linearized per capita rate of population growth: $\log\left(\frac{X_s(t+1)}{X_s(t)}\right)$. The first log difference of trait dynamics (Z_i) were also calculated: $\log\left(\frac{Z_i(t+1)}{Z_i(t)}\right)$.

Using data from replicates at each temperature, we used the "mgcv" package in R to construct generalized additive models (GAM) describing the linked community dynamics (equ.1) (Wood 2006). To account for heavy tailed response variables, we used a scaled-t distribution model. To avoid over-fitting, the model degrees of freedom in the gcv criterion was inflated by a factor of 1.2, following recommendations of (Gu 2013) and (Hiltunen *et al.* 2014). Numerical optimization, using a box constrained variable metric algorithm (Limited-memory BFGS quasi-Newton method), was applied to identify the remaining constants (d_a and α_{ij}).

Formulation and comparison of candidate hypotheses

Competing hypotheses about the trait dependence of demography and community dynamics were formalized by constructing a set of regression models. In these candidate models population growth and consumption rates depended upon different behavioural and morphological traits as well as species abundances. Similarly, hypotheses about the impacts of species abundances on trait dynamics were also formalized. Here regression models were constructed in which the rate of trait change depended upon species. There was a high degree of collinearity between the measurements of several traits. Those showing strong correlation, such as body length and width, were reduced to a single variable. The resulting candidate predictor variables describing prey traits were: body size (Z_{size}; area μm sec^{-1}), swimming speed (Z_{speed}; μm sec^{-1}), displacement rate (Z_{disp}; describing the rate of habitat exploration; μm sec^{-1}) and turning movement variability (Z_{turn}; describing the variability of movement direction; radians turned sec^{-1}). Finally, the composition of the resource population was also used as a putative predictor of changes in species abundances (Z_{comp}), by using the proportion of the total resource that was categorized into the initially rare bacterial class as an additional candidate model covariate. We constructed models including (or excluding) each prey trait individually, and including (or excluding) the resource composition information.

To test whether the inclusion of trait information improved our ability to explain community dynamics at each temperature, the trait-dependent community models were compared with the null model based on abundance only. Cross validation was used to compare the predictive ability of candidate models. All but one replicate was used to parameterize the model and the remaining replicate was used to estimate the models predictive performance. Predicted population changes between each observation were then compared against the observed data and the root mean square error (RMSE) was calculated to quantify model prediction error. This process was repeated, sequentially leaving out each replicate and measuring RMSE. Linear mixed effects modelling was then used to identify which traits produced significant reductions in prediction error. The estimated prediction error scores obtained from leaving out different replicates of each treatment were defined as a random effects.

Constructing a temperature dependent community model of traits and abundance

To quantify how the trait dependence of species interactions varied across the temperature gradient, a temperature dependent community model was constructed, describing species trait dependent interactions across all the experimental temperature gradient. First, the traits dependence of vital rates was identified for each level of the temperature gradient by parameterizing temperature specific community models (as in Chapter.4).

Subsequently, the traits which were found to influence vital rates in the temperature specific community models were used as predictors when parameterizing full temperature dependent community model following equ.1. These full community models describe the dynamics of species abundances across all the experimental temperature conditions. The trait dependence of vital rates was assumed to change smoothly with temperature. Cross validation was again used compare the predictive ability of candidate models.

Results

TEMPERATURE MEDIATED CHANGES IN ABUNDANCE AND TRAIT DYNAMICS

Across the temperature gradient, we observed gradual changes in the dynamics of species abundances and traits and the resource composition (Fig.5.1). At higher temperatures, resource abundance was initially highest and then declined to a lower level than at cooler temperatures. This pattern was mirrored in the abundance of prey. Similarly, at higher temperatures, predators generally reached higher initial abundance but showed rapid declines towards extinction. The traits associated with these species dynamics also showed clear temperature dependent patterns. At higher temperatures, prey size decreased more rapidly over time. This caused prey in warmer environments to be much smaller by the end of the experiment. Prey that were not exposed to predation declined in size as well as those exposed to predation, especially at warm temperatures. However, across temperatures, the size of prey exposed to predation declined substantially more than those in the un-predated treatment (Appendix 5.2). Finally, at higher temperatures, the initially rare resource class became common around day 20 and declined to a low frequency towards the end of the experiment. In

contrast, at lower temperatures, the frequency of the initially rare resource class did not reach as high, but was more stable over time.
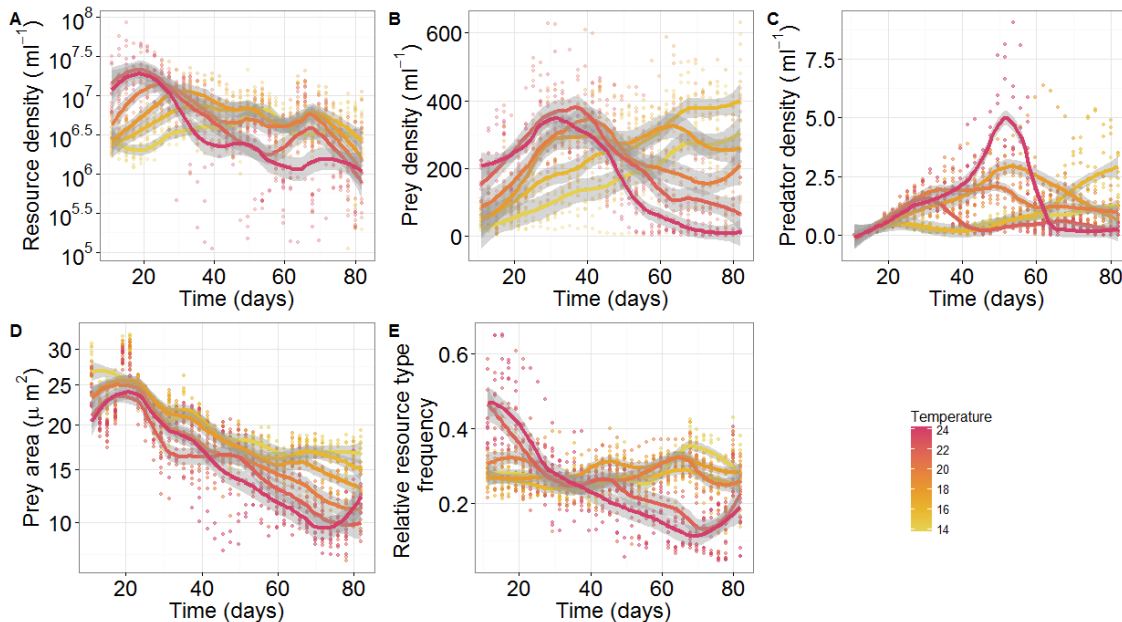
Figure 5.1) The effects of temperature on the dynamics of A) resources, B) prey, C) predators, D) prey body size and E) the resource composition (fraction of resource composed of initially rare resource class). Observations from replicates at each temperature are plotted over time (coloured points) and population trends are indicated by smoothed population trajectories (coloured lines; these are local polynomial regressions and confidence bands).

THE TRAIT DEPENDENCE OF COMMUNITY DYNAMICS ACROSS THE TEMPERATURE GRADIENT

For the six temperature levels, we assessed the predictive ability of community models that included different information about the state of prey traits and the resource composition. At each of the six temperatures, the inclusion of information about prey size and resource composition produced the most significant reduction in the prediction error (Fig.5.2). The inclusion of these factors individually did not greatly reduce prediction error, but together they provided a 17% reduction in prediction error ($Z_{\mathrm{area}} * Z_{\mathrm{comp}}$: $d.f.$(7,6), χ^2=37.74,p<0.001). The other prey traits did not improve the predictive performance of the empirically derived models (trait dynamics are shown in Appendix.5.1).
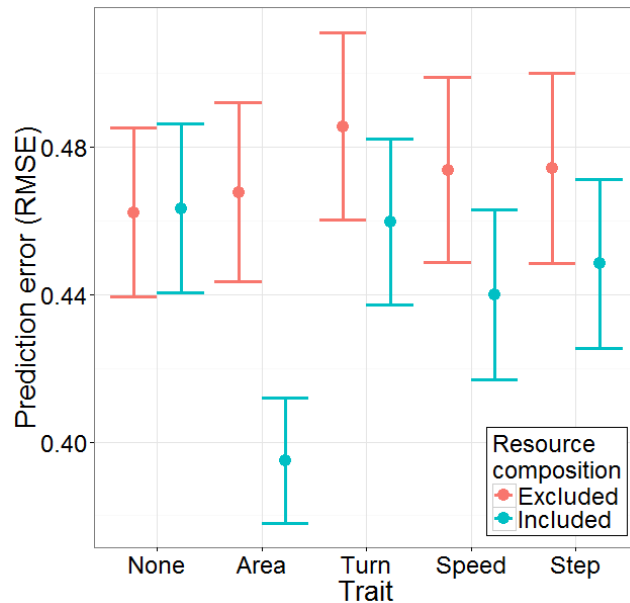
Figure 5.2) Predictability of changes in species abundances in population models containing different combinations of information about prey traits (x-axis) and the resource composition (bar colour). The predictability is quantified by measuring the prediction error between the observed rates of change of species abundances and model predictions, based on the RMSE of predictions during cross validation (lower RMSE is better). Error bars show the standard error of the prediction error observed between rounds of the cross validation, the midpoint being the mean prediction error.

TEMPERATURE & BODY SIZE DEPENDENT CONSUMPTION

The temperature specific community models revealed that, across the temperature gradient, changes in prey body size influenced community dynamics via its effect on the consumption of resources by prey and the consumption of prey by predators (Appendix.5.1). The predictions of the full temperature dependent community model were consistent with the findings from temperature specific models (Appendix.5.1). Allowing the trait dependence of species interactions to vary smoothly with temperature significantly improved the predictive performance of the full community model relative to a model assuming the trait dependence of consumption did not vary with temperature (Table.5.1). Therefore, warming modified the trait dependence of the predator and prey's consumption (Fig.5.3 & Fig.5.4).

Table 5 1) The prediction error (RMSE) of full community models allowing the trait dependence of prey's consumption of resources (f_{RN}) and predator's consumption of prey (f_{NP}) to vary with temperature (+) or to be constant (-). Standard error of RMSE (s.e) shows variability of predictive error. Akaike information criterion (AIC) provides an alternative measure of relative model fit and gives results consistent with those from cross-validation. Selected model, with the lowest prediction error and AIC score is shaded in grey.

Model		Temperature dependence		Prediction error (RMSE)	RMSE s.e	AIC
Term 1	Term 2	f_{RN}	f_{NP}			
$f_{RN}(\mathbf{R}, \mathbf{Z}_i, \mathbf{T})$	$f_{NP}(N, Z_i, T)$	+	+	0.341	0.0178	1616.9
$f_{RN}(\mathbf{R}, \mathbf{Z}_i)$	$f_{NP}(N, Z_i, T)$	-	-	0.360	0.0174	1663.6
$f_{RN}(\mathbf{R}, \mathbf{Z}_i, \mathbf{T})$	$f_{NP}(N, Z_i)$	+	-	0.357	0.0180	1694.6
$f_{RN}(\mathbf{R}, \mathbf{Z}_i)$	$f_{NP}(N, Z_i)$	-	-	0.368	0.0174	1707.0

Across the temperature gradient, the consumption of resources by prey (f_{RN}) was greatest when resource density was high (Fig.5.3). The effect of decreasing prey size on the consumption rate of resources by prey switched as temperature increased. At low to medium temperatures (14 -20 °C), the prey's consumption of resources was reduced when prey body size declined. However, as the temperature increased to the warmest temperatures (22-24°C), this relationship reversed. Consumption of resource was then greatest for prey with a small body size. Populations of these smallest individuals where only observed at the warmest temperatures and these populations had high rates of resource consumption compared to populations at other temperatures and of other sizes.

The temperature, trait and density dependence of consumption of prey by predators (f_{NP}) followed a similar pattern (Fig.5.4). Across the temperature gradient, the consumption of prey by predators increased with prey density. Furthermore, the effect of warming on the trait dependence of consumption varied in a comparable way to that of resource consumption (compare pattern in Fig.5.3 & Fig.5.4). At low to medium temperatures (14 -20 °C), the consumption of prey by predators was lowest when prey body size was small. This relationship was also reversed as the temperature increased to the warmest temperatures (22-24°C). Consumption of prey was then higher when prey had small body size.
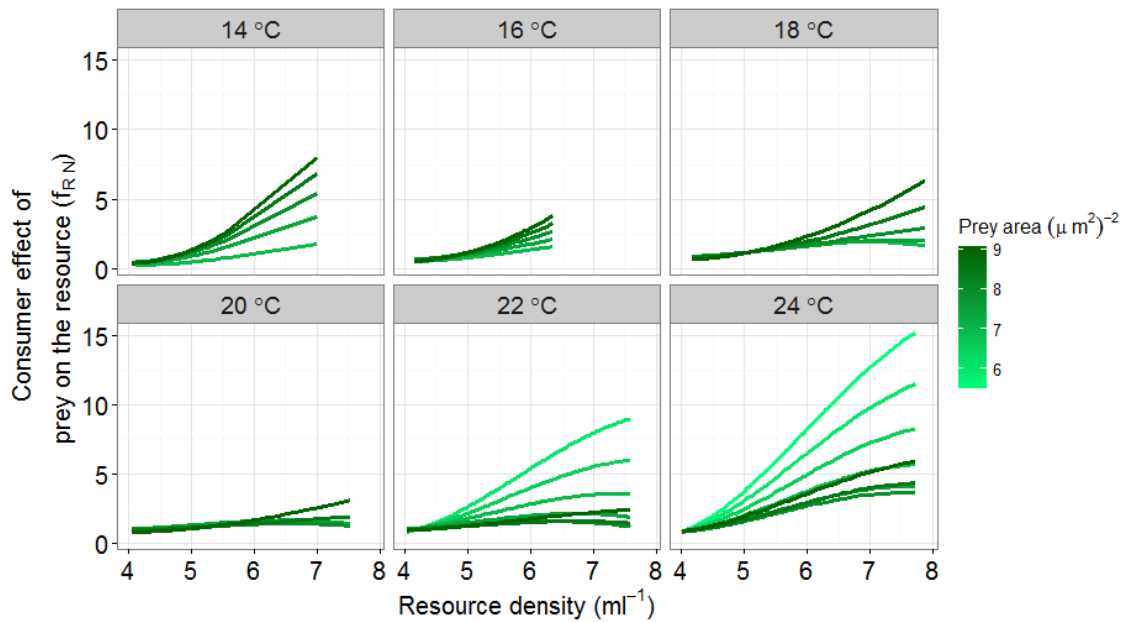
Figure 5.3) The effect of temperature on the trait dependence of resource consumption by prey (f_{RN}). Subpanels show the trait dependence of consumptiion infered for each experimentally observed temperature. Consumption depended on prey body area (Darker green signifies higher consumption). The range of prey body sizes predicted at each temperature corresponds to that observed in replicates under experiencing that environment.
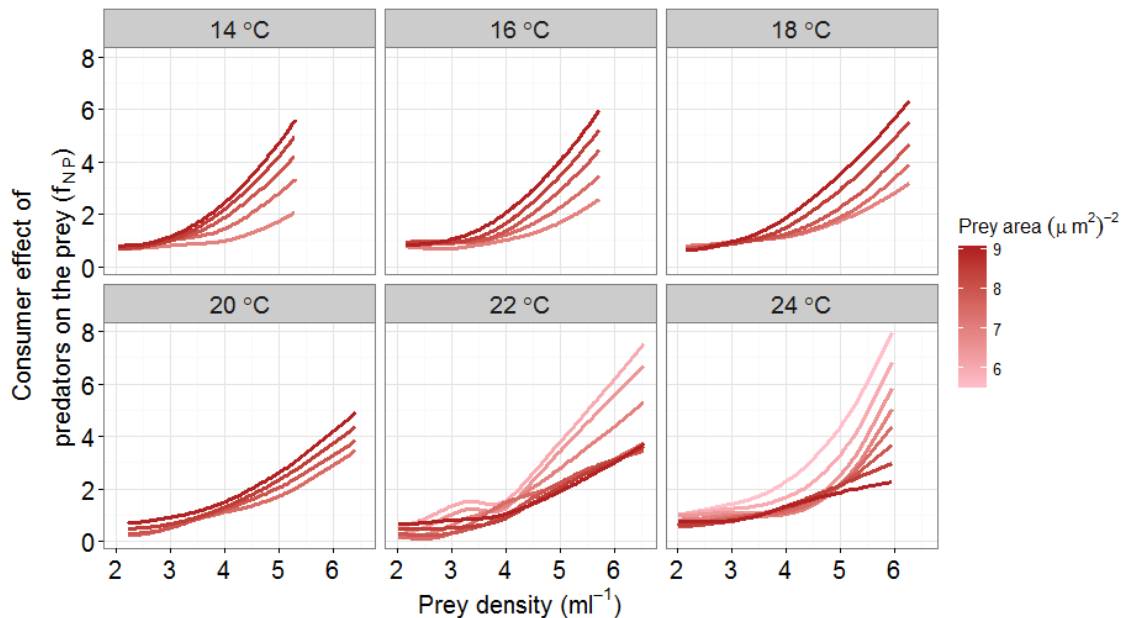
Figure 5.4) The effect of temperature on the trait dependence of predator consumption of prey (f_{NP}). Subpanels show the trait dependence of consumptiion infered for each experimentally observed temperature. Consumption depended on prey body area (Darker red signifies higher consumption). The range of prey body sizes predicted at each temperature corresponds to that observed in replicates under experiencing that environment.

<u>Discussion</u>

In this study, we investigated the link between the dynamics of species traits and their abundances at temperatures along an environmental gradient. We quantified the temperature and trait dependence of the vital rates underpinning community's interactions and species dynamics. We found that across a temperature gradient, community dynamics were influenced by temporal variation in species consumptive interactions which were associated with changes in the prey's body size. We predicted that the traits associated with temporal changes in consumption may have differed across the temperature gradient (as described by Dijk *et al.* 2015). However, this was not to be the case in this system. By modelling community dynamics across the temperature range we revealed that warming modified the trait dependence of consumptive interactions. As a result, the reduction of prey body size, observed in all replicates, had very different impacts on consumption rates in warmer versus cooler environments.

Trait dependent changes in consumption in cool environments

At lower temperatures, decreased prey body size was linked to reductions in both the prey's consumption of resource and also the predator's consumption of prey. Over time, prey shrank and became less vulnerable to predation but also less effective at consuming resources. This indicates the presence of a growth-defence trade-off and suggests selection for individuals allocating resources into defence.

Smaller prey may have attained better defence against predation as a result of a number of mechanisms. Reduced size may not convey defence directly, but may be an indirect consequence of being defended or modifying its life history. Here we outline several mechanisms (see discussion in Chapter 4). Firstly, smaller individuals may have invested resources in physical defence or earlier reproduction rather than growth (Riessen & Sprules 1990; Bolker *et al.* 2003; Travis *et al.* 2014). Secondly, they may have had an improved escape/avoidance behaviour when encountering predators, which comes at a metabolic cost. Thirdly, they may have had a reduced frequency of predator encounters but simultaneously decreased resource intake due to altered behaviour, for example through aggregation (Schmitz *et al.* 1997). Finally, small individuals may have saturated predation by increasing processing

time through production of tough cellular structures. Further work is required to determine the underlying mechanism, but there is support in the literature for a role of several of these factors in microbial communities (Kusch 1993; Hammill *et al.* 2010; Roberts *et al.* 2011).

Trait dependent changes in consumption in warm environments

At higher temperatures, the effect of trait change on consumption rates was reversed. As observed at lower temperatures, prey body size decreased over time. Contrastingly, this was associated with increased consumption by predators and prey. This indicates that, over time, prey became more effective at consuming resources but became more vulnerable to predation. This suggests a modification of the growth-defence trade-off and a switch towards selection for individuals allocating resources into ingestion (growth) instead of defence.

Metabolic theory provides an explanation for this contrasting relationship between body size and consumption rates at higher temperatures. Firstly, it predicts that warming, to within a species thermal range, increases both metabolism and consumption, with metabolic rates rising at a faster rate (Binzer *et al.* 2012; Reuman *et al.* 2013). Secondly, theory predicts that metabolic demands increases with body size and that warming should steepen this scaling relationship (Atkinson 1994; Gillooly *et al.* 2002; Savage *et al.* 2004; Binzer *et al.* 2012; Reuman *et al.* 2013). The "temperature-size-rule" (Atkinson 1994) proposes that the relative performance of smaller individuals will increase with temperature as their lower metabolic demands for survival in warm environments can more easily be met by increased consumption (Ohlberger 2013). Accordingly, recent reviews have found warming-induced declines in the body sizes in a wide range of taxa (Gardner *et al*. 2011; Sheridan & Bickford 2011).

As predicted by metabolic theory, we found that consumption rates were generally higher in warmed environments. Although we did not directly measure metabolic rates across the temperature gradient, there is strong empirical evidence that metabolic rates in this system also increase with warming. In agreement with the "temperature-size-rule", a wide range of protist species, are known to show body size reductions in response to warming (Atkinson *et al.* 2003). It is also known that warming drives earlier reproduction of the prey species (*Colpidium*), as well as increasing respiration rates in this system (Pace & Kimura 1944; Laybourn 1975; Finlay 1977; Fenchel & Finlay 1983; Caron *et al.* 1986). It is therefore likely that the body size declines we observed are a physiological response to warming, rather than a response to predation, as at lower temperatures. Future work could use ecosystem

measurements of oxygen and carbon fluxes to assess the dynamics of metabolic rates and obtain a fuller understanding of the temperature driven changes in body size and the ratio of consumption to metabolism.

Warm environmental conditions may have driven selection for smaller prey with higher feeding rates, due to the high metabolic demands. The increased consumption of small prey by predator, indicates that there was a concurrent reduction in the prey's investment into defence against predation. Therefore, across temperatures, there appears to be a trade-off operating between resource allocation into growth and defence. Under the cooler conditions, resource allocation into defence appears to be increased over time, whilst in the warmest conditions resources appear to be redirected into attaining growth in the metabolically demanding environment.

Our finding that reduced size is associated with increased predator vulnerability at high temperatures but decreased vulnerability at low temperatures, indicates that body size is not the directly influencing predation risk in this system. Instead, body size change is likely an indirect consequence of modifications in life history strategy or resources allocation to growth and defence. A more detailed knowledge of trait underpinning defence would allow a more mechanistic understanding of the impacts of temperature change on trait dependent demography. Nevertheless, we found that across climate conditions, prey body size variation was consistently associated with changes in species interaction strength. Because it provides a measure of resource allocation to growth, it is likely to be a useful trait to study when identifying energetic trade-offs and trait dependent processes. Overall, this study suggest that environmental, density, trait with metabolic measurements may need to be analysed in the context of metabolic and eco-evolutionary and life history theory in order to understand the impacts of environmental change on community dynamics.

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Appendices

Appendix 1) Trait dependence of community dynamics and trait dynamics

A) Evaluation of temperature specific trait dependent community models

At each temperature along the experimental environmental gradient, we measured species abundance and trait dynamics. These observed dynamics of community abundance and trait values were modelled separately for each temperature level. The changes in abundance were modelled as the combined impacts of resource population growth, consumption and predation. These were additively combined smooth functions of density and trait values (Following the methodology developed in Chapter 4). Rival hypotheses about the trait dependence of community dynamics were compared at each temperate and the variables giving with the lowest overall prediction error were identified using cross validation. Here we present summary figures describing the trait dependence of vital rates and the density dependence of trait change (Fig.S5.1:S5.6).

At all temperatures, the inclusion of information about the dynamics of prey body size and resource composition produced the most significant reduction in the prediction error of the empirically derived community models. Changes in prey body size influenced community dynamics by influencing both the consumption of resources by prey and the consumption of prey by predators. Comparing between these model outputs it appears that warming modified the trait dependence of the predator and prey's consumption. At lower temperatures, decreased prey body size generally reduces resource consumption and the loss rate to predation. Conversely at the warmest temperatures (22-24 degrees), decreased prey body size generally increased resource consumption and the loss rate to predation. A full temperature dependent community model was constructed to investigate the effect of warming on the trait dependence of species interactions (see Fig.5.3 & Fig.5.4 in main text).

Across the temperature gradient, changes in prey body size were influenced by species densities, producing feedbacks between trait and ecological dynamics. At low temperatures, high predator density was associated with a reduction in prey body size. This was reversed at the highest two temperatures. High predator density was associated with increased body size. The results indicate that the body size responds differently to changes in predation rates. Across temperatures, a trade-off appears to be operating between growth and defence. This would drive temporal variation in demographic rates.
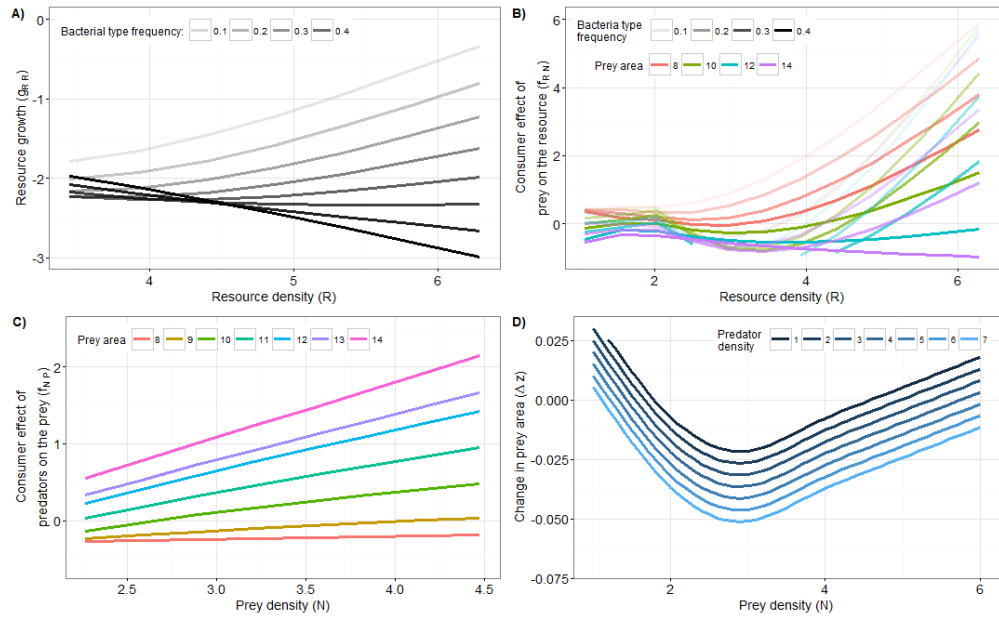
Figure 5.S2) Communities at 14 degrees: Trait and density dependence of community processes and trait change, identified in the empirically derived community model. The model showed: A) density dependent resource growth, which varied with the resource composition (line transparency), B) prey consumption of resources , which varied with prey area (line wavelength colour) and resource composition, C) predator consumption of prey, which varied with prey area, and D) change in prey area trait, which was dependent on predator and prey density (brightness of blue).
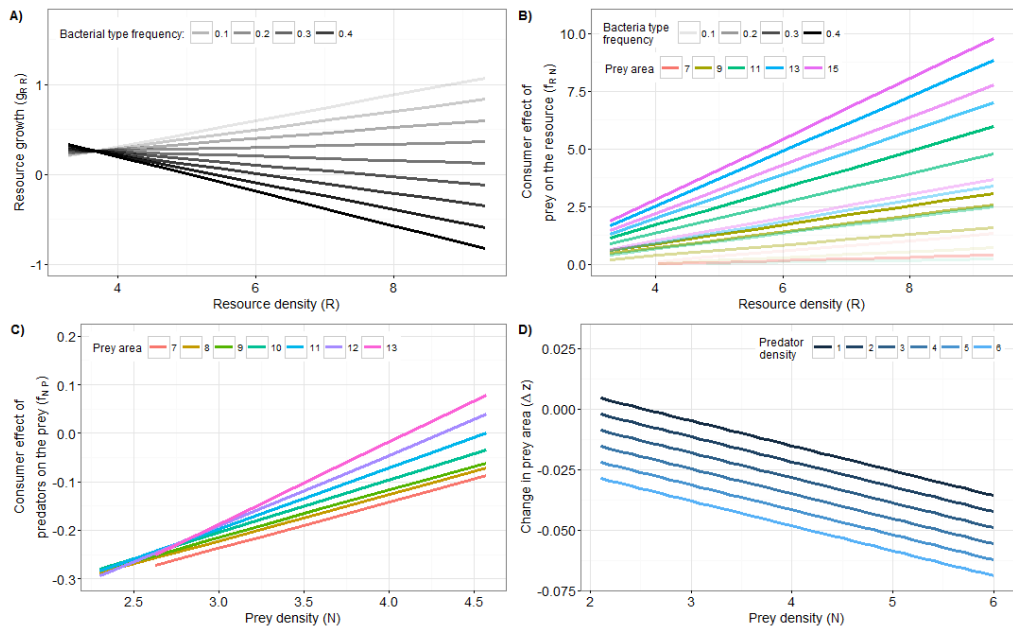
Figure 5.S3) Communities at 16 degrees: Trait and density dependence of community processes and trait change, identified in the empirically derived community model. The model showed: A) density dependent resource growth, which varied with the resource composition (line transparency), B) prey consumption of resources, which varied with prey area (line wavelength colour) and resource composition, C) predator consumption of prey, which varied with prey area, and D) change in prey area trait, which was dependent on predator and prey density (brightness of blue).
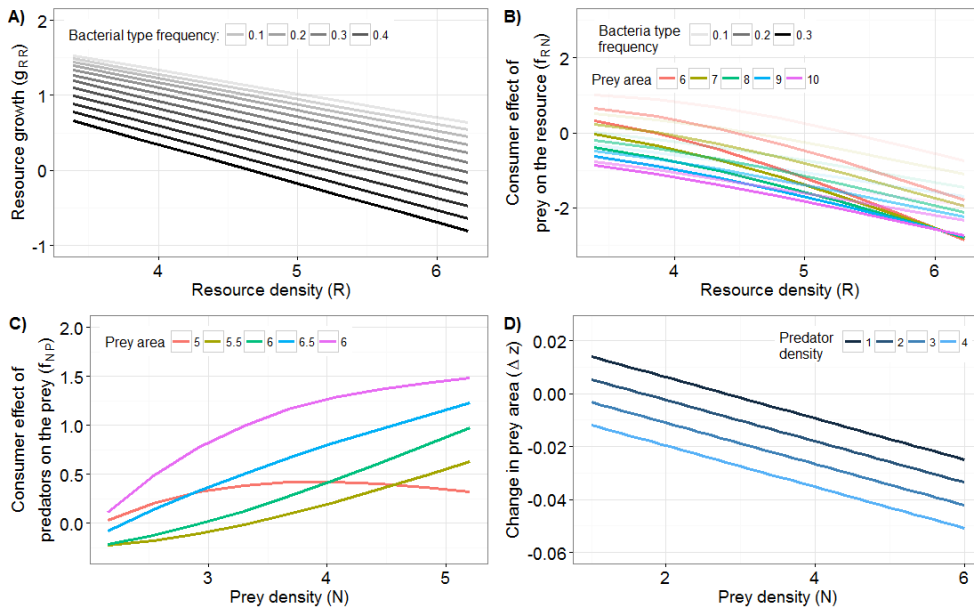
Figure 5.S4) Communities at 18 degrees: Trait and density dependence of community processes and trait change, identified in the empirically derived community model. The model showed: A) density dependent resource growth, which varied with the resource composition (line transparency), B) prey consumption of resources, which varied with prey area (line wavelength colour) and resource composition, C) predator consumption of prey, which varied with prey area, and D) change in prey area trait, which was dependent on predator and prey density (brightness of blue).
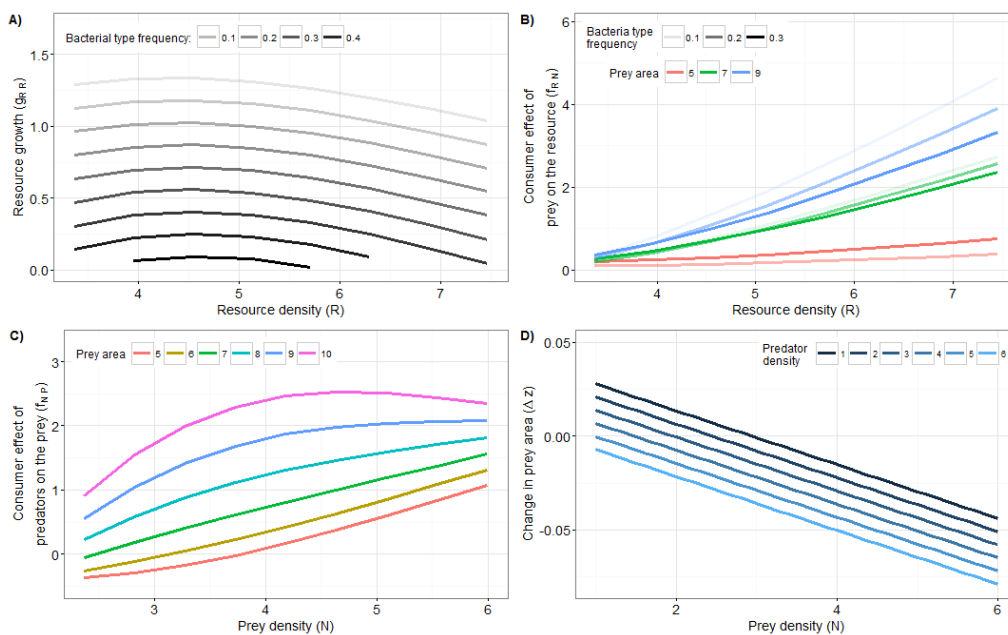
Figure 5.S5) Communities at 20 degrees: Trait and density dependence of community processes and trait change, identified in the empirically derived community model. The model showed: A) density dependent resource growth, which varied with the resource composition (line transparency), B) prey consumption of resources, which varied with prey area (line wavelength colour) and resource composition, C) predator consumption of prey, which varied with prey area, and D) change in prey area trait, which was dependent on predator and prey density brightness of blue).
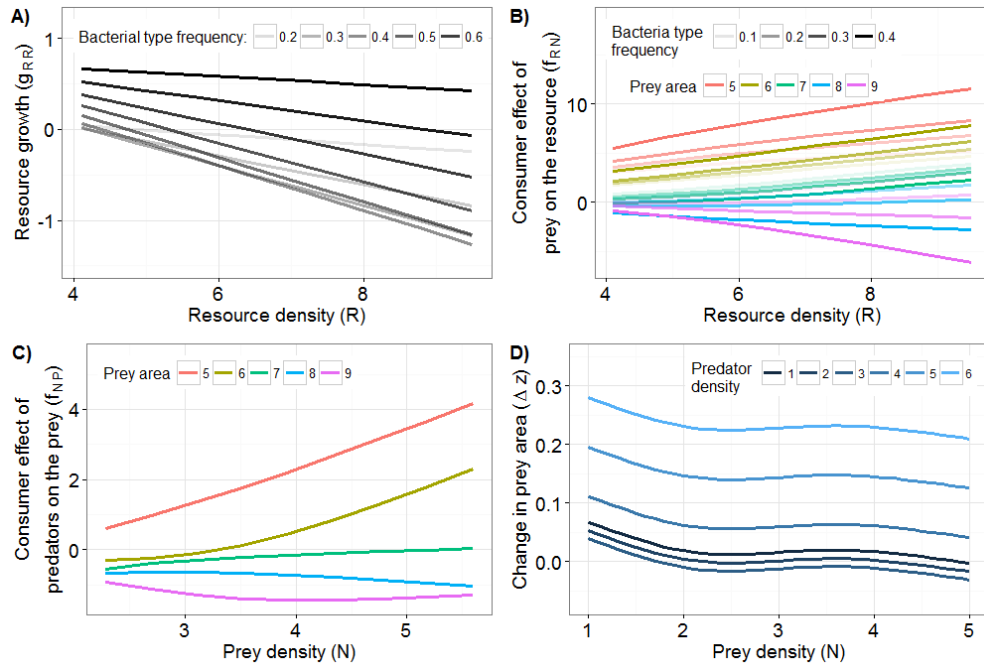
Figure 5.S6) Communities at 22 degrees: Trait and density dependence of community processes and trait change, identified in the empirically derived community model. The model showed: A) density dependent resource growth, which varied with the resource composition (line transparency), B) prey consumption of resources, which varied with prey area (line wavelength colour) and resource composition, C) predator consumption of prey, which varied with prey area, and D) change in prey area trait, which was dependent on predator and prey density (brightness of blue).
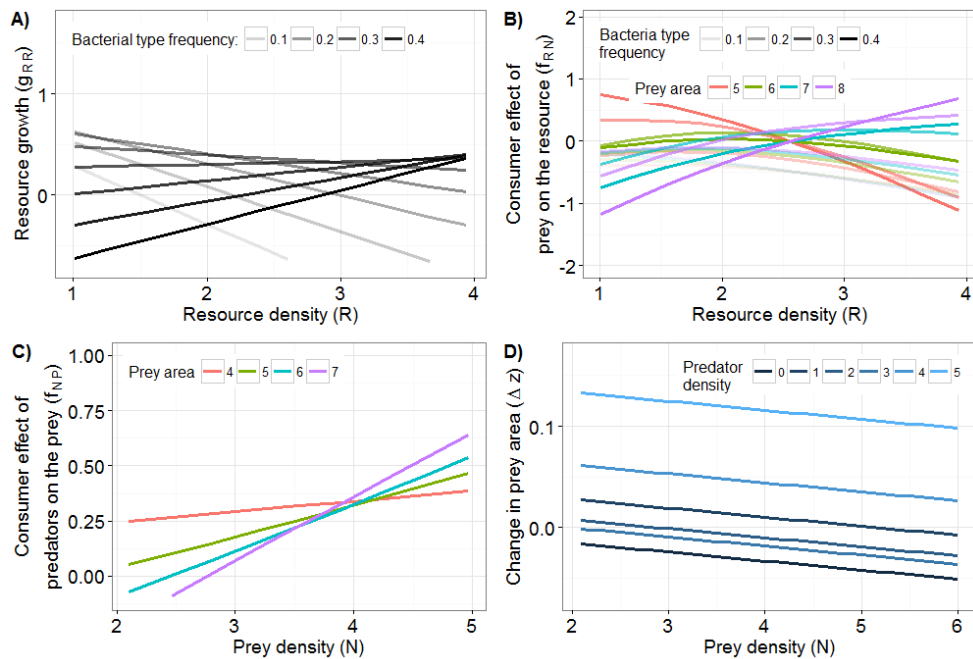
Figure 5.S7) Communities at 24 degrees: Trait and density dependence of community processes and trait change, identified in the empirically derived community model. The model showed: A) density dependent resource growth, which varied with the resource composition (line transparency), B) prey consumption of resources, which varied with prey area (line wavelength colour) and resource composition, C) predator consumption of prey, which varied with prey area, and D) change in prey area trait, which was dependent on predator and prey density (brightness of blue).

B) Additional trait dynamics

In addition to body size, other prey traits were measured for replicate populations over the course of the experiment (Fig.S.7). Their dynamics were not associated with changes in species abundance, however there were some fluctuations in the trait dynamics that were consistent across replicates. A decreasing swimming speed was observed at all temperatures during the first 30 days. A second peak in swimming speed was apparent at lower temperatures around day 50. A similar pattern was observed for the dispersal rate. Finally, the turning variability showed less clear trends over time or across the temperature gradient.
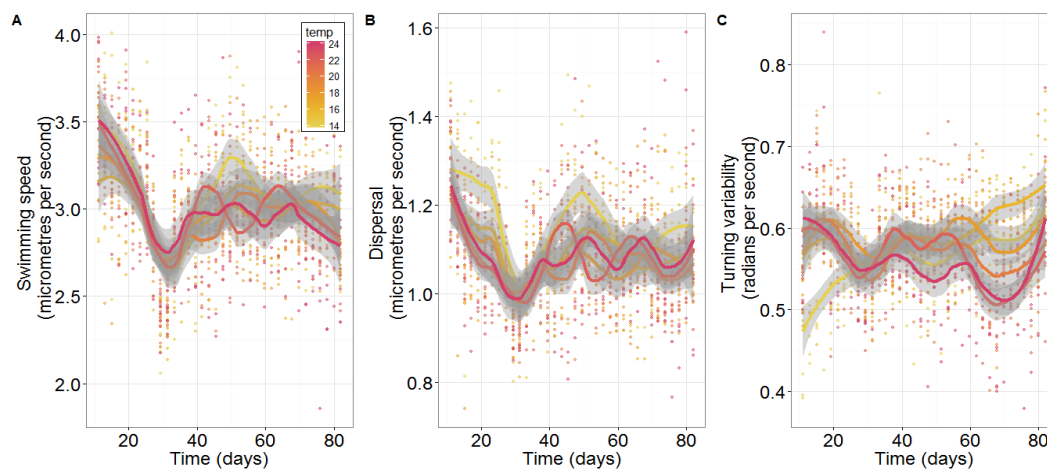
Figure 5.S8) The effects of temperature on the dynamics of A) prey swimming speed, B) prey dispersal rate and C) prey turning variability. Observations from replicates at each temperature are plotted over time (coloured points) and population trends are indicated by smoothed population trajectories (coloured lines; these are local polynomial regressions and confidence bands).

C) Body size in predated and un-predated treatments at different temperatures.

Prey body size dynamics were fairly consistent between replicates that were exposed to the same temperature and predation treatment. Across temperature and predation treatments, prey body size decreased over the duration of the experiment. The body size declines were greatest at high temperatures. Notably, at all temperatures, prey that were exposed to predation decreased in size more than those that were not exposed.
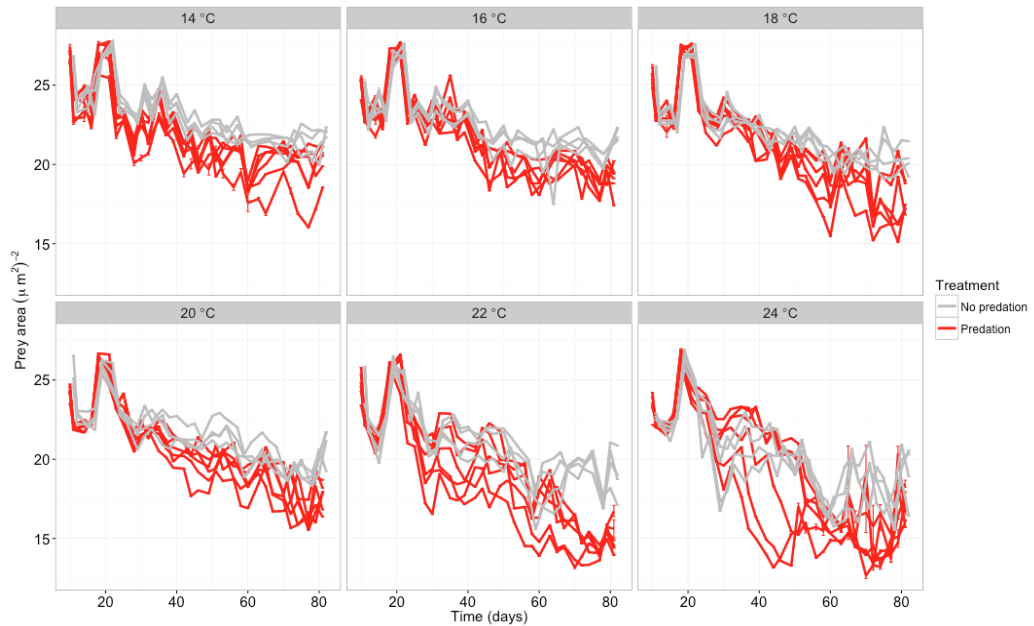
Figure 5.S 9) The effects of temperature on the dynamics of prey body size in treatments that are exposed to predation (red) or not exposed to predation (grey).For each temperature, the dynamics of mean body size are shown for each replicate in the predation treatments (Solid lines). Error bars at each time point (often very small) indicate the standard error of body size measurements in that sample.

Chapter 6: Do mark-recapture analyses using AIC model comparisons produce reliable inferences about the climate dependence of demographic rates?

Jason I. Griffiths, Robert P. Freckleton, Karl L. Evans, Ben J. Hatchwell, Philippa R. Gullett & Dylan Z. Childs

<u>Abstract</u>

1. Impacts of climate on demographic rates (e.g. annual survival) are frequently inferred by comparing the performance of alternative mark-recapture climate models at explaining individual encounter histories. Information theoretic methods, such as AIC, are often used. Reliable inference requires identification of appropriate climate models and accurate estimation of climate effects. Unmeasured processes complicate inferences by introducing random year-specific deviations in survival. Furthermore, non-independence of observations within years complicates definitions of sample sizes, potentially substantially biasing conclusions.

2. A review of recent mark-recapture studies of climatic dependent survival revealed that 87% of information theoretic based investigations use a standard fixed effects likelihood framework. This ignores unmeasured processes that could influence survival. The remaining 13% constructed random effects models to account for these processes using either the method of moments or marginal likelihood random effects frameworks. Such studies usually calculate sample size as the number of released individuals rather than the number of years of study (which is justifiable in the context of climate-survival relationships).

3. We simulated mark-recapture datasets with a known climatic dependence of survival and analysed them using each likelihood framework and sample size definition. The fixed effects analyses rarely supported the data-generating survival model, frequently identified spurious climate variables and underestimated genuine climatic effects. Incorporating random effects using the method of moment was ineffective. Favourably, the marginal likelihood random effects framework consistently allowed identification and unbiased estimation of climatic effects. Defining the effective sample size as the number of year of study, rather than the number of observations, generated more reliable inference.

4. Analyses of a real world long-term dataset highlighted the impact of these methodological choices on our inferences of climatic dependence and future survival projections. Using standard methodologies, spurious climate effects were detected and climate effects misestimated.

5. Overall, we argue that numerous studies use inadequate methods to estimate effects of climate change on survival. To reliably detect climatic dependencies and avoid incorrectly attributing random variation in survival to climate variables, marginal likelihood random effects analyses are required in which the sample size is defined as the number of occasions when survival is estimated.

Key words*:* Climate change, Survival, Capture-mark-recapture, Demographic modelling, Alternative likelihood frameworks, Environmental stochasticity, Information theoretic approach, Marginal likelihood Random effects, Long-tailed tit, Aegithalos caudatus

Introduction

Climatic conditions strongly influence species' demographic rates and local abundances (Sæther *et al*. 2000; Walther *et al*. 2002; Jenouvrier 2013), but identifying and quantifying the climatic variables with the greatest impact is challenging (Sæther *et al.* 2004; Ådahl, Lundberg & Jonzén 2006). Multiple climatic variables may interact to influence demographic rates (Parmesan *et al*. 2013; Griffiths *et al*. 2014), specific seasonal conditions may be highly influential (Ireland *et al*. 2004; Luis *et al*. 2010; Jansen *et al*. 2014) and numerous unmeasured stochastic factors will produce additional chance variation (Sæther 1997; Jonzén *et al*. 2010). Analyses are increasingly undertaken to project species responses to forecasted climate changes (IPCC 2013; Wolf *et al*. 2010; Ruete *et al*. 2012; Jenouvrier *et al*. 2009, 2012), but for inferences and projections to be informative we require methods that can: (1) identify the true climatic dependencies of demographic rates from a suite of possible climatic drivers; and (2) accurately quantify the effect sizes of these climatic impacts.

This paper focuses on methods used when data are observational and there is little prior knowledge of the prevailing climatic dependencies of demography. In this situation, competing potential climate hypotheses must be formalized into statistical models and compared in their relative consistency with data (Burnham *et al.* 2011). Alternatively, when a clearly defined hypothesis can be defined about the climate dependence of demography, well established methodologies are available (Gimenez *et al.* 2007; Gimenez *et al.* 2012; Grosbois *et al.* 2008; Lebreton *et al.* 2012; Frederiksen *et al.* 2014). The reliability of demographic analyses used in the former context remain largely unevaluated.

To study the demography of wild populations, mark recapture field studies are frequently undertaken. To identify climatic drivers of variation in survival rates, capture mark recapture (CMR) models are commonly constructed to describe a suite of hypotheses about climate dependent survival. These can be parameterized using data on the encounters of marked individuals over a study period. A likelihood of each model is obtained, as well as estimates of the postulated climatic effects (Lebreton *et al.* 1992; Maunder *et al.* 2009). The relative strength of support for each climate hypothesis can be measured using information theoretic model comparison, commonly based on Akaike's information criterion (AIC) or a corrected form (e.g. QAIC or AICc) (Akaike 1973; Burnham & Anderson 2002). These criteria penalize a model likelihood term based on the model's complexity.

When information theoretic comparisons are applied to sets of models parameterised using a limited dataset, a finite sample size correction term is required (Burnham & Anderson 2002). This applies stronger penalties to complex models when data is sparse, to prevent overfitting. However, determining the number of effectively independent samples in a CMR climate analyses is not straightforward (Kendall & Bjorkland 2001). Many individuals can be observed each year, suggesting a very large sample size, however they all experience the common year-specific climate conditions and therefore have non-independent survival. When inferring climate drivers of variation in survival over time, we propose that the effective sample size may more justifiably be defined as the number of occasions in which climate variables can be related to survival (Lukacs *et al.* 2004; Grosbois *et al.* 2008). In contrast, mark-recapture programs frequently used to perform climate analyses (MARK and E-SURGE) define the sample size as the number of individuals released prior to the final capture occasion. This implies that each recapture provides independent information to infer the climate dependence of demography. By ignoring the non-independence of individuals' environments, the sample size may be vastly overestimated. This would cause information theoretic corrections to insufficiently penalize overly complex models resulting in the frequent detection of spurious relationships between survival and climate.

Information theoretic approaches are used for two purposes: firstly to identify climatic factors influencing survival and secondly to make predictions of climate dependent survival. However, information theoretic approaches are conceptually designed to produce a good predictive model, and not necessarily to reliably identify climatic impacts on survival, though the latter is the context in which they are frequently applied. Grosbois *et al*. (2008) note that the efficacy of AIC to identify influential climatic variables has received little evaluation. Nevertheless, such

approaches are widely applied as they are thought to alleviate the problems of multiple hypothesis testing and account for model uncertainty through model averaging (Burnham *et al.* 2011).

The construction of CMR models describing the climate dependence of demography is also complicated by the ubiquitous presence of unmeasured environmental factors that have demographic effects (e.g. extreme weather events or disease outbreaks)(Grosbois *et al.* 2008; Frederiksen *et al.* 2014). Reliable inference of the climate dependence of demographic rates requires that inter-annual variation in these rates is correctly attributed to climatic causes and not inflated by attribution of variation resulting from unmeasured factors (Lebreton *et al.* 2012). The consequence of this can be the frequent identification of spurious climate relationships. A review by Grosbois *et al*. (2008) proposed that information theoretic based analyses of climatic impacts upon survival may be greatly improved by utilizing random effects CMR models to describe the impacts of unmeasured factors as random year effects. In contrast, CMR climate implemented in commonly used programs such as MARK (White & Burnham 1999) or E-SURGE (Choquet *et al.* 2009) are usually performed using a fixed effects likelihood framework in which, conditional on measured climate variables, the values of model coefficients, such as mean survival rate, are assumed to be constant across years. This assumes that temporal variation in demographic rates is completely determined by the climatic variables included in the model, with additional variation resulting only from observational uncertainty (Lebreton *et al.* 1992). Since every climatic process influencing demography cannot be measured, the assumption that other stochastic processes are negligible is likely to be frequently violated.

We conducted a review of subsequently published studies (2008-2015) investigating the climatic dependence of survival using mark-recapture methodologies (Supplement 1). We focused on studies where multiple climatic variables were considered as drivers of variation in survival and information theoretic model selection was carried out to identify climate drivers. Of the 71 relevant studies, only 9 used a random effects framework to account for unmeasured environmental causes of inter-annual variation in survival and this has been achieved using two fundamentally different frameworks (Supplement 1b). First, the method of moments (MOM) framework initially fits time-varying survival models, and then uses shrinkage estimation and variance components analyses to attribute variation in survival to explicitly modelled covariates and stochastic effects (Franklin *et al.* 2000; Burnham & White 2002; Loison *et al.* 2002; Royle & Link 2002). Secondly, the marginal likelihood framework specifies a

hierarchical model to describe stochastic inter-annual variation and uses a numerical integration scheme to marginalise the likelihood with respect to these random effects (Maunder *et al*. 2009). The MOM approach is relatively easily implemented, whilst the marginal likelihood framework requires the numerical integration of a high dimensional integral, making it more difficult and rarely employed (but Laake *et al.* 2013 provides a tool to aid this). Furthermore, the sample size in information theoretic model comparison calculations was almost always defined as the number of individuals released (but see Lukacs *et al*. 2004 and Grosbois *et al*. 2008 for alternate calculations).

We evaluated the reliability of the CMR analyses in identifying and quantifying the climate dependent demography. Firstly we examined the performance of the three likelihood frameworks for relating survival to climate: (i) the fixed effects framework, (ii) the method of moments random effects framework, and (iii) the marginal likelihood random effects framework. Secondly, we evaluated whether information theoretic model selection should be applied using corrections for sample size based on either a) the number of individuals released or b) the number of years of study. We use individual based simulations to generate mark-recapture datasets with a known climatic dependence of survival. We then used each combination of likelihood framework and information theoretic sample size correction to perform CMR climate analyses. We assessed the ability of each framework to: distinguish among candidate hypotheses, detect the data-generating model and quantify climatic effects on survival. The concordance of the inferred climate dependencies of survival with the simulated causal processes provides a direct test of the performance of the CMR frameworks (Taper *et al.* 2008). Finally, we use a 19-year real world dataset of avian survival to illustrate how the choice of framework influences our inference about the climatic dependence of avian survival and the projections of future rates.

Methods

We used three types of datasets to test how the choice of likelihood modelling framework and sample size definition influences the reliability of inferences. The first two datasets were derived from two individual-based simulation models in which climatic impacts on survival were either present or absent. We varied the number of years of observations and the number of individuals that were captured and marked each year. This variation reflected the range of research effort observed in the published studies included in our literature review. We also

varied the magnitude of environmental stochasticity, which encompasses the year-specific variation in survival caused by unmeasured sources. The third dataset comprised real-world data on survival rates in an individually colour ringed long-tailed tit (*Aegithalos caudatus*) population in the Rivelin Valley, Sheffield (UK).

We applied three mark-recapture modelling frameworks to each dataset. For all simulations, a common model set that captured climate dependencies was parameterized. To compare the performance of these candidate climatic models, an information theoretic model comparison was used, with a correction applied for small sample sizes. To investigate whether the effective sample size of analyses is more appropriately defined as the number of individual releases or the number of years of study, the model comparison process was repeated under each assumption. Model averaging was then applied over the model set. An equivalent methodology was then applied to the long-tailed tit dataset, and the inferences of each CMR framework were used to predict future climate dependent survival under a range of climate conditions.

SIMULATION ANALYSES

Individual-based simulations were used to generate encounter history datasets with known sources of variation in survival. The first scenario was used to assess the ability of the alternative mark-recapture frameworks to identify support for the data-generating climatic model (test 1). The second scenario was used to determine the accuracy of estimated climatic effects (test 2).

Simulation of climatic dependent survival and encounter history observation

Individual-based simulations of survival and re-sighting were made under a spatially closed population, two-sex Cormack-Jolly-Seber (CJS) model (Appendix.2). Individual survival was simulated based on year-specific survival rates, which depended on climate covariate measurements and also unmeasured stochastic processes. Standardised measurements of four climate variables ($X_{1:4}$) were simulated as independent random variables drawn from a normal distribution, with a mean of zero and standard deviation of one: $X_i \sim N(0,1)$. The logit-linear impacts of climatic conditions on survival were defined by the parameter β_{X_i}.

Under the first simulation scenario, survival and re-sighting were independent of all measured climate variables. Year-specific survival ($\phi(t)$) varied randomly around the mean annual survival probability (μ_ϕ), with the intensity of variation depending on the environmental stochasticity parameter (σ_ϕ). In the second set of simulations, year-specific survival was dependent upon a single climatic variable (X_{dep}) but independent of all other climate variables (X_{indep}) (i.e. only one of the model covariates reflect the causes of variation in survival). Year-specific survival ($\phi(t)$) varied randomly, on the logit scale, around the expected annual survival probability ($\mu_\phi + X_i \beta_{X_i}$). Year-specific impacts of unmeasured processes were incorporated by adding normally distributed year-specific deviation ($\varepsilon_{\phi(t)}$), again with an intensity determined by the environmental stochasticity parameter (σ_ϕ). Therefore, in both simulations the year-specific survival ($\phi(t)$) was calculated as: $\phi(t) = \frac{1}{1+exp^{-\left(\mu_\phi + (\Sigma_{i=1}^{n}(X_i * \beta_{X_i})) + \varepsilon_{\phi(t)}\right)}}$, where $\varepsilon_{\phi(t)} \sim N(0, \sigma_\phi)$.

An individual's annual survival from time t to t+1 ($S_{i,t}$) was simulated, using the year-specific survival probability ($\phi(t)$) as the success probability parameter of a Bernoulli distribution: $S_{i,t} \sim Bern(\phi(t))$. Re-sighting of surviving individuals ($Obs_{i,t}|S_{i,t}$) was then simulated as a second Bernoulli process with a constant probability of re-sighting (μ_p): $Obs_{i,t}|S_{i,t} \sim Bern(\mu_p)$. Encounter histories were simulated for equal numbers of male and female individuals and both sexes had a true mean annual survival probability (μ_ϕ) of 0.5 and re-sighting probability (μ_p) of 0.9.

Defining the candidate model set

A set of 25 candidate CJS models were constructed, under each of the three alternate mark-recapture frameworks: fixed effects, marginal likelihood random effects, and MOM random effects. The models formalise a common set of competing hypotheses about the climatic dependence of survival underlying our simulated datasets. The set included the data-generating climate model and a suite of plausible candidate models defining alternate hypotheses about the climate dependence of survival. Specifically, we first formulated a candidate model, in which year-specific survival ($\phi(t)$) depends on the mean survival rate (μ_ϕ), the additive effects of four climatic variables (β_{X_i}), and the interactions between two pairs of these variables ($\beta_{X_{ij}}$):

$$\mu_\phi + \Sigma_{i=1}^{n} \beta_{X_i} X_i = \mu_\phi + \beta_{X_1} X_1 + \beta_{X_2} X_2 + \beta_{X_3} X_3 + \beta_{X_4} X_4 + \beta_{X_{12}}(X_1.X_2) + \beta_{X_{34}}(X_3.X_4))$$

We then constructed all 24 nested models which can be derived from this model. The re-sighting probability was modelled as a climate independent constant.

Mark-recapture analyses under alternate likelihood frameworks

Under each likelihood modelling framework, the candidate set of 25 models was parameterised for each simulated dataset. The frameworks differ in whether, and how, they account for impacts of unmeasured sources of variation in survival and also the way they calculate the effective number of parameters to be estimated.

Fixed effects models were fitted by assuming that model coefficients are constant across years. Under this approach, encounter histories provide information that M_j individuals are marked at time j and that of these individuals, R_{ji} are first recaptured at time i. Under the assumptions of a CJS model, the probability of an individual marked at time j being recaptured in time i, but not before, can be calculated $(Pr_{j,i} = \phi(i)p(i)\prod_{t=j}^{i-1}\phi(t)(1-p(t)))$ and a likelihood function defined. Fixed effects models were fitted using the widely used mark recapture software MARK and the R package "RMark" (Laake 2013; R Core Team 2014).

The two random effects frameworks account for inter-annual variation in survival that cannot be explained by the covariates, but do so in different ways. Under the MOM random effects framework, a fully time-dependent fixed effects survival model is first parameterised. From this, a conditional maximum likelihood estimate of mean survival $\hat{S}$ can be obtained. Subsequently, variance components estimation is applied using derived estimates of year-specific survival S_i and estimated variance-covariance matrix of temporal deviations in survival. This allows time-varying covariates to be accommodated while simultaneously estimating the environmental stochasticity (Burnham & White 2002). The MOM random effects models were implemented using "RMark".

Under the marginal likelihood random effects framework, year-specific deviations $(\varepsilon_{\phi(t)})$ from the mean survival rate (μ_ϕ) followed a normal distribution (on a logit scale), where $\varepsilon_{\phi(t)} \sim N(0, \sigma_\phi)$ (Royle & Link 2002). The marginal likelihood random effects framework was implemented using the automatic differentiation model builder (ADMB) software which employs the Laplace approximation to approximate the high-dimensional integral associated with the random effects (Fournier *et al.* 2012). Simulations were automated through

generation of input files in R and interfacing with ADMB was then achieved using the "R2admb" R package (Bolker, Skaug & Laake 2013).

Model comparison

An information theoretic approach was used to compare the support for each model in the candidate set in each CMR analysis (Burnham & Anderson 2002). Model comparison was based on AICc scores: $AICc = -2L + 2k + \frac{2k(k+1)}{n-k-1}$. This approach compares model likelihoods (*L*) whilst penalising based on the effective number of estimated parameters (*k*) and correcting for small sample sizes (*n*) (Burnham & Anderson 2002). Notably, the CMR frameworks differ in how they define the number of estimated parameters (*k*). Under the fixed effects framework, *k* is simply the total number of model coefficients, whilst under the marginal likelihood framework, *k* is the number of fixed effects parameters plus one random effect parameter for the variance(σ_ϕ). Finally, the MOM framework estimates *k* based on the dimension of random effects parameter space (Hodges & Sargent 2001; Burnham & White 2002).

The effective sample size (*n*) in AICc comparisons of CMR models can be defined as either: a) the number of individuals released prior to the last capture occasion (*n*=n_{ind}) or b) the number of years of study (*n*=n_{yrs}). If the first definition is used, the sample size will be much larger and the correction for having a limited amount of data will be negligible. For example, consider a situation in which a very large number of individuals were observed over just three years. In a CMR analysis linking inter-annual variation in survival to climatic drivers, there is very little information to determine the factors driving variation between years, as all individuals have experienced the same conditions over just a few years. However, standard analyses implemented using common mark-recapture software would define the sample size to be very large and would not apply a strong AIC penalty term to correct for data limitation. Therefore, when the aim is to identify the climatic drivers of variation in survival between years, it may be more appropriate to define the sample size of effectively independent data points as the number of years of study, rather than the number of individuals released. To determine which definition is most appropriate, we repeated the AICc calculations and subsequent analyses under each assumption.

The relative support for each candidate model in a set was determined by calculating $\Delta AICc$ scores: $AICc_i - AICc_{min}$. This quantifies the difference in information discrepancy of model *i*

(AICc$_i$) relative to that of the "best" model (AIC_{min}). Akaike model weights were calculated as the relative likelihood of model *i* divided by the sum of these values across the candidate set: $\text{Weight}_i = (\exp\left((AIC_{min} - AIC_i)/2\right))/\sum_1^i(\exp\left((AIC_{min} - AIC_i)/2\right))$.

MODEL ADEQUACY TEST 1: What is the probability of detecting support for the data-generating model?

In this first simulation test, the true effect size of measured covariates on survival (β_{X_i}) was zero and inter-annual variation in survival was due to the effects of unmeasured processes. A range of encounter history datasets were simulated which varied in the duration of the mark recapture study (x_{yrs}), the number of individuals marked on each capture occasion (x_{inds}), and the intensity of environmental stochasticity (x_{stoch}) (Table.6.1). Two realisations were simulated at each of the 764 different combinations of these three variables.

Table 6.1) Simulation variables factorially manipulated in simulation test 1, to generate encounter history datasets reflecting a range of mark-recapture study conditions. The values of x_{yrs} and x_{inds} reflect the range of duration and intensity of mark-recapture studies reported from the reviewed literature (Appendix.1). The maximum value of x_{stoch} corresponds to a realised variation in year-specific survival between approximately 0.25-0.75.

Variables	Range	Interval
Number of recapture occasions (x_{yrs})	10 - 40	2 year
Number of individuals released (x_{inds})	10 - 54	4 individuals
Intensity of environmental stochasticity (x_{stoch})	0 - 0.5	0.025 units of deviation

These simulations were used to determine whether CMR frameworks vary in their ability to distinguish the amount of support for different hypotheses, and to identify the data-generating model. Successful detection of support for the data-generating model was defined conservatively as this model having an AICc score less than 4 units more than the "best" fitting model. This follows the commonly used rule of thumb proposed by Burnham & Anderson (2002) and Richards (2005). We fitted generalized additive models (GAMs) to summarise the relationships of the probability of successful detection of the data-generating model with the duration of mark-recapture study (x_{yrs}), the numbers of individuals released annually (x_{inds}),

and the intensities of environmental stochasticty (x_{stoch}). Models were implemented in the "mgcv" R package (Wood & Augustin 2002). The conclusions are not affected by choice of the AICc threshold for defining sufficient support (Appendix.2).

MODEL ADEQUACY TEST 2: Which frameworks give unbiased estimates of climatic effects on survival?

In the second simulation test, encounter histories were generated in which year-specific survival was dependent upon one climatic variable ($\beta_{X_{dep}}$) but independent of all other climate variables ($\beta_{X_{indep}}$ = 0). Stochastic inter-annual variation in survival was also introduced. We simulated 480 encounter histories for subsequent analysis with each mark-recapture framework. Within these simulations, we followed a two-way factorial design, varying: i) the duration of the mark recapture study and ii) the size of the climatic effect impacting survival ($\beta_{X_{dep}}$) (Table.6.2).

Table 6.2) Simulation variables factorially manipulated in simulation test 2, to generate encounter histories that are relatively long for ecological datasets, representing a gradient towards the best case scenarios of data availability. Two realizations were simulated at each combination of $\beta_{X_{dep}}$ and x_{yrs}. In all simulations, x_{inds} and x_{stoch} were the same.

Variables	Range	Interval
Climate effect impacting survival ($\beta_{X_{dep}}$)	± (0.1 - 0.45)	0.05
Number of recapture occasions (x_{yrs})	30 - 50	5 year
Number of individuals released (x_{inds})	20	-
Intensity of environmental stochasticity (x_{stoch})	0 .3	-

Under each framework, CMR analyses were performed on the simulated encounter histories, AICc model comparison was applied and model weights were used to perform model averaging. This multi-model inference provides overall estimates of the effect size of the climate covariates on survival ($\beta_{X_{est}}$), taking into account model structure uncertainty (Burnham *et al.* 2011). Each candidate model contributes to the overall estimate of the effect size, based on the relative weight of support for that model. Linear regression was used to

model the estimation error of climate effect under each inference framework as a function of the actual climatic effect size in the simulation. The observed estimate ($\beta_{X_{est}}$) was first regressed against the actual climate effect size ($\beta_{X_{dep}}$). The true climatic effect was then subtracted from the model average estimates and model prediction in order to calculate the magnitude and direction of bias in parameter inference ($\beta_{X_{est}} - \beta_{X_{dep}}$).

REAL WORLD MARK-RECAPTURE EXAMPLE: COMPARING ANALYSIS FRAMEWORKS

Study system: Long-tailed tit Mark-Recapture Analyses

We explored how real world conclusions about the climatic dependence of survival are influenced by our choice of CMR framework, by comparing their inferences regarding the climatic dependence of survival in a long-tailed tit population (Location: Rivelin Valley, Sheffield, England (53°38'N 1°56W; altitude =168 m a.s.l., range = 150–270 m). Encounter histories of 985 individuals of genetically confirmed sex were constructed following mark-recapture protocols during the breeding seasons of 1994–2012 (Gullett *et al.* 2014). To investigate how inter-annual variation in seasonal climate influences survival, four seasonal variables relevant to long-tailed tit biology were defined: 1. Spring (March-May), 2. Summer (June-August), 3. Autumn (September-November) and 4. Winter (December-February). For each season, measurements of average local daily temperature and total precipitation were calculated and these climatic covariates were then standardized (mean=0, s.d.=1).

Previous results, using the fixed effects framework, suggested that survival is largely determined by temperature and precipitation levels in Spring (t1 & p1) and Autumn (t3 & p3) and the interaction of these two climate variables in Spring (Gullett *et al.* 2014). A candidate model set was developed to formalize different hypotheses about the climatic dependence of long-tailed tit survival. This set included all 24 nested models of the full model which characterized the seasonal interactions of these two variables:

$$\Phi_t = \frac{1}{1+exp^{-\left(\mu_\Phi + \beta_{X_{t1}}X_{t1} + \beta_{X_{p1}}X_{p1} + \beta_{X_{t3}}X_{t3} + \beta_{X_{p3}}X_{p3} + \beta_{X_{t1.p1}}(X_{t1}.X_{p1}) + \beta_{X_{t3.p3}}(X_{t3}.X_{p3})\right)}}.$$

The same structural model was used as in the original publication by Gullett *et al.* (2014) (Appendix.3). Candidate climatic models were parameterised under each CMR framework: fixed effects, marginal likelihood random effects, and MOM random effects. Model comparison and averaging was applied, to provide overall estimates of the impacts of

temperature and precipitation in Spring and Autumn on long-tailed tit survival. Future long-tailed tit climate dependent survival probability was then predicted using the model average parameter estimates from each framework and based upon different climate change scenarios (as in Gullett *et al.* 2014; see Appendix.3).

Results

Model adequacy test 1 - What is the probability of detecting support for the data-generating model?

Using each combination of CMR framework and sample size definition, the probability of finding support for the data-generating climate model was assessed under a range of mark-recapture study conditions. Regardless of how the AICc score was calculated, the fixed effects framework typically performed poorly compared to the marginal likelihood random effects framework, only having a high probability of supporting the data-generating model under very low levels of environmental stochasticity (Fig.6.1 grey lines). As the amount of environmental stochasticity increased, the probability of successful model detection declined substantially. Analyses of simulated datasets that were longer or had a larger number of individuals released annually exhibited increasingly worse performance. These large datasets contained more information about variation in inter-annual survival which could be falsely attributed to spurious climatic variables.

The MOM framework performed badly (Fig.6.1 blue lines). When the AICc sample size was defined as the number of years of study, it often failed to find support for the data-generating climate model and was not robust to the presence of environmental stochasticity. Alternatively, when the AICc sample size was defined as the number of individuals released, the MOM approach was unable to distinguish rival hypotheses and rarely allowed rejection of any models. Therefore the data-generating model could not actually be detected despite its ΔAICc value being low (Appendix.2). We therefore do not present this method in Figure 6.1A.

The marginal likelihood random effects framework (Fig.6.1 green lines) gave the most reliable inference, having a substantially higher probability of successfully identifying support for the data-generating climate model. Its performance was also robust to the introduction of environmental stochasticity. When the AICc sample size was defined as the number of years of study, the marginal likelihood framework provided the highest probability of detecting the

data-generating model, and this probability did not depend strongly upon the number of years of study or individuals released annually.

A) AICc sample size = number of individuals released

B) AICc sample size = number of years of study
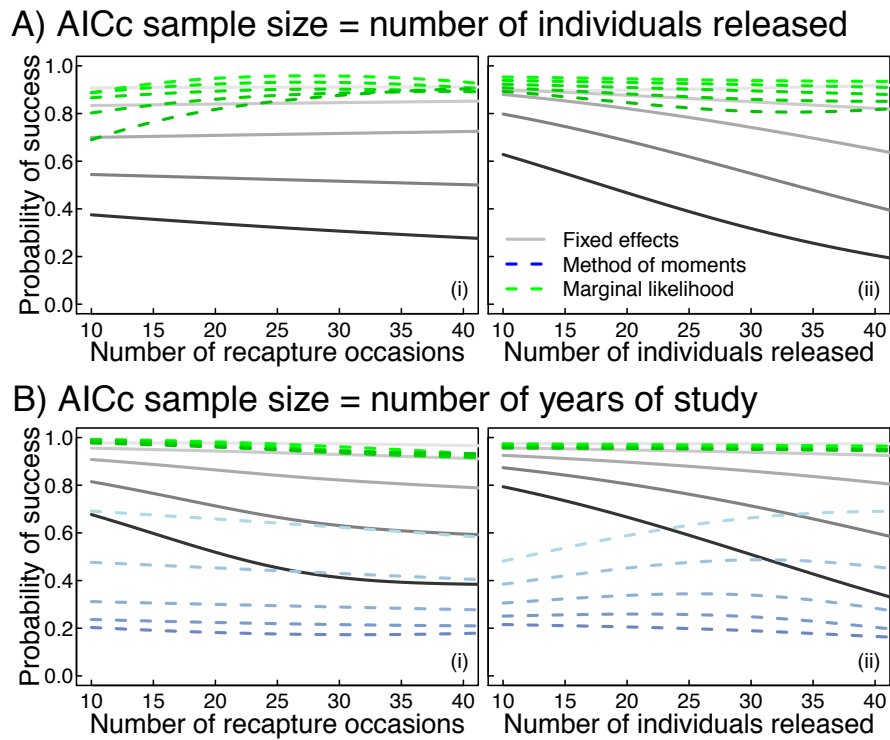
Figure 6.1) The probability of mark-recapture analyses successfully finding support (ΔAICc < 4) for the data-generating model of climatic dependent survival. AICc calculations are made under the assumption that the sample size is: the number of individuals released (subpanel A) or the number of years of study (subpanel B). The probability of success is characterized for each framework: the fixed effects framework (grey lines), the marginal likelihood random effects framework (green dashed lines) or the MOM framework (blue dashed lines). Successful detection is related to the gradient of environmental stochasticity, darker shades indicate higher levels of stochasticty (σ_ϕ 0 - 0.5) and i) the duration of the mark-recapture study, and ii) the number of individuals released at each occasion. The MOM approach is not presented in subpanel A (sample size=n_{inds}) as this approach does not allow models to be distinguished.

Model adequacy test 2 - Estimation bias of alternative mark-recapture frameworks

Using the results of the second simulation study, in which survival was dependent on a single climatic variable, we assessed the estimation accuracy of the alternative CMR frameworks by comparing the model averaged climate effect estimate obtained from the CMR analysis of each simulation to the known climate effect size underlying simulations (Fig.6.2). The bias of the three frameworks was independent of how the AICc score was calculated, probably because the number of years of study in these simulations was reasonably large ($n_{yrs} \geq 30$) and greater than the number of years in many datasets generated in the first simulation ($10 \leq n_{yrs} \leq 40$). Both the fixed effects and the MOM frameworks showed strong systematic

estimation bias, consistently underestimating the magnitude of the actual climatic effect ($\beta_{X_{dep}}$). The MOM framework performed the worst, inferring only very small climatic effects, even when the true effects were large. The marginal likelihood framework performed substantially better, with no biologically significant bias occurring across the range of true effect sizes. These results did not depend on the duration of the synthetic dataset.
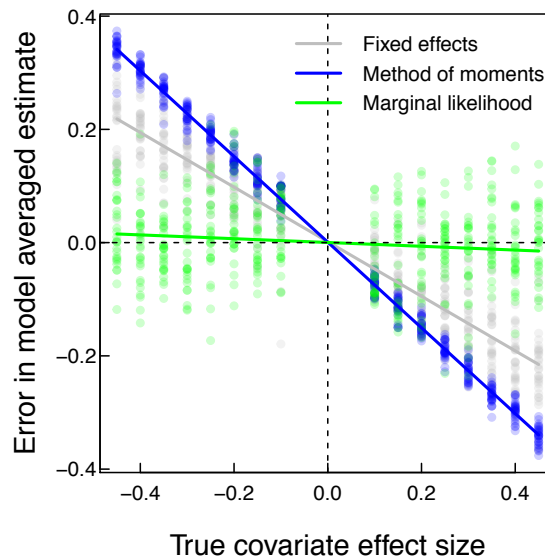
Figure 6.2) The accuracy of inferred climatic effects under the alternative frameworks as a function of the simulated climatic effect size. The error in model averaged estimates of the true climatic effect size ($\beta_{X_{est}} - \beta_{X_{dep}}$) is shown for: fixed effects framework (grey), the method of moments framework (blue) and the marginal likelihood random effects frameworks (green). Each point represents the error for a single simulation and solid lines show linear model fits. Patterns of bias are independent of the choice of AICc sample size definition and here we present results assuming the sample size is the number of years of study.

Real world example - Impact of framework choice on inferences about climate dependence of survival and future projections

Depending on how AICc scores were calculated and the CMR framework applied, we obtained contrasting inferences about the climate factors related to long-tailed tit survival (see Appendix.3 for details). When AICc scores were calculated by assuming that the sample size is the number years of study, as our simulations and reasoning suggests, the fixed effects and random effects analysis give strikingly different results. Fixed effects analysis suggested that survival was likely influenced by the interaction between temperature and precipitation in Spring and possibly also Autumn. In contrast, the marginal likelihood random effects analysis strongly indicated that survival was related only to the level of Autumn precipitation. Other

hypotheses received minor support (ΔAICc > 4). These inferences are supported by a diagnostic regression tool which we present in Appendix.4. As in the simulations, the methods of moments analyses do not allow hypotheses to be distinguished.

After model averaging, the magnitude of climatic effects on survival strongly differed depending on the CMR framework and the AICc sample size definition (Fig.6.3). As in our simulation studies, larger effects of the climate variables on survival were identified under the marginal likelihood random effects framework compared to the fixed effects framework, suggesting that the fixed effects framework underestimates genuine climate effects whilst also identifying several spurious relationships. This probably results from the inability of fixed effects analyses to attribute variation in survival caused by unmeasured factors to a source other than those unrelated measured variables included in the model. The projections of future climate-dependent long-tailed tit survival therefore depended strongly on the CMR framework applied and how AICc sample size was defined (Appendix.3).
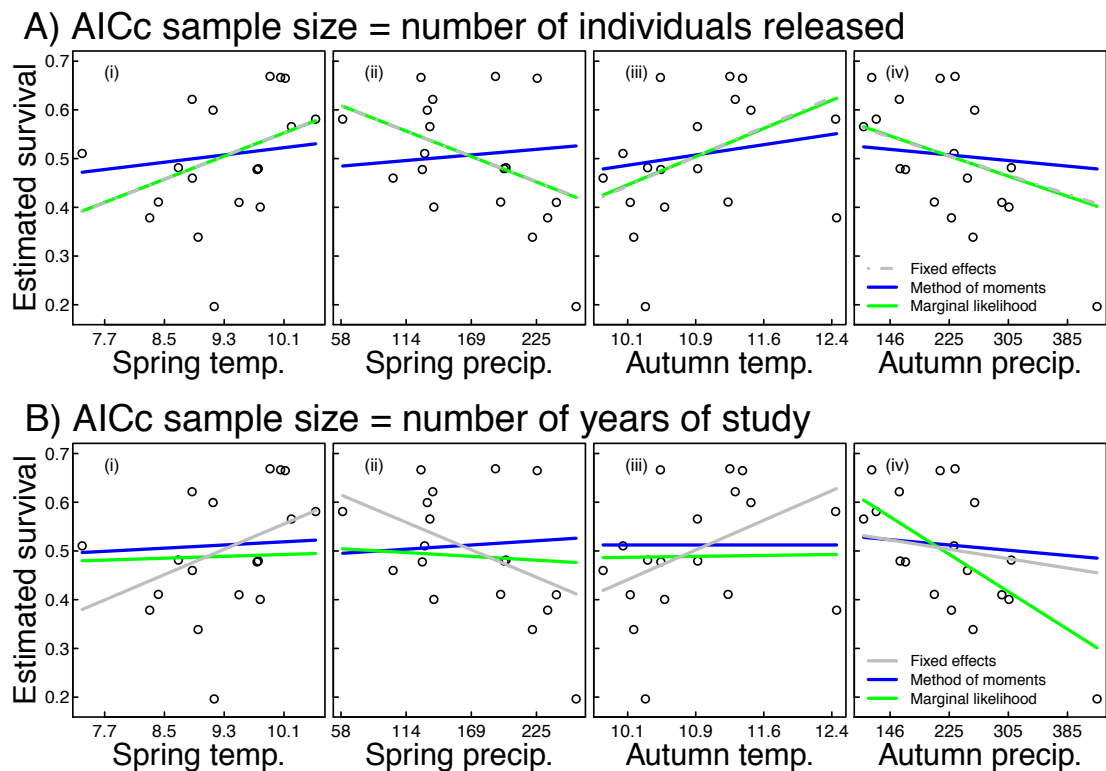
Figure 6.3) Relationships between estimated long-tailed tit survival (open circles) and four climate variables: (i) mean Spring temperature (°C), (ii) total Spring precipitation (mm), (iii) mean Autumn temperature (°C) and (iv) total Autumn precipitation (mm). Solid lines indicate model-averaged survival estimates, predicted over the range in climate experienced during the study. Model comparison and averaging calculations are made under the assumption that the sample size is the number of individuals released (A), or the number of years of study (B). The relationships between survival and climate is predicted for each framework: the fixed effects framework (grey lines), the marginal likelihood random effects framework (green lines) or the method of moments framework (blue lines).

Discussion

We present a thorough evaluation of the reliability of mark-recapture methods at inferring the climatic dependence of demographic rates. Three mark-recapture analysis frameworks were compared under two definitions of sample size in AICc model comparisons. We demonstrate that both the choice of likelihood framework, used to account for unmeasured processes, and the assumptions made about the extent of data independence substantially influence conclusions about past survival and quantitative projections into the future.

Simulation analyses showed that the fixed effects analyses generally performed poorly, having a low probability of identifying the true climate variables influencing survival. Unless a suitable random effects framework is applied, spurious climatic relationships are likely to be detected. The magnitudes of genuine climatic effects are also likely to be increasingly underestimated as the true effect size increases. Biased inferences likely occur because the impacts of unmeasured factors, responsible for significant inter-annual variation in survival, are not appropriately partitioned. The marginal likelihood framework provides a robust method to incorporate year-specific random effects of unmeasured factors into CMR models, allowing accurate and unbiased inference of the climatic dependence of survival. Conversely, the method of moments random effects framework is not appropriate for inferring the effects of climate on survival as candidate hypotheses were not reliably distinguished, the probability of identifying data-generating model was low and estimated climatic effects were heavily biased.

In CMR analyses of limited datasets, AICc comparisons are routinely applied assuming the effective sample size, in the correction term, to be the number of individuals released prior to the final occasion. Our analyses revealed that this definition produced unreliable inferences about the climatic dependence of survival. More reliable inferences are obtained by defining the AICc effective sample size as the number of years of study. Switching to this latter definition generally favours simpler climate models. In our case study this substantially altered inferences about the climatic drivers and future projections of long-tailed tit survival.

Statistical justification

The statistical literature offers insight into the appropriate types of questions that can reliably be addressed by the two alternative CMR random effects frameworks. Vaida & Blanchard (2005) reviewed an analogous problem of using linear mixed-effects models and AIC model

comparison in the analysis of non-independent data. They stressed that when random effects are present AIC comparisons are not straightforward. It is difficult to determine: a) what likelihood calculation should be used, b) how the effective degrees of freedom (number of parameters) of a model should be determined and c) how to define the effective sample size of the finite sample size correction term when data is limited. To help determine the appropriate likelihood framework and definition to calculate the effective degrees of freedom, Vaida & Blanchard (2005) emphasize the need to distinguish two different levels of focus of analyses and argue that the same likelihood structure and AIC calculation is not appropriate in both cases.

Firstly, there are questions relating to individual clusters of data, which in a CMR setting equates to inferences such as survival in a specific year. For such questions, where the random effects are themselves of interest, conditional likelihood inferences should be applied, meaning that the probability of a given set of climate parameters is dependent upon the model, the data and the estimated year-specific random deviations in expected survival. Random effects then count towards fractions of degrees of freedom and the effective degrees of freedom calculation is estimated based on the leverage of each year-specific deviation in survival on the estimated mean survival (Hodges & Sargent 2001). The MOM framework is more closely aligned with conditional inference approaches in the way that it calculates the likelihood and the number of effective parameters. It may therefore be more suitable for learning about individual clusters in the data, potentially providing more accurate estimates of year-specific survival probability, for example.

Secondly, there are population-level questions regarding the difference between clusters i.e. explaining differences in survival between years. For such questions, the random effects are not themselves the focus, but simply a device to model correlation of responses within clusters. Therefore, marginal likelihood inferences should be applied, allowing calculation of the probability of a given set of climate parameters given a climate model and data once the random year specific nuisance effects are partitioned out (marginalized/integrated). Here, the degrees of freedom of a model can be simply defined as the sum of the number of fixed parameters and variance components. Investigations of the impacts of climate on inter-annual survival have a population-level focus, as we are interested in inferring population mean survival and the effect sizes of important climatic drivers. This justifies the use of a marginal likelihood random effects framework with a degrees of freedom calculation based on the number of fixed and random parameters.

The AICc finite sample size correction term, can have important impacts on inferences in the linear mixed-effects modelling context (Vaida & Blanchard 2005), and we also found this to be true in CMR analyses. The magnitude of the correction depends on the sample size, however, in many types of ecological analyses of non-independent data, appropriate definitions of effective sample size have to be fully investigated (Kendall & Bjorkland 2001; Burnham & Anderson 2002; MacKenzie 2006). Considering the level of focus of an analysis elucidates the appropriate definition of the effective sample size, for investigations of the impacts of climate on demographic rates. The theoretical sample space is the set of all combinations of the climate conditions which influence demography. Given that demographic rates are usually estimated and related to the climate sample space only once per year, the sample size is simply the number of years of study.

Following this logic, it becomes apparent why overfitting is common when the sample size is defined as the number of individuals released. The AICc finite sample size correction is intended to penalize models with a high number of estimated climate parameters relative to the number of independent observations. This prevents complex models from simply describing random variation in inter-annual survival. Defining the effective sample size as the number of individuals released falsely implies that models with as many parameters as there are independent estimates of climate dependent demography have only a modest flexibility with which to explain inter-annual variation in survival. This underestimation of the degrees of freedom:sample size ratio, possibly by an order of magnitude, results in the AICc data limitation penalty term being far too small (approximately zero in the case of the long-tailed tit dataset).

Practical application of random effects mark–recapture methods

Although the necessity to account for data non-independence has been widely documented in statistical literature, methods to achieve this have not been widely adopted in the CMR literature (but see Gimenez *et al*. 2012 & Appendix.1 for examples of the implementation of random effects models). The large number of studies failing to identify their assumptions of negligible unmeasured processes and independent data, indicates that ecologists are unclear about the limitations of classical fixed effects CMR analyses. When a few climatic factors have overwhelming impacts on demographic rates and other sources of inter-annual variation are small, the fixed effects framework may provide reasonable estimates. This may be true in

extreme ecosystems, such as polar regions, where the effect of sea surface temperature is overwhelmingly important (Jenouvrier *et al*. 2009), or arid ecosystems, where precipitation is a key driver (Jonzén *et al.* 2010). However, in temperate ecosystems, numerous climatic factors may have important impacts at key seasonal times (Robinson *et al.*2007). We advise that the marginal likelihood random effects framework is utilised in future analyses of climate dependent demography, as the stochastic variability in inter-annual survival is not known beforehand and must be established during the analysis.

Our literature review revealed that marginal likelihood random effects analyses are not applied in the overwhelming majority of CMR climate studies, perhaps because different procedures to account for unmodelled stochastic processes have been proposed (Burnham & White 2002; Royle & Link 2002; Barry *et al.* 2003). A major factor limiting the application of marginal likelihood analyses has likely been the technical difficulty of their implementation. Fortunately, software is increasingly available to aid these analyses (Fournier *et al.* 2012). The R package "marked", by Laake *et al.* (2013), offers a user friendly way to implement several categories of CMR models, although manual implementations in ADMB provide substantial computational gains.

Alternative inference approaches

Although we have focused on maximum likelihood inference, analogous Bayesian inferences can also be conducted by constructing hierarchical models and using a numerical sampling scheme to integrate out the random year effects (Brooks *et al.* 2002; Grosbois *et al.* 2008; Gimenez *et al.* 2012; King 2012). Likewise, although we focus on AIC model comparison, other information theoretic approaches such as BIC and DIC can also be used. Notably, if prior knowledge is strong enough that the effects of only a few climatic drivers need to be evaluated, the classical null hypothesis testing (NHT) approach can be used to detect significant impacts of a specific climatic driver without constructing random effect models (Lebreton *et al.* 2012). In this situation, the analysis of deviance (ANODEV) test has been developed to be simple and yet robust to the effects of unmeasured variables (Grosbois *et al.* 2008; Lebreton *et al.* 2012). However, it should not be applied when many combinations of putative climatic factors are examined as this will lead to multiple significance testing problems and high rates of false detections.

Considering the results of our literature review and simulation studies together, it appears that a substantial proportion of inferences of published studies about the climatic dependence of demographic rates may be biased or entirely spurious. Analyses conducted under the fixed effects framework are likely to generate misleading predictions of the ecological impacts of climate change. These analyses readily find support for an impact of many unrelated variables and also underestimate the effects of genuine climatic variables that they do identify. This issue will be particularly severe when there are large stochastic sources of variation in survival. Unfortunately, under these conditions having more data (more individuals or more years studied) intensifies these problems. Future ecological studies that aim to infer the abiotic sources of temporal variation in biotic rates should recognize the importance of accounting for unmodelled factors causing random temporal variation in the biological response variable and should ensure that a marginal likelihood framework is implemented, with appropriate AICc correction.

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Appendices

Appendix.1) Literature review of method recently used to detect the climatic dependence of survival with mark-recapture models

We examined the mark recapture methods used to detect the climatic dependence of species survival by conducting a systematic literature search. We surveyed the literature using the Web of Knowledge database and the search topics "mark recapture" and "climate or environment". Of the articles returned by this search, we evaluated the relevance of all peer-reviewed manuscripts published between 2008 and 2014 (n=553). This covers the period since the review paper of Grosbois et al. (2008) until present. We found 111 studies that used mark-recapture models to relate climatic or environmental covariates to temporal changes in the survival of animals using mark-recapture data. For each paper, we determined the model comparison approach used to test for statistical support for a climatic effect hypothesis and also determined whether the analysis used a method to account for variation in survival between years not attributable to climatic covariates (process variation) (Fig.6.S1).

In the ecological literature, one of two different model comparison methodologies has generally been use to examine the support for hypotheses about the climatic dependence demographic rates (Grosbois et al. 2008). These approaches are: a) the null hypothesis testing (NHT) approach and b) the information theoretic approach of model comparison. The distinction between these approaches lies in their enterprise. The first aims to detect whether

there is a significant effect of a specific climatic driver (versus the null hypothesis of no effect). The latter compares relatively how well a set of models, hypothesizing the climatic dependence of demography, describe the data. Lebreton *et al*. 2012 developed and evaluated a procedure for testing for effect of a climate variable (using NHT), when constructing fixed effects models but in the presence of stochastic interannual variation. This can be implemented in the software package U-CARE. Our review shows that many studies using the NHT approach now adopt this method. The use of information theoretic model comparison was very common, especially when there is little prior knowledge of the climatic drivers of demography.

There are some recent papers indicating that unmeasured inter-annual variation in survival should be accounted for by incorporating random yearly deviations in survival into model structures (Grosbois *et al.* 2008; Frederikson *et al.* 2014). Despite this, our review shows that this is rarely attempted (only 9/71 studies) and has been done using two differing frameworks.

Firstly, the method of moments framework can be applied, by using shrinkage estimation and variance components analysis to account for process variation without explicitly incorporating it into the model structure. This method of implementing random effect mark-recapture models represents an intermediate level of technical difficulty. It can be implemented within MARK, although additional work is required to fit these models and understand their working. The second methodology is to explicitly incorporate temporal random effects into the structural equation of the mark recapture model and to then marginalize these random effects back out during the inference process. This can be achieved under either a hierarchical Bayesian approach or a maximum likelihood approach (King 2012; Gimenez et al. 2012; Jansen et al. 2014; Maunder et al. 2009) These techniques are the most difficult as they are not yet implemented in common mark-recapture programmes (e.g. MARK and U-CARE). Instead models must be constructed using more technically challenging software such as winBUGS, JAGS or ADMB. However the marginalization of random effects is being increasingly applied under both the NHT and information theoretic approach and more user friendly software is becoming available (Laake et al. 2013).
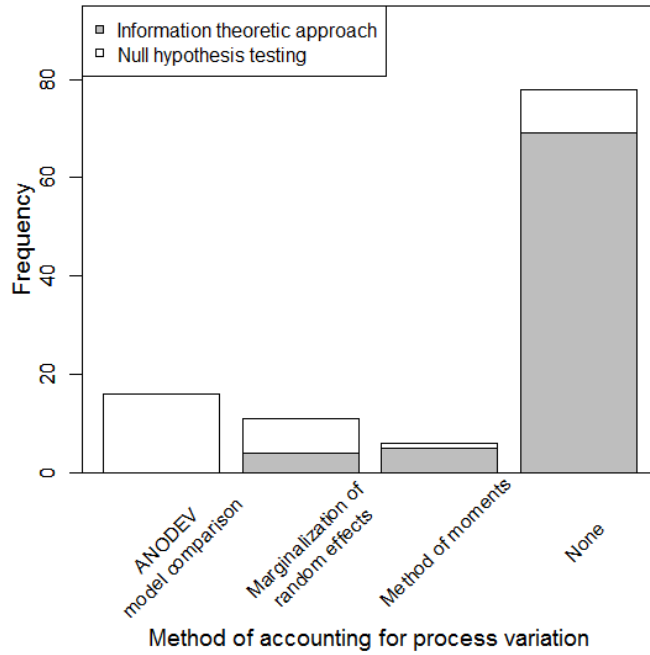
Figure 6.S1)Summary of the frequency with which published studies use different methodologies to account for process variation when using the information theoretic approach (grey bars) or using classical null hypothesis testing (white bars) to infer climatic effects on survival.

Table 6.S 1) Summary information of methodological details extracted from the studies examined in the literature review.

Author	Year	Model comparison approach	Model selection tool	Framework to model process variation	Software	Number of years
Muncy et al.	2014	Information theoretic	AICc	None	MARK	5
Inoue et al.	2014	Information theoretic	AICc	None	MARK	15
Horswill et al.	2014	Null hypothesis testing	LRT	Method of moments	MARK	9
Hovenin et al.	2014	Information theoretic	AICc	None	MARK	7
Wellicome et al.	2014	Information theoretic	AICc	None	MARK	15
Soldatini et al.	2014	Information theoretic	AIC	None	U-CARE	21
Gullett et al.	2014	Information theoretic	AICc	None	MARK	18
Jansen et al.	2014	Null hypothesis testing	Analysis of 95% Credible region	Marginalization of random effects	R (R2jags) JAGS	12
Jergenson et al.	2014	Information theoretic	AICc	Method of moments	MARK using MCMC	16
Altwegg et al.	2014	Null hypothesis testing	LRT	ANODEV model comparison	MARK	17
Garcia-Perez et al.	2014	Information theoretic	AICc	None	MARK	10
Troyer et al.	2014	Information theoretic	AICc	None	MARK	36
Lovich et al .	2014	Information theoretic	AIC	None	R	34
Jonker et al.	2014	Information theoretic	AICc	None	MARK	22
Phillott et al.	2013	Information theoretic	AICc	None	MARK	15
Pavón-Jordán et al.	2013	Information theoretic	AICc	Method of moments	MARK	29
Lok et al.	2013	Information theoretic	AICc	Method of moments	MARK	22

Earl & Semlitsch	2013	Information theoretic	AICc	None	MARK	6
Zylstra et al.	2013	Information theoretic	AICc	None	MARK	22
Winkler et al.	2013	Information theoretic	AICc	None	MARK	11
Dybala et al. a.	2013	Null hypothesis testing	LRT	ANODEV model comparison	MARK	30
Boulanger et al.	2013	Information theoretic	AIC or AICc	None	MARK & U-CARE	10
Patil et al.	2013	Information theoretic	AIC	None	MARK	6
Schwarz et al.	2013	Information theoretic	DIC	None	MTG	12
Chambert et al.	2013	Null hypothesis testing	posterior predictive checking	Marginalization of random effects	OpenBUGS & R	30
Dybala et al. b.	2013	Null hypothesis testing	LRT	ANODEV model comparison	MARK	30
Brown et al.	2013	Information theoretic	AICc	None	MARK & RMARK	14
Salewski et al.	2013	Null hypothesis testing	LRT	ANODEV model comparison	MARK & UCARE	33
Hedger et al.	2013	Information theoretic	AIC	None	MARK	8
Genovart et al.	2013	Null hypothesis testing	LRT	ANODEV model comparison	E-SURGE & U-CARE	7
Pardo et al.	2013	Null hypothesis testing	LRT	ANODEV model comparison	MARK and R	43
Seward et al.	2013	Information theoretic	AIC	None	MARK & RMARK	4
Aubry et al.	2013	Information theoretic	AICc	None	MARK & RMARK	27
Peacock et al.	2012	Information theoretic	AICc	None	MARK & RMARK	30
Korfanta et al.	2012	Information theoretic	AICc	None	MARK	22
Juillet et al.	2012	Null hypothesis testing	LRT	ANODEV model comparison	E-SURGE & U-CARE	13
Reichert et al.	2012	Information theoretic	AIC	None	MARK	14
Fordham et al.	2012	Information theoretic	AICc	None	MARK	12
Hiert et al.	2012	Information theoretic	AICc	None	MARK	24
Jenouvrier et al.	2012	Information theoretic	AIC	None	M-SURGR	28
Price et al.	2012	Information theoretic	AICc	None	MARK	5
Campbell et al.	2012	Information theoretic	AICc	Method of moments	MARK	13
Robert et al.	2012	Null hypothesis testing	LRT	ANODEV model comparison	MARK	18
Blomberg et al.	2012	Null hypothesis testing	LRT	ANODEV model comparison	MARK	7
Gimenez et al.	2012	Null hypothesis testing	Posterior model probability	Marginalization of random effects	WinBUGS & U-CARE	17
Heard et al.	2012	Null hypothesis testing	Posterior model probability	Marginalization of random effects	OpenBUGS	4
Calvert et al.	2012	Null hypothesis testing	Analysis of 95% Credible region	Marginalization of random effects	WinBUGS	19
Russell & Ruffino	2012	Null hypothesis testing	Analysis of 95% Credible region	Marginalization of random effects	WinBUGS	10
Jørgensen et al.	2012	Information theoretic	AICc	None	MARK & U-CARE	18
Melnychuk et al.	2012	Information theoretic	AIcc	None	MARK	12
Smith et al.	2012	Information theoretic	AICc	None	MARK & RMARK	2
Hegg et al.	2012	Information theoretic	DIC	Marginalization of random effects	WinBUGS	25
Emmerson & Southwell	2011	Null hypothesis testing	LRT	ANODEV model comparison	MARK & RELEASE	16
Morris et al.	2011	Information theoretic	AICc	None	MARK	5
Bergeron et al.	2011	Information theoretic	AICc	None	MARK	5

Gamelon et al.	2011	Information theoretic	AICc	None		E-SURGE	22
Péron et al. a.	2011	Null hypothesis testing	LRT	ANODEV model comparison	E-SURGE	20
Keech et al.	2011	Information theoretic	AICc	None	MARK	6
Péron et al. b.	2011	Information theoretic	DIC	Marginalization of random effects	WinBUGS & R	20
Muths et al.	2011	Information theoretic	AICc	None	MARK	6
Lebl et al.	2011	Information theoretic	AICc	None	MARK & RMARK	12
Goswami et al.	2011	Information theoretic	AICc	None	MARK	25
Lee	2011	Information theoretic	AIC	None	MARK	20
O'Shea et al.	2011	Information theoretic	AIC	None	MARK	4
Heulin et al.	2011	Information theoretic	AIC	None	M-SURGE & U-CARE	7
Reid et al.	2011	Information theoretic	AICc	None	MARK	3
Welch et al.	2011	Information theoretic	AICc	None	MARK & RMARK	13
Cubaynes et al.	2011	Null hypothesis testing	LRT	ANODEV model comparison	E-SURGE	19
Martins et al.	2011	Information theoretic	AICc	None	MARK & RMARK	5
Arizaga et al.	2011	Information theoretic	AICc	None	MARK & U-CARE	46
Glenn et al.	2010	Information theoretic	AICc	None	MARK & RELEASE	14
Macdonald et al.	2010	Information theoretic	AICc	None	MARK	21
Le Galliard et al.	2010	Null hypothesis testing	Combined AICc and LRT	ANODEV model comparison	MARK M-SURGE	16
Mabille et al.	2010	Information theoretic	AICc	None	M-SURGE & U-CARE	32
Shueller & Peterson	2010	Information theoretic	AICc	None	?	3
Dugger et al.	2010	Information theoretic	AICc	None	MARK & U-CARE	12
Oro et al.	2010	Null hypothesis testing	Combined AICc and LRT	ANODEV model comparison	M-SURGE & U-CARE	16
Luis et al.	2010	Information theoretic	AICc	None	MARK, RMARK & U-CARE	15
Previtali et al.	2010	Information theoretic	AICc	None	MARK	17
Nevoux et al.	2010	Null hypothesis testing	LRT	ANODEV model comparison	MARK & U-CARE	15
Boano et al.	2010	Null hypothesis testing	LRT	ANODEV model comparison	RELEASE, U-CARE & MARK	18
Frick et al.	2010	Information theoretic	AICc	None	MARK, RMARK & RDSURVIV	16
Schorcht et al.	2010	Information theoretic	AICc	None	MARK & U-CARE	20
Grosbois et al.	2009	Information theoretic	DIC	Marginalization of random effects	WinBUGS &U-CARE	11
Altwegg et al.	2009	Information theoretic	AICc	None	MARK	11
Hall et al.	2009	Information theoretic	AICc	None	MARK	6
Liu et al.	2009	Information theoretic	AICc	None	MARK & U-CARE	12
Ballerini et al.	2009	Information theoretic	AICc	None	MARK U-CARE & M-SURGE	11
Low & Pärt	2009	Information theoretic	AICc	None	MARK	8
Catry et al .	2009	Information theoretic	AICc	None	MARK	12
Lescroel et al.	2009	Null hypothesis testing	Combined AICc and LRT	None	MARK & U-CARE	9

Vasconcellos & Colli	2009	Information theoretic	AICc	None	MARK	34
Hario et al.	2009	Information theoretic	AICc	None	MARK, U-CARE & RELEASE	47
Calvert et al.	2009	Information theoretic	AICc	None	MARK AND R lmer	11
Schorr et al.	2009	Information theoretic	AICc	None	MARK	11
Bakker et al.	2009	Null hypothesis testing	AICc and LRT	None	MARK & U-CARE	5
Jenouvrier et al.	2009	Null hypothesis testing	AICc and LRT	None	MARK, U-CARE & M-SURGE	24
Gimenez & Barbraud	2009	Information theoretic	DIC	Marginalization of random effects	U-CARE, M-SURGE, WinBUGS and R	40
Nevoux et al.	2009	Null hypothesis testing	LRT	None	M-SURGE & U-CARE	22
Gruebler & Naef-Daenzer	2009	Information theoretic	AICc	None	MARK	15
Barbraud et al.	2008	Null hypothesis testing	LRT	None	M-SURGE & U-CARE	13
Focardi et al.	2008	Information theoretic	AIC	None	U-CARE & SURGE	8
Frederiksen et al.	2008	Null hypothesis testing	LRT	None	MARK	43
Votier et al.	2008	Null hypothesis testing	LRT	None	U-CARE & M-SURGE	20
Scherer et al.	2008	Information theoretic	AICc	None	MARK & RELEASE	7
Plummer et al.	2008	Null hypothesis testing	LRT	None	MARK & RELEASE	10
Grosbois et al.	2008	Null hypothesis testing	LRT AICc & DIC	Marginalization of random effects	MARK, M-SURGE, U-CARE, WinBUGS	16
Reid et al.	2008	Information theoretic	AICc	Method of moments	MARK	25
Guitiérrez et al.	2008	Information theoretic	AICc	None	MARK & CONTRAST	13
Taylor et al.	2008	Information theoretic	AICc	None	MARK	5
Nevoux et al.	2008	Null hypothesis testing	LRT	None	U-CARE & M-SURGE	12

Appendix.2) Simulation of individual encounter histories and additional details of analysis of simulation study

A) Individual based simulation

The encounter history of an individual in a mark recapture study consists of a time series of '1's and '0's, with '1' indicating that the animal has been sighted and is alive in a year, whilst '0' indicates that the animal was not encountered. We used individual-based simulations to generate encounter histories, by specifying a model underlying temporal variation in survival and using this to calculate year-specific survival and re-sighting probability. These two probabilities were then used to simulate the survival of each individual from one year to the

next. Once these stochastic realizations of individual survival between years was simulated, individual sighting of survivors was simulated.

<u>B) Influence of ΔAICc threshold level on the probability of detecting support for the data-generating climate model</u>

Under a range of mark recapture study conditions we assess the probability of finding support for the data-generating climate model, for each CMR framework. The relative level of support of each model is measured as the difference in AICc score of that model relative to the best supported model (the minimum AICc model). A threshold level of support has to be defined at which a model is defined as being supported. In the main manuscript we present the findings for the case when support is conservatively defined as an AICc value within 4 units of the best supported model (ΔAICc < 4). This represents just a moderate amount of support, but is still distinguishable from the most supported model. A framework failing to identify even this moderate amount of support for the true climatic model can therefore be classified as failing to infer the climatic dependence of survival.

As this threshold criteria for sufficient support is to some extent arbitrary, we provide output for the analysis when the threshold is set to 2 AICc levels above the best supported climatic model (Fig.6.S2) and when we simply assess whether the data-generating climate model is the best supported model (Fig.6.S3). Under these different support threshold criteria we see that the qualitative relationships between success probability and A) the number of recapture occasions and B) the number of individuals released annually remains the same as presented in the main text. At each threshold the marginal likelihood random effects framework allows more robust inference in the presence of environmental stochasticity. The main difference of having a lower ΔAICc score defining sufficient support, is simply that the probability of identifying the data-generating model is decreased. This is an inevitable consequence of having more stringent criteria for support.
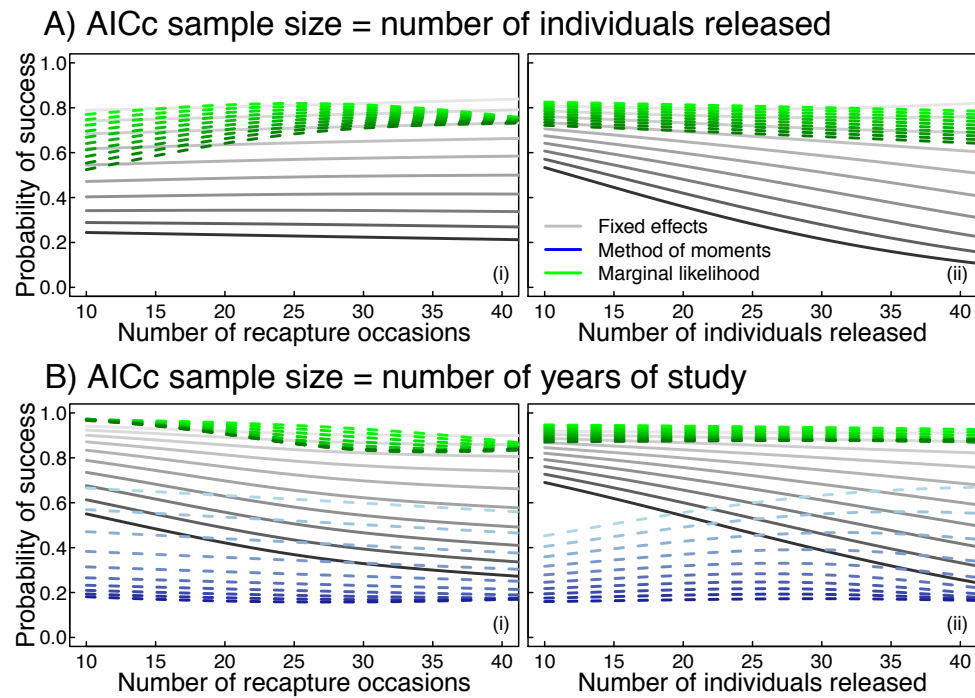
A) AICc sample size = number of individuals released

B) AICc sample size = number of years of study

Figure 6.S2) The probability of mark-recapture analyses successfully finding support for the data-generating climate model at the support threshold of ΔAICc < 2. AICc calculations are made under the assumption that the sample size is: the number of individuals released (subpanel A) or the number of years of study (subpanel B). The probability of success is characterized for each framework: the fixed effects framework (grey lines), the marginal likelihood random effects framework (green lines) or the method of moments framework (blue lines). Successful detection is related to the gradient of environmental stochasticity, darker shades indicate higher levels of stochasticty (σ_ϕ 0 - 0.5) and i) the duration of the mark-recapture study, and ii) the number of individuals released at each occasion. The method of moments approach is not presented in subpanel A ($sample\ size = n_{inds}$) as this approach does not allow models to be distinguished.

A) AICc sample size = number of individuals released
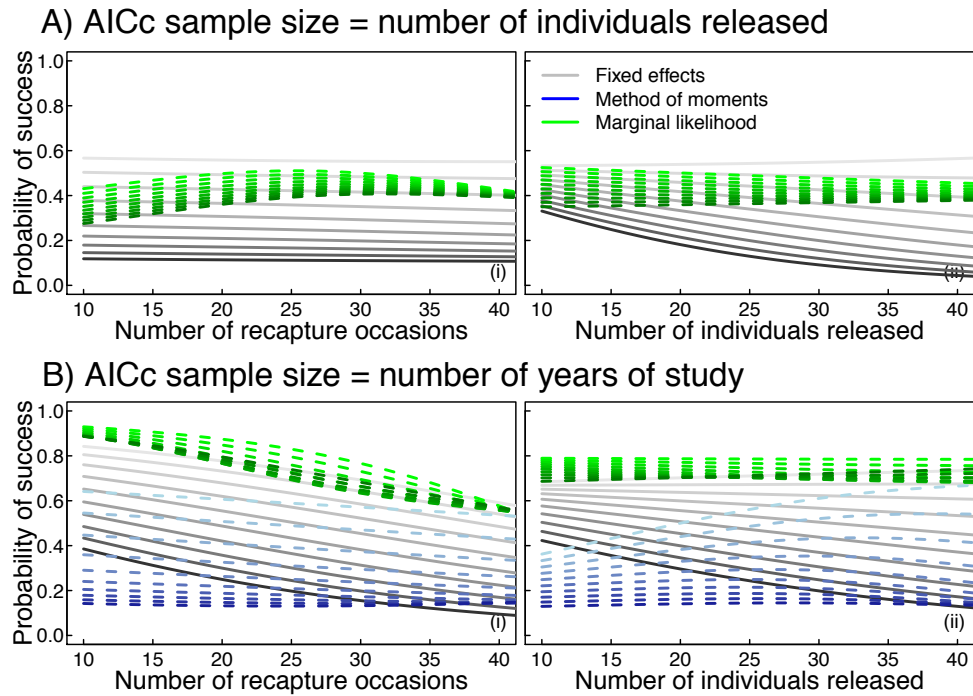
B) AICc sample size = number of years of study

Figure 6.S3) The probability of mark-recapture analyses successfully finding support for the data-generating climate model at the support threshold of ΔAICc=0. AICc calculations are made under the assumption that the sample size is: the number of individuals released (subpanel A) or the number of years of study (subpanel B). The probability of success is characterized for each framework: the fixed effects framework (grey lines), the marginal likelihood random effects framework (green lines) or the method of moments framework (blue lines). Successful detection is related to the gradient of environmental stochasticity, darker shades indicate higher levels of stochasticty (σ_ϕ 0 - 0.5) and i) the duration of the mark-recapture study, and ii) the number of individuals released at each occasion. The method of moments approach is not presented in subpanel A ($sample\ size = n_{inds}$) as this approach does not allow models to be distinguished.

Appendix.3) Long-tailed tit example: Comparison of inferences from alternate mark-recapture analysis of climatic-dependent survival.

A candidate set of models was produced relating to hypotheses about the main seasonal climatic factors influencing long-tailed tit survival and how these climatic factors interact within seasons. The candidate set includes 25 models, with the most complex model reflecting the findings of Gullet *et al.* (2014): $\Phi_t = \dfrac{1}{1+exp^{-\left(\mu_\phi + \beta_{X_{t1}} X_{t1} + \beta_{X_{p1}} X_{p1} + \beta_{X_{t3}} X_{t3} + \beta_{p3} X_{p3} + \beta_{X_{t1.p1}} (X_{t1}.X_{p1}) + \beta_{X_{t3.p3}} (X_{t3}.X_{p3})\right)}}$. Each logical nested model of this full model was also considered (presented in the first column of table 6.S2). The simplest model was therefore an intercept only model. The number of climatic predictors included in the candidate models varied between zero and six.

Alternate mark-recapture analyses of the long-tailed tit encounter histories were performed by parameterizing the candidate set under each CMR framework: fixed effects framework, the methods of moment's random effects and the marginal likelihood random effects frameworks. AICc model comparison was applied and a full model comparison table of results for each framework is shown in Table.6.S2 and Table.6.S3. We adopt the short hand notation of Lebreton et al. (1992) to refer to each candidate model in a concise way. In this scheme, the full climatic model of survival defined above is denoted: φ (~t1 x p1 + t3 x p3). Correspondingly, the simple intercept only model is denoted φ (~1). Models are presented from most to least complex. The relative support for each model is given by the ΔAICc scores and model weights. Cell colouring reflects the degree of support for each model under a given framework. Models with: a) ΔAICc <2 are most supported and roughly equivalent (darkest grey), b) 2> ΔAICc <4 are marginally less supported (dark grey), c) 4> ΔAICc <7 are distinguishably less supported (mid grey), d)7> ΔAICc <10 are rather less supported (light grey) and e) ΔAICc > 10 are very poorly supported (white). This classification follows the rules of thumb recommended by Burnham & Anderson (2002) and Richards (2005).

The outcome of the AICc model comparison depends strongly upon the CMR framework used to analyse the data but is also strongly influenced by the definition of the AICc sample size. Firstly, consider the differences between the inferences of CMR frameworks when the AICc sample size is assumed to be the number of individuals released (Table.6.S2). This is the default assumption of analyses in standard mark-recapture software (e.g. MARK). When applying fixed effects and marginal likelihood analyses, strong support is found for the hypothesis (model) that long-tailed tit survival is largely influenced by the seasonal effects of temperature and precipitation in Spring and Autumn, as well as the interactions between the two climatic drivers within each season. Specifically, strong support was found for the climatic model: φ(~t1*p1+ t3+p3)(AIC weight: fixed=0.51, marginal likelihood =0.47) and the full climatic model φ(~t1*p1+ t3*p3) (AIC weight: fixed=0.35, marginal likelihood =0.36). Dissimilarly, under the method of moments framework, the support was distributed between most of the models rather evenly (all models have similarly low AICc scores: dark grey). This large uncertainty in the choice of best model indicates that the competing hypotheses could not be sufficiently distinguished based on the available data using this framework.

Now consider the differences between the inferences of CMR frameworks when the AICc sample size is assumed to be the number of years of study (Table.6.S3). The inference of the fixed and marginal likelihood frameworks about the climatic variables related to variation in

survival become much more dissimilar. In fact, the fixed and marginal likelihood frameworks identify completely different climate models. The fixed effects framework found support for two complex climate models, indicating that survival was influenced by: a) the interaction between Spring temperature and precipitations plus an additive effects of Autumn temperature (AIC weight: fixed=0.63) or b) the interaction between Spring temperature and precipitations plus the additive effects of Autumn temperature and precipitation (AIC weight: fixed=0.24). In contrast, the marginal likelihood random effects framework did not support these complex models. Instead a high weight of support was given to a single climate model, hypothesising that survival is related just to the level of Autumn precipitation (AICc weight= 0.69). Other simple models received minor amounts of support, but were distinguishably less well supported (daicc >4). The method of moments random effects framework gave support to a single climate model, however the extreme difference in AICc relative to nested models indicates that this framework is not providing reliable inferences. This inadequacy stems partially from the relatively large effective number of parameters relative to the sample size. This causes the AICc correction term to penalize in an overly extreme way.

Table 6.S2) Information theoretic comparisons of the performance of alternate climate models for alternate CRM frameworks under the assumption that the AICc sample size is the number of individuals released. Models describe hypotheses of drivers in variation in long-tailed tit survival (ϕ). Climate covariates of temperature (t) and precipitation (p) during both Spring (t1 and p1) and Autumn (t3 and p3) were used to explain variation in survival. Cell colouring reflects the degree of support for each model under a given framework. Darker colours indicate stronger support (see main appendix text for details).

| Survival model | Fixed effects Framework | | | | | Random effects Framework | | | | | | | | | |
| | | | | | | Method of moments | | | | | Marginal likelihood | | | | |
	Deviance	npar	AICc	ΔAICc	weight	Deviance	npar	AICc	ΔAICc	weight	Deviance	npar	AICc	ΔAICc	weight
ϕ ~ t1 x p1 + t3 x p3	459.3	10	2422.16	0.74	0.35	443.2	18.31	2423.02	1.38	0.05	414.5	11	2402.41	0.53	0.36
ϕ ~ t1 x p1 + t3 + p3	460.6	9	2421.43	0.00	0.51	443.2	18.14	2422.67	1.03	0.06	418.0	10	2401.88	0.00	0.47
ϕ ~ t1 + p1 + t3 x p3	472.2	9	2433.02	11.59	0.00	442.0	18.94	2423.08	1.44	0.05	426.	10	2410.88	9.00	0.01
ϕ ~ t1 x p1 + t3	466.2	8	2424.99	3.56	0.09	442.8	18.54	2423.06	1.42	0.05	428.4	9	2405.26	3.37	0.09
ϕ ~ t1 x p1 + p3	471.9	8	2430.74	9.32	0.00	442.4	18.01	2421.64	0.00	0.11	430.0	9	2407.9	6.01	0.02
ϕ ~ t1 + t3 x p3	481.2	8	2440.05	18.62	0.00	442.0	19.78	2424.88	3.24	0.02	432.8	9	2412.66	10.77	0.00
ϕ ~ p1 + t3 x p3	477.1	8	2435.93	14.50	0.00	442.0	19.81	2424.90	3.26	0.02	430.7	9	2411.56	9.67	0.00
ϕ ~ t1 + p1 + t3 + p3	481.8	8	2440.65	19.22	0.00	442.1	19.63	2424.64	2.99	0.02	434.7	9	2413.60	11.71	0.00
ϕ ~ t1 x p1	483.5	7	2440.32	18.90	0.00	441.8	18.94	2422.90	1.26	0.06	444.6	8	2413.45	11.57	0.00
ϕ ~ t3 x p3	485.2	7	2441.97	20.54	0.00	442.1	19.61	2424.58	2.93	0.02	438.5	8	2412.37	10.49	0.00
ϕ ~ t1 + p1 + t3	489.3	7	2446.08	24.65	0.00	442.1	19.62	2424.58	2.94	0.02	445.9	8	2415.71	13.83	0.00
ϕ ~ t1 + p1 + p3	482.5	7	2439.36	17.93	0.00	442.2	19.50	2424.43	2.78	0.03	440.9	8	2411.75	9.87	0.00
ϕ ~ t1 + t3 + p3	486.0	7	2442.84	21.41	0.00	442.0	19.71	2424.65	3.00	0.02	441.0	8	2412.81	10.93	0.00
ϕ ~ p1 + t3 + p3	483.7	7	2440.51	19.09	0.00	442.2	19.50	2424.42	2.78	0.03	439.5	8	2412.33	10.45	0.00
ϕ ~ t1 + p1	492.1	6	2446.92	25.49	0.00	441.9	18.91	2422.91	1.27	0.06	454.1	7	2414.91	13.03	0.00
ϕ ~ t1 + t3	502.0	6	2456.84	35.41	0.00	441.5	20.36	2425.55	3.91	0.02	456.8	7	2418.65	16.77	0.00
ϕ ~ t1 + p3	486.3	6	2441.08	19.65	0.00	442.1	19.44	2424.27	2.63	0.03	448.0	7	2410.85	8.97	0.01
ϕ ~ p1 + t3	496.2	6	2451.03	29.60	0.00	442.0	19.71	2424.66	3.02	0.02	451.6	7	2415.39	13.50	0.00
ϕ ~ p1 + p3	484.5	6	2439.34	17.91	0.00	442.2	19.34	2424.17	2.53	0.03	445.8	7	2410.57	8.69	0.01
ϕ ~ t3 + p3	488.0	6	2442.81	21.38	0.00	442.1	19.50	2424.35	2.71	0.03	445.7	7	2411.55	9.67	0.00
ϕ ~ t1	504.1	5	2456.89	35.46	0.00	441.6	20.29	2425.45	3.80	0.02	462.9	6	2417.73	15.85	0.00
ϕ ~ p1	501.1	5	2453.91	32.48	0.00	441.9	18.79	2422.73	1.09	0.06	459.3	6	2415.11	13.23	0.00
ϕ ~ t3	515.6	5	2468.39	46.97	0.00	441.5	18.38	2423.50	1.85	0.04	462.2	6	2419.01	17.13	0.00
ϕ ~ p3	488.4	5	2441.14	19.71	0.00	442.3	18.21	2421.87	0.23	0.10	451.8	6	2409.63	7.75	0.01
ϕ ~ 1	520.9	4	2471.66	50.23	0.00	441.5	20.30	2425.45	3.80	0.02	442.0	5	2418.74	16.86	0.00

Table 6.S3) Information theoretic comparisons of the performance of alternate climate models for alternate CRM frameworks under the assumption that the AICc sample size is the number of years of study. Models describe hypotheses of drivers in variation in long-tailed tit survival (ϕ). Climate covariates of temperature (t) and precipitation (p) during both Spring (t1 and p1) and Autumn (t3 and p3) were used to explain variation in survival. Cell colouring reflects the degree of support for each model under a given framework. Darker colours indicate stronger support (see main appendix text for details).

| Survival model | Fixed effects Framework | | | | | Random effects Framework | | | | | | | | | |
| | | | | | | Method of moments | | | | | Marginal likelihood | | | | |
	Deviance	npar	AICc	ΔAICc	weight	Deviance	npar	AICc	ΔAICc	weight	Deviance	npar	AICc	ΔAICc	weight
ϕ ~ t1 x p1 + t3 x p3	459.3	10	2449.52	10.22	0.00	443.2	18.31	2784.17	1656.34	0.00	414.5	11	2446.24	29.02	0.00
ϕ ~ t1 x p1 + t3 + p3	460.6	9	2441.31	2.01	0.24	443.2	18.14	2746.73	1618.90	0.00	418.0	10	2433.17	15.95	0.00
ϕ ~ t1 + p1 + t3 x p3	472.2	9	2452.90	13.60	0.00	442.0	18.94	1127.829	0.00	1.00	426.0	10	2442.17	24.95	0.00
ϕ ~ t1 x p1 + t3	466.2	8	2439.30	0.00	0.65	442.8	18.54	3209.89	2082.06	0.00	428.4	9	2427.66	10.42	0.00
ϕ ~ t1 x p1 + p3	471.9	8	2445.05	5.75	0.04	442.4	18.01	5625.278	497.45	0.00	430.0	9	2430.28	13.06	0.00
ϕ ~ t1 + t3 x p3	481.2	8	2454.35	15.05	0.00	442.0	19.78	1655.96	528.13	0.00	432.8	9	2435.04	17.82	0.00
ϕ ~ p1 + t3 x p3	477.1	8	2450.24	10.94	0.00	442.0	19.81	1505.44	377.61	0.00	430.7	9	2433.94	16.72	0.00
ϕ ~ t1 + p1 + t3 + p3	481.8	8	2455.00	15.70	0.00	442.1	19.63	1830.65	702.82	0.00	434.7	9	2435.98	18.76	0.00
ϕ ~ t1 x p1	483.5	7	2450.43	11.13	0.00	441.8	18.94	1911.61	783.78	0.00	444.6	8	2429.36	12.14	0.00
ϕ ~ t3 x p3	485.2	7	2452.08	12.78	0.00	442.1	19.61	1719.88	592.05	0.00	438.5	8	2428.28	11.06	0.00
ϕ ~ t1 + p1 + t3	489.3	7	2456.19	16.89	0.00	442.1	19.62	2050.63	922.80	0.00	445.9	8	2431.62	14.40	0.00
ϕ ~ t1 + p1 + p3	482.6	7	2449.47	10.17	0.00	442.2	19.50	1653.49	525.66	0.00	440.9	8	2427.66	10.44	0.00
ϕ ~ t1 + t3 + p3	486.0	7	2452.96	13.66	0.00	442.0	19.71	1785.92	658.09	0.00	441.0	8	2428.72	11.50	0.00
ϕ ~ p1 + t3 + p3	483.7	7	2450.62	11.32	0.00	442.2	19.50	1745.26	617.43	0.00	439.5	8	2428.24	11.02	0.00
ϕ ~ t1 + p1	492.2	6	2453.86	14.56	0.00	441.9	18.91	2054.08	926.25	0.00	454.0	7	2426.04	8.82	0.01
ϕ ~ t1 + t3	502.0	6	2463.79	24.49	0.00	441.5	20.36	2163.52	1035.69	0.00	456.8	7	2429.78	12.56	0.00
ϕ ~ t1 + p3	486.3	6	2448.02	8.72	0.01	442.1	19.44	1571.74	443.91	0.00	448.0	7	2421.98	4.76	0.06
ϕ ~ p1 + t3	496.2	6	2457.97	18.67	0.00	442.0	19.71	2066.68	938.85	0.00	451.6	7	2426.52	9.30	0.01
ϕ ~ p1 + p3	484.5	6	2446.28	6.98	0.02	442.2	19.34	1545.26	417.41	0.00	445.8	7	2421.70	4.48	0.07
ϕ ~ t3 + p3	488.0	6	2449.75	10.45	0.00	442.1	19.50	1705.872	578.04	0.00	445.7	7	2422.68	5.46	0.05
ϕ ~ t1	504.1	5	2461.47	22.17	0.00	441.6	20.29	2165.23	1037.40	0.00	462.9	6	2425.32	8.10	0.01
ϕ ~ p1	501.1	5	2458.48	19.18	0.00	441.9	18.79	2093.20	965.37	0.00	459.3	6	2422.70	5.48	0.04
ϕ ~ t3	515.6	5	2472.97	33.67	0.00	441.5	18.38	2187.15	1059.31	0.00	462.2	6	2426.60	9.38	0.01
ϕ ~ p3	488.4	5	2445.72	6.64	0.03	442.3	18.21	1461.91	334.08	0.00	451.8	6	2417.22	0.00	0.69
ϕ ~ 1	520.9	4	2474.49	35.19	0.00	441.5	20.30	2200.89	1073.06	0.00	442.0	5	2423.70	6.48	0.03

Chapter 7: General Discussion

Evaluation of aims and objectives

The aim of this thesis was to examine the impacts of abiotic and biotic environmental changes on species vital rates and population dynamics. In the introduction, two main objectives were outlined: A) to examine how environmental changes influences species dynamics and their interaction with other community members and B) to determine how inferences can be made about the impacts of biotic and abiotic environmental change on species vital rates. In the subsequent chapters we utilized data driven modelling approaches to address these questions, deriving quantitative insights into the ecological impacts of changes in temperature, salinity, productivity and precipitation. We examined the impacts on species dynamics of: i) combined environmental change, ii) inducible defence, iii) trait-abundance feedbacks and iv) temperature dependent species interactions. We developed improved methodologies to: i) partition the pathways by which environmental changes altered species invasion rates, ii) identify growth-defence trade-offs, iii) quantify the trait, density and temperature dependence of species interactions and iv) identify and measure the impacts of climate change on the demography of wild populations.

Influences of combined environmental change on community dynamics

It is expected that environmental change will have a multifaceted impact on a species population's dynamics, influencing multiple demographic rates simultaneously (Doney *et al.* 2012; Régnière *et al.* 2012; Jenouvrier 2013). The findings of Chapter 2 show that the effects of combined environmental changes on species vital rates can have non-additive impact on population dynamics. In agreement with our population level findings, previous individual level studies also indicate that the cumulative effects of multiple environmental changes on birth, survival and death rates are frequently non-additive (Darling & Côté 2008). Together, these results show that the dynamical consequences of changes in one environmental variable will often be modified by concurrent changes in the state of other environment conditions. Therefore, multiple aspects of the environment may need to be incorporated into population prediction models. There is also a need to investigate the functional mechanisms by which environmental changes influences vital rates in order to understand the non-additivity of their effects. The interactive effects of environmental change that were observed in Chapter 2 appeared to be relatively simple, given the vast number of possible synergisms, but this may

not be a general result. However, the underlying mechanisms driving these interactions appeared to be interpretable based on metabolic theory and a consideration of nutrient cycling, suggesting that it may indeed be possible to anticipate and generalize about such synergies(Savage *et al.* 2004; Beveridge, Petchey & Humphries 2010)

Factors complicating predictions of species responses to environmental change

Perhaps the most fundamental question in our endeavours to understand population responses to environmental change concerns the level of biological detail that must be modelled in to give sufficiently accurate representations of the system. That is, in which situations do we need to include information about: a) genetic, physiological or trait differences at the individual level, b) differences between subgroups of a population (age, stage, or trait-based), c) population level changes in mean trait values (physiological performance, resource consumption and defence), d) community level interspecific interactions, e) ecosystem level processes (oxygen or carbon fluxes)?

Our findings from Chapter 2 support the view that it is crucial to model the coupled dynamics of interacting species when investigating the dynamic consequences of environmental change. The impacts of environmental change on a species invasion rate was found to be strongly influenced by environmental impacts on the performance of the resident population and its interaction strength. Recent reviews examining climate change impacts on species interactions and population declines have also indicated that changes to species interactions frequently play an important role (Tylianakis *et al.* 2008; Cahill *et al.* 2013). As species do not occur in isolation but instead interact with other community members, predictions of species performance and range shifts under future environmental conditions will likely be inaccurate if interspecific interactions are not incorporated (Bradley *et al.* 2010; Gilman *et al.* 2010).

In Chapter 3 and 4 we provide evidence that it is also important to consider phenotypic responses of individuals to environmental change, in order to understand the dynamics of even simple microbial systems. Considering changes in consumptive interactions between species in response to individual level morphological and behavioural trait change significantly improved our ability to explain community dynamics of a predator-prey-resource system. The trait dependencies of consumption could then be interpreted using life history theory and through consideration of resource allocation trade-offs (Coley *et al.* 1985; Abrams *et al.* 1993). Exposure to predation was found to cause a gradual decline in prey vulnerability and was

associated with a reduction in body size. The defensive response of prey came at a cost of reduced allocation to population growth. Removal of the predatory pressure permitted resources to be redirected to growth. Similar growth defence trade-offs have been identified in other systems, but usually at the individual level (Tollrian & Harvell 1999; Andersson & Hughes 2010; Travis *et al.* 2014). To our knowledge, our approach in Chapter 4 was the first to show a complete feedback between trait dependent vital rates, species dynamics and density dependent trait change. The implications of this work is that over multigenerational timescales the vital rates underpinning population dynamics should not be assumed to be constants, but instead variables that will likely depend on individual's morphology, behaviour or condition.

The feedbacks between species' traits and abundances may have been driven by either evolutionary selection or phenotypic plasticity (Thompson 1998; Tollrian & Harvell 1999; Agrawal 2001; Yoshida *et al.* 2004; Fordyce 2006; Cortez 2011; Kasada *et al.* 2014). Further empirical work is required to determine the relative contribution of these mechanisms. In natural systems, such experiments are not possible, however the use of decomposition analyses may allow a partitioning of contribution of each of these mechanisms in driving observed changes in individuals' traits (Ozgul *et al.* 2009). Current eco-evolutionary theory indicates that both evolution and plasticity can drive trait dependent variation in vital rates, permitting a wide range of complex dynamical behaviours that would not occur in purely ecological systems, due to temporal variation in species growth and consumption rates (Abrams *et al.* 1993; Cortez 2011; Tien & Ellner 2012; Kasada *et al.* 2014; Koch *et al.* 2014). Resultantly, inferring the drivers of population dynamics may be challenging without considering the feedbacks between defence traits, predation pressures, population growth and trait change (as suggested by Hiltunen *et al.* 2014*).* This provides strong motivation for long-term population studies to concurrently assess the dynamics of abundance, ecologically relevant traits and metabolic cost and benefits.

In Chapter 5, we identified that the nature of trait dependent consumptive interactions varied across an environmental gradient. At all temperatures, temporal variation in the strength of predation was associated with decreases in prey body size. However, environmental change reversed the impact of trait changes on consumption rates. Reduced body size was associated with prey defence against predation at low temperatures (as described above), but increased vulnerability at high temperatures. From this we inferred that body size was probably not the trait directly influencing predation risk. Instead, changes in prey body size was likely an indirect

result of a modified life history strategy or altered resources allocation to growth and defence (as described in Travis *et al.* 2014).

Although body size may not directly mediate defence, it is nonetheless a very useful trait to measure as it provides a simple surrogate measure of allocation to cell growth. It may therefore be useful for identifying how energetic trade-offs and trait dependent processes vary with respect to environmental conditions. It also allows calculation of changes in population biomass which may in some cases be more predictable. The changes in body size and the trait dependence of consumption rates observed at warm temperatures were consistent with metabolic theory and were interpreted as a response to the energetic demands of the warm conditions (Atkinson 1994; Savage *et al.* 2004; Binzer *et al.* 2012a; Reuman *et al.* 2014). These findings highlight a potential challenge in understanding the response of communities to gradual environmental change. If interactions are trait-dependent, but the dependence varies across environments, it may be difficult to anticipate changes in prey vulnerability in novel conditions, without a detailed knowledge of the defence traits driving variation in species interactions. Predicting how environmental change will influence the defence traits may require a detailed understanding of the physiological and life history consequences of environmental change (Coley *et al.* 1985; Abrams *et al.* 1993; Gillooly *et al.* 2002; Savage *et al.* 2004; Bassar *et al.* 2010; Binzer *et al.* 2012b; Travis *et al.* 2014; Reuman *et al.* 2014). The use of dynamic energy budget theory and physiologically structured population models may improve predictability (de Roos 1997; Kooijman 2000).

Methods to reliably infer impacts of environmental change

Throughout this thesis we developed new approaches to infer the causes and consequences of environmentally-driven changes in vital rates. In Chapter 2, we developed a novel variance decomposition method, to partition the contribution of different mechanisms by which species invasion rates were influenced by environmental changes. This revealed the importance of inter-specific interactions in determining invasion dynamics. We argue that similar decomposition analyses should be more widely adopted to quantify the roles of the many processes that often influence ecological systems (Price 1970; Rees *et al.* 2004; Hairston *et al.* 2005; Ellner *et al.* 2011; Griffiths *et al.* 2015).

In Chapter 3, we constructed a novel methodology for quantifying the impacts of exposure to predation on the key ecological rates of prey population growth and defence against

consumption. Data collected in prey growth trials and predator feeding assays could then be combined, allowing changes in species abundances caused by population growth to be clearly distinguished from changes driven by predator consumption. By constructing models that reflect the observation process as well as the biological processes, we revealed clear evidence of a trade-off between growth and defence. This trade-off is predicted by theories of resource allocation and is an assumption underpinning eco-evolutionary theory (Coley *et al.* 1985; Abrams & Matsuda 1997; Mougi 2012a).

In Chapters 4 and 5, we established an innovative approach to empirically model the link between the dynamics of species traits and their abundances. This improved our ability to explain community dynamics and provided clear evidence of a feedback between trait dependent vital rates and density dependent trait change. The approach was derived from models underpinning theories of trait dependent ecological interactions and density dependent trait change (Abrams & Matsuda 1997; Ellner & Becks 2010; Cortez 2011; Mougi 2012b; Cortez & Weitz 2014). The theoretical frameworks are now well developed, however there is a clear gap between theoretical predictions and empirical tests. Data-driven modelling is required to test theoretical expectations and gain quantitative insights into the strength of feedbacks between trait and abundance dynamics.

Finally in Chapter 6 we conducted formal tests of the performance of information theoretic methods used to infer the climate dependencies of demographic rates. By simulating demographic datasets, with a known climatic dependence of survival, and using alternate mark-recapture frameworks to analyse them, we evaluated the performance of several common approaches. We demonstrate that both the choice of likelihood framework, used to account for unmeasured processes, and the assumptions made about the extent of data independence substantially influence conclusions about past survival and quantitative projections into the future. Continued efforts are needed to ensure that robust methods are developed and distributed to allow reliable ecological inferences to be made and theories tested. The use of simulation and verification of model performance, using a model structure adequacy approach should be more widely adopted (Taper *et al.* 2008).

Utility of small scale experiments to address questions about the prediction of population responses to environmental change.

A large fraction of the empirical data used in this thesis was generated using microbial microcosm experiments. The use of microcosm experiments provides several advantages when addressing questions about population responses to environmental change. Firstly, they allow rapid and replicated generation of data sets which follow the dynamics of species abundance for many generations. Their small size permits large populations to be examined and manipulated experimentally. Finally, their cellular simplicity removes complexity that is present in size structured organisms with complex life histories.

This allows the identification of fundamental factors which complicate the prediction of population dynamics, without the complexity of natural climate regimes or community complexity. Even in these simple unicellular organisms, we uncovered a network of relationships among traits and resource, prey and predator abundances and showed that modelling the feedbacks between these processes improved our ability to explain community dynamics. To understand and predict population dynamics, there is a need for high temporal resolution data on the dynamics of species physiology, traits and abundances as well as ecosystem processes. Current studies rarely contain more than 100 temporal samples and usually do not measure individual characteristics. Fortunately, it is becoming increasingly viable to collect such data sets with the invention of novel automated monitoring and measuring equipment (e.g. Pennekamp 2015). To make powerful inferences, it would be beneficial to increase sampling frequency by an order of magnitude, allowing measurement uncertainty to be substantially reduced (due to temporal autocorrelation) and allow high frequency dynamic changes to be identified.

Future research developments

The multifaceted and complex nature of environmental change may mean that the forecasting horizon of many systems is a rather short amount of time (Selvam 2007; Petchey *et al.* 2015). Research effort should therefore focus on identifying the potential prediction horizon of important systems and identify predictive frameworks appropriate for this time scale. To make truly predictive forecasts will be much more challenging than explaining previously observed dynamics, but provides an extremely strong test of our understanding of a system's biology. This predictive understanding will likely require detailed demographic data relating

environmental conditions to individual birth, growth and survival rates rather than phenomenological time series approaches. Methods linking demographic and abundance data into a single model inference framework are increasingly well developed (Lee 1985; He, Ionides & King 2010; Ozgul *et al.* 2010; Hartig *et al.* 2011; González & Martorell 2013; González *et al.* 2016; Barthold *et al.* 2016), but forecasting species responses to environmental change is still a nascent field (Jenouvrier *et al.* 2013).The dynamical consequences of environmental change are also likely to be dependent on the strength of species interactions. In such cases, multi-species forecasting methodologies will be needed to model the coupled dynamics of strongly interacting species. Parameterizing such forecasting model will requiring substantially more empirical data to quantify the functional forms of the interactions and the potential trait dependencies.

Our findings indicate that concepts from several related fields within evolution, physiology and ecology may need to be unified to understand the behavioural and morphological responses of individuals to environmental change. Identifying the feedbacks between the dynamics of species abundance and traits influencing defence, or altering other vital rates, appears to be an important prerequisite for predicting environmental impacts on population dynamics. The complex dynamics, anticipated by eco-evolutionary theory, may not easily be explained using classical ecological theory, based purely on species abundance. Detailed physiological and metabolic analyses, and modelling frameworks to integrate the resulting data are need. There is also a need to make these complex mathematical models more widely applicable (de Roos 1997).

Continued work is required to unify theories of population and community ecology with those of physiology, life history and evolutionary biology. Concerted effort is needed to develop methods to obtain inferences from empirical data related to each of these fields and combine the findings to make powerful dynamics forecasts. Finally we need to identify types of forecasting approaches that can probabilistically combine theoretical expectations from different levels of biological detail and empirical data from multiple sources into a common predictive framework.

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