EXTINCTION AND ENVIRONMENTAL CHANGE: TESTING THE PREDICTABILITY OF SPECIES LOSS

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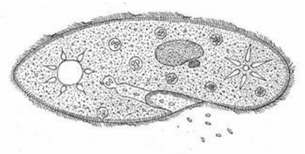

There is a tide in the affairs of men,
Which, taken at the flood, leads on to fortune;
Omitted, all the voyage of their life
Is bound in shallows and in miseries.
On such a full sea are we now afloat;
And we must take the current when it serves,
Or lose our ventures.

William Shakespeare, c. 1599

The beauty and genius of a work of art may be re-conceived, though its first material expression be destroyed; a vanished harmony may yet again inspire the composer; but when the last individual of a race of living beings breathes no more, another heaven and another earth must pass before such a one can be again.

Charles William Beebe, 1906

* * *

For Jasmin

Acknowledgements

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Declaration

Several chapters of this thesis are based on published manuscripts that were jointly authored and have been edited to comply with the format of this thesis, namely:

Chapter 2: **Clements, C.**, Worsfold, N., Warren, P., Collen, B., Blackburn, T., Clark, N., Petchey, O. *Experimentally testing an extinction estimator: Solow's Optimal Linear Estimation model.* Journal of Animal Ecology (2013). The original ideas were conceived jointly. Experimental work was carried out primarily by CC, with help from NC, and the first draft of the manuscript was written by CC.

Chapter 4: **Clements, C.**, Collen, B., Blackburn, T., Petchey, O. *Recent environmental change may affect accurate inference of extinction.* Conservation Biology, *preliminarily accepted.* The original ideas, experimental work, and first draft of the manuscript were done by CC.

Chapter 5: **Clements, C.**, Collen, B., Blackburn, T., Petchey, O. *Effects of directional environmental change on extinction dynamics in experimental microbial communities are predicted by a simple model.* Oikos, *in press.* The original ideas were conceived jointly. Experimental work and first draft of the manuscript were done by CC.

Chapter 6: **Clements, C.**, Warren, P., Collen, B., Blackburn, T., Worsfold, N., Petchey, O. *Interactions between assembly order and environmental change can alter both short and long-term community composition.* Ecology and Evolution (2013). The original ideas were conceived by CC, and the experimental work and first draft of the manuscript were also done by CC.

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Summary

Current extinction rates are thought to be significantly higher than at any point in the last 65 million years. Such a loss of species could have serious implications for human well being, as humanity depends upon services that are derived from diverse, functioning ecosystems. Given the predicted increase in human alterations to the global biosphere in the next century, minimising the loss of species, both now and in the immediate future, has become a prime concern, and has led to policy driven initiatives designed to halt or slow biodiversity loss. Tackling this loss of diversity requires an understanding of current, and recent, extinction events, as well as how future environmental change may alter the probability of a species persisting.

In this thesis I explore, using small-scale aquatic microcosms, modelling, and data from real-world extirpations, the predictability of extinction events, both historic and future, and how environmental change may alter the persistence of populations alone and in a community context.

I present results that suggest our ability to accurately infer the current rate of species loss will depend upon the method used to infer extinction, the amount of a habitat historically searched when looking for a species, and also the underlying population dynamics of that species, which can be altered by environmental change.

I demonstrate that the timing of extinction events, driven by various rates of environmental change, can be predicted using a simple phenomenological model, if a detailed knowledge of how the environment will change over time is known.

Lastly, I show that environmental change can interact with community assembly processes to alter the probability of a species persisting, and thus community composition.

The results of this work contribute to our understanding of current, and future, extinction events, and provide a basis for using quantitative approaches to inform conservation decision-making.

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THE fossil record shows that extinction is common and is the ultimate fate of all species; 99% of all the species that have ever existed are thought to now be extinct (Novacek 2001). Extinction, and the opposite process, speciation, create a continuous turnover of species that, along with abundance, determines local and global biodiversity (Newman & Sibani 1999; Chamberlain & Fuller 2000). From the temporal distribution of fossils it is possible to estimate the average length of time species persist for, generally acknowledged to be $\sim 10^6$-10^7 years (May, Lawton & Stork 1995), which equates to a background rate of species loss in marine systems, for example, of about 0.1-1 species per year (Rockström *et al.* 2009). However, at a few key points during the history of life on earth this relatively slow rate of species turn-over has accelerated, with a large proportion of species (>75%) being lost over a relatively short period of time (typically 1-2 million years Barnosky *et al.* 2011). These "mass extinction" events have been documented only five times over the last 600 million years, the most recent (and infamous - the Cretaceous/Tertiary mass extinction responsible for the loss of the dinosaurs) being c. 65.5 million years ago (Schulte *et al.* 2010). Evidence suggests, however, that the current rate of extinction, despite significant uncertainty (Thuiller *et al.* 2004; Stork 2010), may be similar to those five devastating periods in the history of life on earth when mass extinction events occurred (Pereira *et al.* 2010; Barnosky *et al.* 2011), and these estimates and comparisons have lead to suggestions that a sixth mass extinction event is occurring (Wake & Vredenburg 2008; Barnosky *et al.* 2011).

Much of this increase in the rate of extinctions has been linked to recent, all-pervasive, anthropogenic alterations to the global biosphere (Chapin *et al.* 2000; Thomas *et al.* 2004) which have coincided with a period of extremely high biodiversity, probably the highest ever seen in the history of life on earth (Fig. 1.1). The effect has been, and is predicted to be, the staggering loss

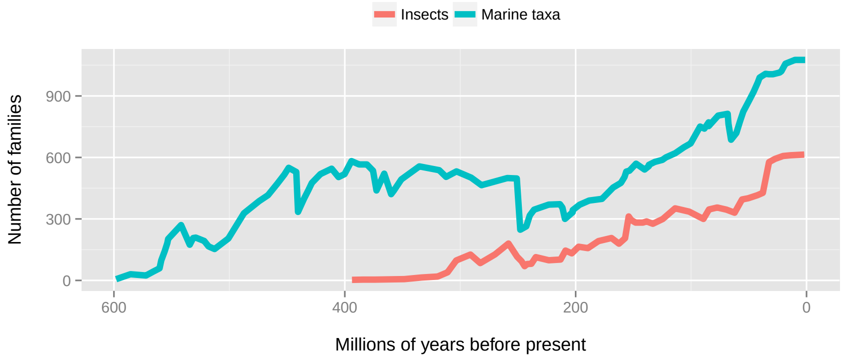

Figure 1.1: The change in the number of families of marine taxa and insects over the past 600 million years (adapted from Labandeira & Sepkoski 1993; Sepkoski 1993).

of species at rates 100's to 1000's of times higher than that found in the fossil record (Pereira *et al.* 2010). Between 18 and 35% of species globally are thought to be committed to extinction (Thomas *et al.* 2004), and in some instances (such as birds on oceanic islands) 28-56% of species will be functionally extinct (see below) by 2100AD (Şekercioğlu, Daily & Ehrlich 2004). However, the uncertainty associated with such figures is often large, as by necessity such estimates are based on extrapolations from limited data (see, for example, Thuiller *et al.* (2004) in response to Thomas *et al.* (2004)). Additionally, for many other less well studied groups the number of species at risk of extinction is simply unknown (for invertebrates, the conservation status of less than 1% of species is documented (Collen *et al.* 2012), a worry given the critical role they play in the functioning of ecosystems (e.g. Folgarait 1998)). Thus, the true scale of current species loss is to a large extent unknown, and could be lower, or, more worryingly, higher, than current estimates suggest.

The loss of species diversity at the scale of a mass extinction event could have serious implications for ecosystem stability and functionality (Dunne, Williams & Martinez 2002; Worm *et al.* 2006; Lecerf & Richardson 2010), and consequently human well being, as we rely heavily on the services derived from ecosystems (Díaz *et al.* 2006). Thus predicting how future global change will affect global diversity is a prime concern. Because extinction is a non-random process (and may be dependant on species-specific traits such as body size (Purvis *et al.* 2000)), understanding why, and when, future extinction events will manifest themselves is facilitated by a good understanding of the current extinction crisis (Collen & Turvey 2009). Of key importance is quantifying *if and when a species has gone extinct*, and *how extinction events may be driven by environmental change*, information which can be used in the development of metrics of extinction, and estimates of extinction risk.

1.1 Defining extinction

EXTINCTION has classically been viewed as the global loss of all the individuals of a species, also termed "true extinction" (Ladle & Jepson 2008). This is the interpretation employed by the International Union for Conservation of Nature (IUCN) in their Red List of Threatened Species (IUCN 2012), and typically when people allude to extinction it is this event they are referring to. For many animal species a global extinction event can be regarded with some certainty as the point of no return, and a species is categorised as extinct when the last individual dies. However, this is a rather simplistic view, and issues with this definition arise as extinction is rarely, if ever, observed and therefore must be inferred. This is particularly problematic when long-lived dormant-but-viable forms of a species persist, for example the seeds of some plant species that can remain viable for hundreds or even thousands of years (Shen-Miller *et al.* 1995; Leino & Edqvist 2010).

Extinction events more commonly occur at the local, population level. Such local extinction (often termed "extirpation" (Ladle & Jepson 2008)) occurs when a population or sub-population of a species ceases to exist in a given area, but persists elsewhere. For some species the continued extinction and re-establishment of local populations is viewed classically as part of their metapopulation dynamics (Harrison 1991), whilst for others the loss of a population may have more serious consequences (such as a reduction in genetic variation, and thus a species's gene pool (Mccauley 1991)). It is worth noting that global extinctions are really a special subset of local extinctions, where the population being lost is the last (globally) of that species. Consequently, understanding the drivers and dynamics of local extirpations can help inform our understanding of global extinction events. Because such extirpation events are far more common than global extinction events, they can provide some of the only data available on extinction dynamics in wild populations (e.g. Fagan & Holmes 2006).

In addition to the more commonly considered local and global extinctions, it has been recognized for over 150 years that there may be stages preceeding these events where a species could be considered "extinct", even though some individuals persist (Murray 1860). The conceptual example given by Murray (1860) highlights one of these cases: if only one sex of a sexually reproducing species is still extant, that species is "committed to extinction". Such a scenario can also be produced by, for example, low genetic variation, as inbreeding depression can condemn a species to extinction at some point in the near future (Bijlsma, Bundgaard & Boerema 2000; O'Grady *et al.* 2006; Wright, Tregenza & Hosken 2007).

Low population size can also produce "ecologically" or "functionally"[1]extinct species (Ladle & Jepson 2008; Säterberg, Sellman & Ebenman 2013), where population densities have fallen to the point that a species no longer interacts to any great extent with other species in a community (Estes, Duggins & Rathbun 1989; Ladle & Jepson 2008). A simple example of this is a species that has no wild populations still extant, and is only present in captivity; the species is not extinct but forms no part of a natural community. Such ecological extinctions can have significant cascading effects, for example the well-documented decline in Sea-Otter numbers in the Northeast Pacific resulted in huge increases in Sea Urchin numbers, and consequently the loss of whole kelp forests due to overgrazing (Estes & Duggins 1995). More recently, work has shown that the functional extinction of a species can significantly affect the survival of other species that interact with it: primary extinctions are more often caused by the functional extinction of a threatened species than by the rapid decline to extinction of the threatened species (Säterberg, Sellman & Ebenman 2013).

In this thesis I will concentrate on the extinction of local populations (i.e. with no spatial element, such as metapopulation or metacommunity dynamics), but with no explicit differentiation between extirpation and global extinction. Population level extinctions are fundamental ecological processes, and the concepts, techniques, and findings presented here are applicable whether those extinctions are local or global.

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[1] It should be noted that "functionally extinct" has been used to refer to species that are both "ecologically extinct" and "committed to extinction". Here I use it only to refer to species that are "ecologically extinct", as this is how it has been used most frequently in recent times (e.g. Säterberg, Sellman & Ebenman 2013).

1.2 Causes of species loss

The manifold drivers of population extinction can be grouped into three broad categories (Griffen & Drake 2008): (i) Demographic effects, (ii) Genetic effects, and (iii) Environmental stressors (both biotic and abiotic).

Demographic drivers of extinction derive from the size, variability, and connectivity of a population (Griffen & Drake 2008). Small populations are especially susceptible to extinction, not only because there are few individuals left, and so catastrophic events are more likely to wipe out the entire population, but also because small population size can cause a host of deleterious effects, such as increased population stochasticity (May 1972). Such variability in population size is caused by chance mortality and reproduction events which are driven by variation between individuals (Shaffer 1981), and the magnitude of these events are predicted to scale with population size by the inverse square law (May 1972). Demographic effects become more important as a population size decreases, as stochastic events affect proportionally more of the population (Forney & Gilpin 1989; Lande, Engen & Saether 2003; Desharnais *et al.* 2006). The magnitude of this stochasticity can determine the minimum viable population size of a species (Shaffer 1981); the greater the magnitude of population stochasticity, the greater the probability that random population fluctuations will drive the population to extinction. Migration into and out of a population can mitigate some of the detrimental effects of low abundance, however when migration rates are high there is an increased probability that connected populations will simultaneously fall to low abundances, and thus lead to the extinction of all the populations (Earn, Levin & Rohani 2000).

Genetic effects influencing extinction risk include all debilitating genetic variation that reduces the fitness of a population (for a review see Frankham 2005). Such effects are usually driven by small, highly-connected populations (although see Lynch 1991), where inbreeding, low genetic variation, and the accumulation of deleterious mutations are common (Novella *et al.* 1995; Hansson & Westerberg 2002; Frankham 2005). Saccheri *et al.* (1998) showed that inbreeding in the Glanville Fritillary butterfly (*Melitaea cinxia*) led to an increased chance of extinction, and Lynch *et al.* (1993) suggested the uptake of deleterious mutations can potentially lead to negative population growth, termed “mutational meltdown”. Low genetic diversity means that populations are less able to cope with stressful events, such as a changing biotic or abiotic environment, as they have less evolutionary potential, and so are at greater risk of extinction (Bijlsma *et al.* 2000; Reed, Briscoe & Frankham 2002; Acevedo-Whitehouse *et al.* 2003; Spielman *et al.* 2004). Frankham *et al.* (1999) clearly demonstrated this process in populations of *Drosophila melanogaster*: low genetic diversity, caused by population bottlenecks, reduced a population's ability to adapt to cope with abiotic stress, leading to more rapid extinction.

Environmental effects, both biotic and abiotic, arguably have the greatest effect on the extinction risk of a population, although this depends, critically, on population size (Lande 1993; Wootton & Pfister 2013). Within this category can be included such high-profile stressors as habitat deterioration, habitat fragmentation, climate change, exploitation, disease, and invasive species, many of which are directly or indirectly driven by human activity (Chapin *et al.* 2000; Thomas *et al.* 2004). Environmental conditions can directly and indirectly determine whether a species can survive in a habitat, and consequently environmental change can lead to the loss of large numbers of individuals, or in extreme cases the loss of a population. For example, Thomas *et al.* (1996) describe the effects of environmental perturbations on a butterfly (*Euphydryas editha*) metapopulation, where a single weather event (a late summer frost, that killed the butterfly's plant food source) led to the extinction of several populations.

Environmental drivers of extinction risk typically interact with one another, and also with genetic and demographic effects (e.g. Coulson *et al.* 2001). Environmental conditions may also drive demographic and genetic effects, by reducing a species's population size to the extent that demographic and genetic effects become important. The potential interactions between these three broad categories mean assigning a single cause to an extinction event can be problematic. As a conceptual example, a population might go extinct due to a disease, which it was susceptible to because of low genetic diversity caused by a population bottleneck, which was the result of a cataclysmic series of extreme weather events. However, whilst in many cases there may be uncertainty as to the specific drivers of species loss, it is unquestionable that humanity is dramatically changing the global environment, both biotic and abiotic, and with this change comes unprecedented threats to global biodiversity (Chapin *et al.* 2000).

Over the last 500 years the global human population has risen from ∼500

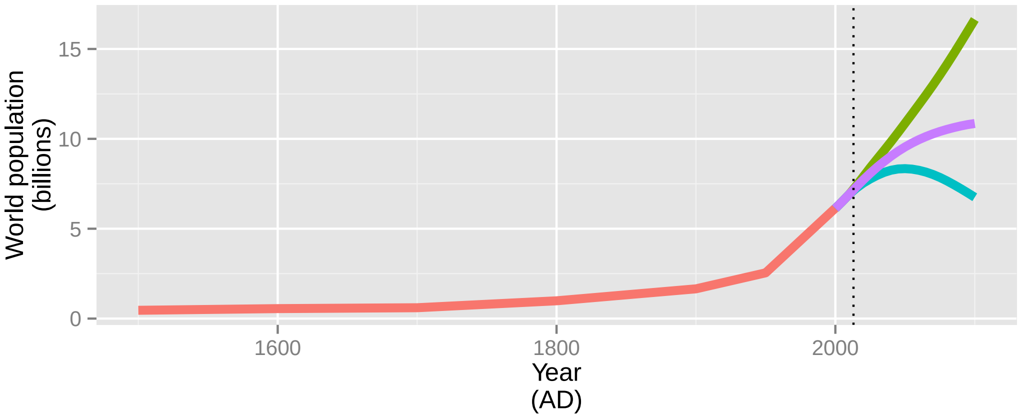

Figure 1.2: Historic global human population estimates (1500AD - 2000AD from Klein Goldewijk *et al.* 2010), and low, medium, and high population projections from 2000AD until 2100AD (from UN Population Division 2013). Vertical dashed line indicates the year 2013.

million to over 7 billion, an increase of 1400% (Klein Goldewijk, Beusen & Janssen 2010; UN Population Division 2013), and this trend is projected, although with a large degree of uncertainty, to continue until around 2100 (Fig. 1.2). The knock-on effects of this explosion of the human population on the biotic and abiotic environment have been dramatic: we consume ~1/3 of the net primary productivity of terrestrial ecosystems (Chapin *et al.* 2000), by 2050 we are expected to use 74% of the world's available freshwater (Postel, Daily & Ehrlich 1996), levels of CO_2 and other highly active greenhouse gasses are rising (Meinshausen *et al.* 2009; Ramanathan & Feng 2009) with knock-on effects to the planet's climate and weather systems (Meehl *et al.* 2000; Gastineau & Soden 2009; Pall *et al.* 2011), and exploitation of resources (both mineral and organismal) has never been higher. These human driven alterations are so profound that we may have ushered in a new geological epoch, the anthropocene (Crutzen 2002), where anthropogenic effects dominate how the global biome functions.

1.3 Consequences of species loss

The extinction, both "true" and "functional", of a high number of species will change the composition, function, and stability of ecosystems worldwide (Dunne *et al.* 2002; Gaston & Spicer 2004; Hooper *et* *al.* 2005; Worm *et al.* 2006; Lecerf & Richardson 2010), and has the potential to threaten human well-being (Díaz *et al.* 2006). Humanity relies on stable, functioning ecosystems for a diverse range of services, including soil fertility, pest and disease control, protection from natural hazards, water quality, and food (Díaz *et al.* 2006). Of particular concern may be the loss of critical services such as pollination which we rely heavily upon (Şekercioğlu *et al.* 2004; Johnson *et al.* 2010); in 2006 the ecological services, including pollination, provided by insects alone to the United States of America were estimated to be worth $57 billion annually (Losey & Vaughan 2006). The loss of key species, such as pollinators, can also influence the extinction risk of those dependent upon them, destabilising communities and increasing the risk of further extinction events (Bond 1994). Examples of this are common: a study into the loss of primate-dispersed trees in Peru found that extirpation of all large primates, and a 61% reduction of medium sized primates, has led to a 46% reduction in the number of trees whose seeds rely on primate dispersal, and a 248% increase in abiotically dispersed trees (Nunez-Iturri, Olsson & Howe 2008).

At a less pragmatic level, many people intrinsically value biodiversity. For years non-governmental organizations (NGOs) have strived, using public donations, to minimize the loss of flagship species (Walpole & Leader-Williams 2002). Regardless of the reasons, minimizing biodiversity loss in the face of the continued exploitation and alteration of natural ecosystems has become a key scientific and political goal, however doing so requires the ability to gauge accurately which species have gone extinct, when, and how predicted changes to the environment are likely to alter species persistence.

1.4 Quantifying extinction - the challenges of getting it right, and consequences of getting it wrong

CURRENT best estimates put the number of species on Earth between 8 and 9 million (Chapman 2006; Mora *et al.* 2011), however of these less than 1.8 million have been formally described (Chapman 2006; IUCN 2013), and significantly less have been assessed to find out whether they are either extinct, or at risk of extinction. The IUCN's Red List of Threatened Species (Mace *et al.* 2008; IUCN 2013) provides the most complete evaluation of the conservation status of plant and animal species, but in 2013 the Red List had assessed and categorized only 70,289 species, or about 4% of

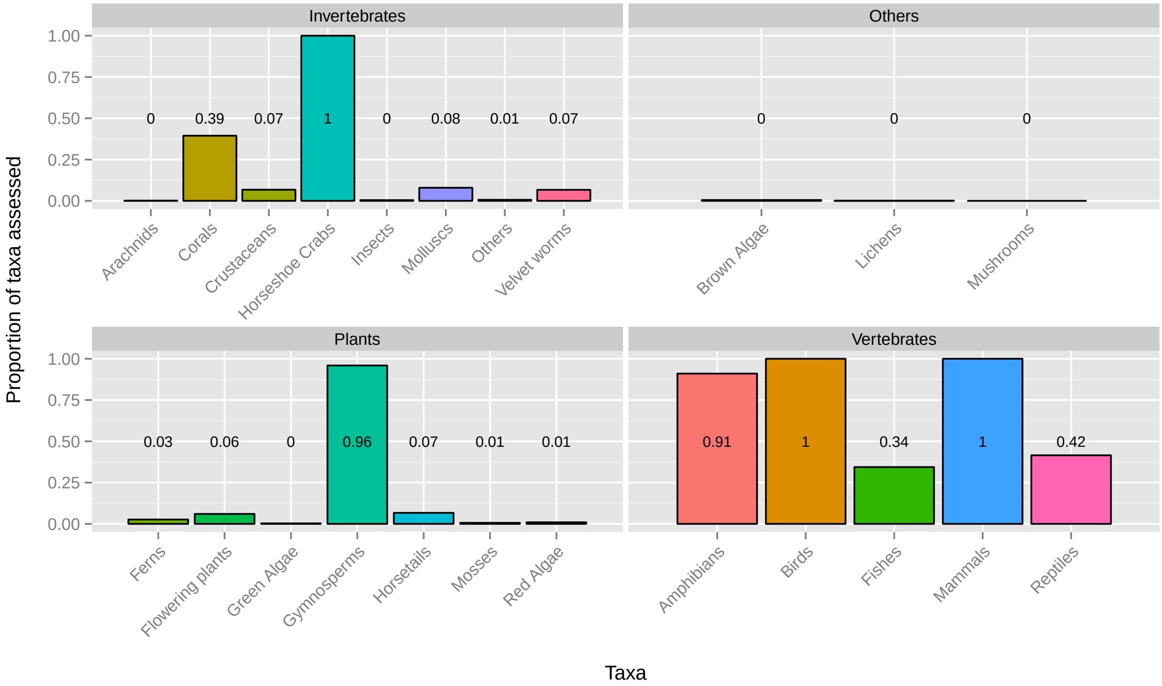

Figure 1.3: Proportion of each species from each taxon that are assessed by the IUCN Red List of Threatened Species (IUCN 2013).

formally described species (IUCN 2013). Of the assessed species 20,219 (28.7%) were threatened with extinction, 20% of animal and 7% of plant species were classified as Data Deficient (had insufficient data to determine whether they were under threat), and 799 plant and animal species were thought to have gone extinct in the last 500 years (IUCN 2013, Fig. 1.3). The number of species assessed by the IUCN has steadily increased (Fig. 1.4), and whilst this is clearly a significant step in the right direction, given the relatively small number of species assessed so far, the true number of extinct and threatened species is likely to be far higher than 799 (Dirzo & Raven 2003; Dunn *et al.* 2009). Indeed, the number of extinctions over the last 500 years is probably somewhere in the tens of thousands (Dunn *et al.* 2009), and it is thought we may be responsible for 5-20% of extinctions in some groups of organisms (Chapin *et al.* 2000). However, many of these numbers are strongly biased, with the majority of assessments carried out on terrestrial vertebrates and plants as these taxa are typically better documented (Collen *et al.* 2012, Fig. 1.3).

Uncertainty in the true rate of biodiversity loss arises from, amongst other

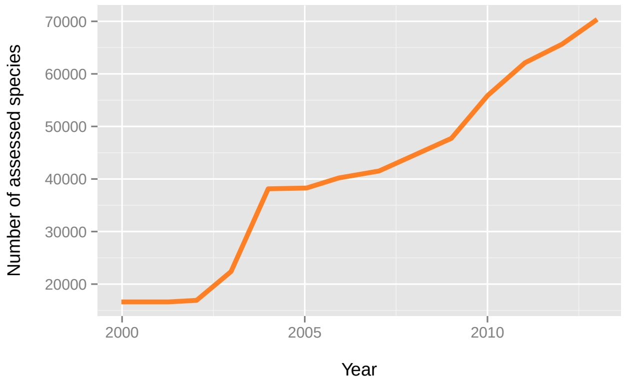

Figure 1.4: The number of species assessed by the IUCN since 2000 (IUCN 2013).

things, the ambiguity associated with classifying a species as extinct. For some species the exact date and location of the last individual to perish is known, as well as the causes of extinction (e.g. *Ectopistes migratoruis*, the Passenger Pigeon, driven to extinction by hunting on an industrial scale (Schorger 1936; Halliday 1980)), however for the vast majority of species this is not the case (see the above example of Data Deficient species). Species-specific traits may affect the ease with which a species can confidently be classified as extinct; for example, if the species has a habitat inaccessible to observers (Scott *et al.* 2008), is un-described, or is cryptic (Reed 1996) individuals are likely to be overlooked. This difficulty has led to the rediscovery of more than 1/3 of mammal species that have previously been classified as extinct by the IUCN and in the scientific literature (Fisher & Blomberg 2011).

The incorrect designation of a species as extinct can have far reaching consequences; if a species is declared extinct then conservation efforts to help the species will cease (Collar 1998), and legal protection for the species and its habitat could be withdrawn (Scott *et al.* 2008). There may therefore be reluctance to designate a species as extinct in an attempt to minimize the risk of Romeo error - declaring a species extinct before it actually is (Collar 1998) - and Lazarus taxa - species declared as extinct being found extant (Keith & Burgman 2004).

On the other hand, waiting to declare a species as extinct also has its drawbacks; it lessens the perceived impacts we are having on the world around us (Diamond 1987), and may continue to commit funds to a species that has already been lost, when those funds could, perhaps, be used to save an endangered species. The recent example of the "rediscovery" of *Campephilus* *principalis*, the Ivory-Billed Woodpecker (Fitzpatrick *et al.* 2005), is a case in point; the US Department of the Interior and the US Agriculture Department immediately injected $10 million to preserve and aid conservation of its habitat (Wilcove 2005), however subsequent studies have shown no evidence at all of the persistence of *C. principalis*, with search efforts now suspended (Dalton 2010). A retrospective analysis by Gotelli *et al.* (2011) suggested this mistake could have been avoided by applying methods to infer whether a species is likely to still be extant, with their work suggesting that *C. principalis* was unlikely to have survived past 1980 (see also Scott *et al.* 2008).

Clearly it is of paramount importance to positively identify the actual extinction status of a species. The IUCN states that a species should be considered extinct when "exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), throughout its historic range have failed to record an individual" (IUCN 2012). However this is far from fool-proof, as one is unlikely to be able to search 100% of a species's habitat, and even if it were possible it requires significant input of time and effort for each and every species. Turvey *et al.* (2007) documented the recent search (in 2006) for any remaining individuals of the Yangtze River Dolphin (*Lipotes vexillifer*), a six week, multi-boat acoustic and visual survey of 3338km of the Yangtze River, concluding that the species was "likely to be extinct", but conceding that "it is conceivable that a couple of surviving individuals were missed".

To circumvent the enormous and costly expenditure of effort required to conduct adequate surveys to confirm extinction, techniques have been developed in an attempt to infer whether a species is currently extinct or extant (e.g. Robson & Whitlock 1964; Strauss & Sadler 1989; Solow 1993a, b, 2005; Burgman, Grimson & Ferson 1995; Reed 1996; McCarthy 1998; Solow & Roberts 2003; Gotelli *et al.* 2011). Many of these utilize historic sighting data, which is often all that is recorded of little known species (Solow 1993a). Such techniques may provide relatively simple ways of accurately quantifying the extinction status of large numbers of organisms quickly, and one has already been put forward as being potentially suitable to inform a proposed IUCN category of "Critically endangered - possibly extinct" (Butchart, Stattersfield & Brooks 2006; Collen, Purvis & Mace 2010). This would be a significant step forward in quantifying biodiversity loss. The applicability of such techniques resides, however, in their accuracy, and meaningful tests of these methods remain rare as there are often significant issues with the approaches used to test them (Clements *et al.* 2013).

1.5 Approaches to extinction research

THREE main approaches have been used to attempt to understand the drivers, consequences, and predictability of extinction events: (i) Theoretical models (e.g. Mosimann & Martin 1975; Lande 1993; Foley 1994; Allen & Allen 2003; Lande *et al.* 2003; Dunne & Williams 2009; Rivadeneira, Hunt & Roy 2009), (ii) Observational studies of wild population extinctions (e.g. Estes *et al.* 1989; Laurance, McDonald & Speare 1996; Thomas *et al.* 1996; Brooks *et al.* 2002; Fagan & Holmes 2006; Pounds *et al.* 2006), and (iii) Experimental approaches using, usually, small scale communities (e.g. Philippi *et al.* 1987; Burkey 1997; Belovsky *et al.* 1999; Drayton & Primack 1999; Vucetich *et al.* 2000; Bancroft & Turchin 2003; see Griffen & Drake 2008 for a review). Analysis of the fossil record, the spatial and temporal distribution of fossil finds, has also given important insights into, for example, previous climate driven extinctions (Lister & Stuart 2008), the effects of human settlement on species survival (Wroe *et al.* 2004), and the effects of species loss (Lopes dos Santos *et al.* 2013). However such data suffer from the same disadvantages as observational studies (see below), with the added complexity that the fossil record is inherently incomplete, and this approach is not considered further in this thesis.

Whilst all three have their merits, they are also each flawed: mathematical models are, by design and necessity, simplifications of real world processes (e.g. Lotka 1920; Volterra 1926), data from wild population extinctions are often limited both spatially and temporally (e.g. Laurance *et al.* 1996), whilst small scale experimental systems have, due to their simplicity, a perceived lack of relevance to the real world (e.g. Carpenter 1996). Such approaches can, however, be complementary to one another, and understanding the weaknesses of each, and combining these methods to validate findings, should be the goal of all ecological research.

1.5.1 Studying extinction using mathematical models

MATHEMATICAL models are ubiquitous in ecology, and have a long history of use (arguably the first being Fibonacci's prediction of how rabbit numbers would increase, published in 1202AD (Gillman 2009), 667 years before Ernst Haeckel coined the term "Ecology" (Begon, Townsend & Harper 2006)). Commonly, models may be employed to describe the potential dynamics of populations under a range of future scenarios (e.g. Ovaskainen & Meerson 2010). Statistical approaches may also prove useful, for example to help predict which types of species may be at risk of extinction (e.g. Brook *et al.* 2000), or infer if, or when, a species has gone extinct (e.g. Solow 2005). Seminal papers using these approaches have been able to dispel commonly held misconceptions; for example Lande (1993) showed that small populations with high growth rates may persist for long periods of time, even in the face of environmental stochasticity and random catastrophes.

Such theoretical models may provide mechanistic understanding upon which empirical data can build, but key is the validation of such models, be they statistical or dynamical, with data.

1.5.2 Studying extinction using data from observational studies

WHILST the fossil record is full of the remains of species no longer extant, actually observing the extinction of a wild population remains a very rare event, a function of the uncertainty of knowing in advance if a population is at risk of extinction, and the low probability of observing the demise of the last individual. Historic records of extinction events often come in the form of sporadic recorded sightings of individuals (e.g. the infamous Dodo (Roberts & Solow 2003)), usually produced because of some endeavour other than searching for that species (Solow 2005). Such records provide little or no indication of population size, or if, or when, a species has finally gone extinct, only the times at which the species is known to be extant (assuming the sightings are trustworthy (Solow *et al.* 2011)). Whilst this information may be useful for gauging whether a species is likely to still be extant (e.g. Solow 2005), it is of limited use in understanding how or why species go extinct. The exception to this is the use of such data to identify the traits that predispose species to extinction, so called "correlates of extinction risk". These include large body size, limited geographic range, complex social mating systems or structures, and high trophic level (Purvis *et al.* 2000; Jones, Purvis & Gittleman 2003; O'Grady *et al.* 2004), and often relate back to demographic threats to a population's persistence. For example limited geographic range inherently limits population size, a factor known to influence extinction risk (Purvis *et al.* 2000). Correlating species traits with extinction risk forms an important tool for predicting what species might be especially at risk of future extinction, but relies on accurately determining what species are currently extinct, without which correlates of extinction risk cannot be quantified.

Rarely, significant input of effort has yielded detailed data on wild populations that have declined to extinction, usually gained by targeted surveys of species or populations thought to be at risk (e.g. Laurance *et al.* 1996). In many instances observations are restricted both temporally and spatially as large-scale surveys are time consuming, costly, and logistically difficult, especially if the habitat is sizeable, or the organism hard to identify (see Turvey *et al.* 2007). Where such data does exists, it can provide invaluable information on the dynamics of species declines in wild populations, but is subject to the limitations of collecting data in an uncontrolled setting, chief amongst these being uncertainty as to whether a species is truly extinct (Turvey *et al.* 2007). This lack of certainty limits the usefulness of the data, as the event you are seeking to quantify and understand (extinction) cannot be confirmed.

1.5.3 Studying extinction using experimental communities

A wide variety of both natural and artificial systems, ranging from relatively large scale field manipulations (e.g. Drayton & Primack 1999; Ferraz *et al.* 2007) to small-scale experimental communities (e.g. Philippi *et al.* 1987; Belovsky *et al.* 1999; Petchey *et al.* 1999), have been used to investigate the processes driving, and consequences of, extinction events. Field manipulations are often affected by a variety of factors (natural environmental variation, immigration and emigration, heterogeneous environments (Griffen & Drake 2008)), such that it may be impossible, or logistically unreasonable, to disentangle links between the hypothesized drivers of observed extinction events (Griffen & Drake 2008). In addition, there are ethical issues associated with observing, or forcing, extirpation in wild communities.

Laboratory based experimental systems, characterized by their relative simplicity, replicability, rapid dynamics, and tractability, have consequently been employed widely in extinction research (Lawton 1995; Griffen & Drake 2008). Of particular interest is the facility to accurately record population dynamics, and to observe extinction events with a high degree of certainty.

Due to their simplicity, and, to a greater or lesser extent, perceived lack of realism, small-scale experimental systems have often become whipping posts for the ire of field-ecologists; Carpenter (1996) called them "irrelevant and diversionary". Whilst such setups are clearly simplifications of the complexity of real-world systems, their use of living organisms (with their associated stochasticity), in moderately complex scenarios, means they are able to provide a bridge point between theory and real world observations or large-scale field manipulations. Due to the short-replication times of the organisms used in such studies, and consequently the long ecological time frames that can be achieved over short periods of time in the real-world, microcosms have recently been championed as experimental systems for unpicking global problems, such as the responses of ecosystems to climatic change (Benton *et al.* 2007). As with any simplification of real-world systems (be they controlled, small-scale field manipulations, mathematical models, or microcosm experiments) care should be taken in extrapolating findings to more complex situations. However, careful design of experimental communities, and linking the results generated within them to theory, larger-scale experimentation, and observational studies, can yield significant benefits.

1.6 Aims and methods of subsequent chapters

THE discussion above highlights the need to quantify current biodiversity loss, and understand how future human-mediated global change may alter species persistence and community structure. Whilst significant effort has been applied to these questions, they are all far from answered, and, given the prevalence of current anthropogenically driven stressors, we are at a critical stage of biodiversity research. Of paramount importance will be the synthesis of different techniques (theory, experimentation), sources of data (simulated, experimental, and real-world observational), and modes of inquiry (observation, experimentation). In this thesis I aim to advance our understanding of real world biodiversity issues by utilising the flexibility of laboratory based microcosm experiments, in conjunction with both theoretical approaches and data from real world extirpations.

The current extinction crisis, and our seeming inability to accurately declare a species as extinct, even after large inputs of effort (Fisher & Blomberg 2011), has driven the development of probabilistic based statistical metrics for inferring if or when a species has gone extinct (e.g. Robson & Whitlock 1964; Strauss & Sadler 1989; Solow 1993a, b; Burgman *et al.* 1995; Reed 1996; McCarthy 1998; Solow & Roberts 2003; Gotelli *et al.* 2011; Fisher & Blomberg 2012). Recent efforts have assessed how well such metrics perform (Rivadeneira, Hunt & Roy 2009; Collen, Purvis & Mace 2010), however limitations exist in the approaches taken (Clements *et al.* 2013).

The work presented in Chapter 2 introduces a novel approach to testing such methods for inferring extinction, which addresses many of the issues encountered previously (such as uncertain extinction events, and temporally limited data) by utilising data from small-scale experimental communities. Specifically, I test Optimal Linear Estimation (Roberts & Solow 2003; Solow 2005), as this technique has previously been suggested as a candidate for informing the proposed IUCN Red List category “Critically endangered - Possibly extinct” (Butchart, Stattersfield & Brooks 2006; Collen, Purvis & Mace 2010).

Whilst conducting the work in Chapter 2, it became clear that that whilst numerous methods to infer extinction have been proposed over the last 20 years, these methods are not readily available. To facilitate future testing and development of such methods I collected together six commonly used metrics into a package for the statistical programming language R (R Development Core Team 2013). Chapter 3 introduces this package, called “sExtinct”, and walks the reader through how to use the package, what it is capable of, and some troubleshooting of common mistakes.

Whilst Chapter 2 set a precedent for testing methods for inferring extinction using microcosm data, of particular use to conservation practitioners is knowing what methods perform better than others and whether these methods are robust to different potential drivers of error. To this end, Chapter 4 builds on the results of Chapter 2 by investigating how various rates of population decline can affect our ability to infer extinction events accurately, and how robust the different techniques are to these rates of population decline. This work uses data from a microcosm experiment where temperature was manipulated through time to alter the rate of extinction of replicate populations, and also data on real world extirpations recorded within the Living Planet Index (Collen *et al.* 2009), in Fagan and Holmes (2006), and in Laurance *et al.* (1996).

Whilst assessing whether certain species are currently extinct or extant is clearly important, and has serious implications for funding and policy (Wilcove 2005), the ability to understand how projected anthropogenic environmental alterations may alter a species's risk of extinction is central to our ability to minimise biodiversity loss in the future. The first step towards understanding such effects in natural communities is to first investigate them under controlled conditions. In Chapter 5 I look at how the rate of temperature change over time, an important component of anthropogenic environmental change (IPCC 2007), can alter the timing of extinction events in replicate populations, and whether it is possible to predict when these extinction events will occur using a simple, four-parameter phenomenological model that incorporates metabolic theory.

Species do not, however, reside individually but in assemblages of species. Having shown how different temperature conditions can alter the growth rate and persistence of a single species (Chapter 5), in Chapter 6 I investigate how environmental conditions can alter the persistence of a species in a community context. The survival of a species is known to be altered not only by abiotic conditions such as temperature, but also by the strength of species-species interactions, which can be altered by on-going invasion processes in wild communities (Shorrocks & Bingley 1994). I present the results of a microcosm experiment where temperature and the order in which species invade a habitat are factorially manipulated, to show how future climatic change may interact with stochastic processes that drive community composition, and potentially influence the extinction risk of a species.

1.7 References

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2 Experimentally testing the accuracy of an extinction estimator: Solow's Optimal Linear Estimation model

2.1 Abstract

MATHEMATICAL methods for inferring time of extinction have been widely applied but poorly tested. Optimal Linear Estimation (also called the "Weibull" or "Weibull extreme value" model) infers time of extinction from the temporal distribution of sighting events. Previous papers have suggested Optimal Linear Estimation provides accurate estimates of extinction time for some species, however an in-depth test of the technique is lacking.

The use of data from wild populations to gauge the error associated with estimations is often limited by very approximate estimates of the actual extinction date and poor sighting records. Microcosms provide a system in which the accuracy of estimations can be tested against known extinction dates, whilst incorporating a variety of extinction rates created by changing environmental conditions, species identity, and species richness.

We present the first use of experimental microcosm data to exhaustively test the accuracy of one sighting based method of inferring time of extinction under a range of search efforts, search regimes, sighting frequencies, and extinction rates.

Our results show that the accuracy of Optimal Linear Estimation can be affected by both observer-controlled factors, such as change in search effort,

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as well as inherent features of the system, such as species identity. Whilst Optimal Linear Estimation provides generally accurate and precise estimates, the technique is susceptible to both overestimation and underestimation of extinction date.

Microcosm experiments provide a framework within which the accuracy of extinction predictors can be clearly gauged. Variables such as search effort, search regularity and species identity can significantly affect the accuracy of estimates, and should be taken into account when testing extinction predictors in the future.

2.2 Introduction

Species are being lost at an unprecedented rate (Barnosky *et al.* 2011). This loss of biodiversity could have huge impacts both on ecosystem function and the survival of co-dependent extant species (Memmott, Waser & Price 2004). Biodiversity loss is one measure used to infer human impacts on ecosystems, however the exact rate of species loss is unclear, and the fate of many species remains unknown (e.g. Rös & Pineda 2009). This has been highlighted by the re-appearance of 36% of mammal species (67 species) classified as extinct since A.D.1500 (Fisher & Blomberg 2011), the so-called "Lazarus effect" (Keith & Burgman 2004).

Categorising a species as extinct with confidence is deceptively difficult, since searching an entire species range is often unfeasible, and detection probabilities may be low, for example if the species is cryptic, small, has a large range or low population density (Solow 2005). Inferring extinction from data produced by less than exhaustive searching would reduce the effort spent attempting to classify a species as extinct. Recent examples, such as the search for *Campephilus principalis* (Fitzpatrick *et al.* 2005; Fitzpatrick 2006), the Ivory Billed Woodpecker, highlight the advantage of quantitatively estimating the extinction status of a species, prior to a large investment of time and money in the search for any remaining individuals (Jackson 2006; Rout, Heinze & McCarthy 2010). Consequently a considerable number of extinction predictors have been proposed over the last 20 years (e.g. Solow 1993a, b; Mccarthy 1998; Solow & Roberts 2003; Solow 2005; McInerny *et al.* 2006; Gotelli *et al.* 2011). A large proportion of these aim to infer extinction from sighting only (as opposed to abundance) data in an attempt to deal with the many species for which only a few sightings have been recorded (Fisher & Blomberg 2012).

Clearly inferring the date of extinction of a species could be of considerable practical value, but if such techniques are to be widely applied to the critical question of extinction status, thorough testing to identify their limitations is essential (Collen, Purvis & Mace 2010). Gauging how well these predictors of time of extinction will cope under real world conditions has historically been tackled by using either data from modelled populations or sighting data from species thought to be currently either extinct or on the verge of extinction (Rivadeneira, Hunt & Roy 2009; Elphick, Roberts & Reed 2010; Collen, Purvis & Mace 2010; Fisher & Blomberg 2011). Collen, Purvis & Mace (2010) applied "Optimal Linear Estimation" to assess the probability of extinction in 10 mammal and 10 bird species from Oceania and Asia respectively. For those species with more than 5 reported sightings the technique appeared to provide accurate predictions. However species with few or widely spaced sightings were assigned extinction times with upper confidence intervals often millennia into the future, even when the last recorded sighting of the species was over 100 years ago. Rivadeneira, Hunt and Roy (2009) assessed the accuracy of several sighting based estimators of time of extinction using data from model populations. Accuracy was defined as the date of extinction of the model population falling within the upper 95% confidence interval produced by each method. Predictions tended to be more accurate when populations fell rapidly (as opposed to gradually) to extinction. Unfortunately given the often-wide confidence intervals, the use of confidence intervals as a metric of accuracy appears unhelpful (e.g. Collen, Purvis & Mace 2010). Fisher and Blomberg (2012) attempted to assess the accuracy of three sighting-only extinction estimators (stationary Poisson, non-parametric, and the Weibull/Optimal Linear Estimation) by comparing predicted probabilities of extinction with current IUCN classification, using data on mammal species currently thought to be extinct or possibly extinct. However the inherent problem with using data from wild populations is that there are very few examples where the time of extinction has been accurately observed (Collen, Purvis, and Mace 2010). Data such as those published in Fagan and Holmes (2006), where species have been monitored for population abundances preceding a local extinction event, offers the opportunity to test extinction indices on extinctions in natural communities. Unfortunately such data sets are few and far between, and often only deal with very small populations just prior to extinction.

Small-scale experimental communities offer an opportunity to test specific methods for inferring extinction on data generated by living organisms, under a range of biotic and abiotic treatments. Given the replicability, ease of manipulation of a variety of environmental variables, short generation time and large number of readily culturable species, microcosms can be used to bridge the gap between modelled and field data. Where an experimental microcosm approach provides particular advantages in testing extinction estimators is the ability to search an entire habitat of known size and accurately assess whether a species is extinct or not. With regular sampling, population declines and extinction dates can be quantified to a level of accuracy that is simply not feasible in field systems.

Here we concentrate on exhaustively testing test a single method for inferring extinction date based on sighting only data - Optimal Linear Estimation (Roberts & Solow 2003; Solow 2005), hereafter OLE, and outline a new framework within which extinction predictors can be tested in the future. Our aim is not to compare multiple indices, which has been addressed previously (e.g. Rivadeneira, Hunt & Roy 2009), but to use the combination of experimental microcosm systems, with a specific method for predicting extinction, to investigate whether varying search efforts and population dynamics are likely to impact our ability to infer extinction. OLE has received attention for several reasons: i) OLE does not assume that detection probability or abundance prior to extinction is stable (assumptions that are made by other models, specifically the stationary Poisson model, Fisher & Blomberg 2012), ii) it has been found to perform well even when a species is declining in abundance gradually through time, and when the probability of observing the species is low (Rivadeneira, Hunt & Roy 2009), and iii) it has been proposed as a method to inform an IUCN category of "Critically Endangered - Possibly Extinct" (Collen, Purvis & Mace 2010). Given the importance of IUCN categorization, and the potential perils of wrongly classifying a species as extinct (or extant), thorough testing of this method is critical.

Using a time series of historic sighting events, OLE can be used to infer the date of extinction of a species which is currently thought (or assumed) to be extinct (Roberts & Solow 2003; Solow 2005). Observations of an individual (or multiple individuals at the same point in time) are recorded as a single sighting event; the species has been observed as being extant at a given point in time. OLE assumes that the joint distribution of the most recent sighting events has the form of a Weibull extreme value distribution, and estimates the shape parameter of this distribution from the temporal spread of the sighting events. In a simple example, a vector of sighting events at times 10, 14, 17, 25 and 59 forms the input data for OLE. From this OLE produces a point estimate of the extinction date (for the example given above this would be at t=143) as well as a lower and upper 1-alpha confidence interval (in this case these would be t=99 and t=1100 respectively, with a 95% confidence interval). Because OLE estimates the time of extinction from the gaps between sighting events, OLE assumes search effort never drops to zero, and so gaps in sighting record are not simply a product of irregular search forays, a situation that clearly occurs when remote or hostile habitats are rarely searched (e.g. the infrequent search for the Alaotra Grebe, *Tachybaptus rufolavatus*, in 1989, 2004 and 2009, when the species was declared extinct (BirdLife International 2010)). Although OLE makes no other assumptions, change in the "observability" of individuals of a species as the population becomes smaller caused by, for example, a change in behaviour, could have significant impacts on the accuracy of predictions. On top of this, intrinsic factors (e.g. the life history of a species) or outside pressures (e.g. overhunting) could alter the rate at which a species falls to extinction. Further details are provided in Roberts & Solow (2003) and Solow (2005).

Here we utilise some of the features of microcosm systems to generate long runs of abundance data, preceding eventual extinction, in replicate populations. Varying environmental conditions and community compositions allows us to produce variation in the rate of population decline and timing of extinction. Knowing both the abundances of the populations through time and the size of the habitat in which they live, we generate search regimes to produce data sets of sighting events that vary both in frequency and temporal spacing. We then examine the effect of both observer controlled (e.g. search effort) and intrinsic (e.g. species identity) factors on the accuracy of extinction estimates made using OLE.

2.3 Methods

FIFTEEN different communities were constructed using every possible composition of four bactiverous protist ciliate species (*Paramecium caudatum*, *Loxocephalus sp.*, *Colpidium striatum* and *Blepharisma* *japonicum*), i.e. four single-species communities, six two-species communities, four three-species communities, and one four-species community. Five replicates of each community were kept at 15^{o}C for the duration of the experiment and five replicates at 20^{o}C, giving a total of 150 microcosms. The microcosms were housed in two incubators, each containing 75 microcosms, with one replicate of each community on each of five shelves, and the position on a shelf randomized. Shelves were rotated vertically within the incubators every sampling day, and the microcosms were swapped between incubators (but kept at a constant temperature) every seven days to minimize any possible incubator effect. Microcosms consisted of lidded plastic petri dishes (diameter 100mm, height 20mm) containing 50ml of liquid medium. The medium solution contained 0.1g of crushed protozoa pellets (Carolina Biological Supply, USA) per litre of Chalkley's medium (Thompson *et al.* 1988). The medium was inoculated with the bacteria *Bacillus cereus*, *B. subtilis* and *Serratia marcescens* on day -2. Communities were established on day 0 by adding a volume of medium containing ~100 individuals of each species from high-density stock cultures. Extinctions were driven by competition for limited resources, and so were due to competitive exclusion or starvation. Evaporative loss over time was replaced with distilled water to avoid any increase in the concentration of salts within the microcosms.

2.3.1 Sampling

SAMPLING was undertaken three times a week for 163 days, at which point the rate of population loss had dropped to only 1-2 extinctions per week and so, given that a high proportion of *Loxocephalus* and *P.caudatum* populations had gone extinct (86%), the experiment was stopped. To assess presence or absence of a species, each microcosm was placed under a stereoscopic microscope and surveyed for each species at 7.5-30x magnification. If a species was not seen within a 5-minute search period it was assumed to be extinct. In this event the species was then explicitly searched for during the next sampling period to confirm this. No species that had been recorded as absent in two consecutive samples was observed again.

Sampling to estimate population sizes was based on the methods of Lawler and Morin (1993) and closely mirrored that of Worsfold, Warren and Petchey (2009). Microcosms were mixed and a sample of nine drops was then trans-

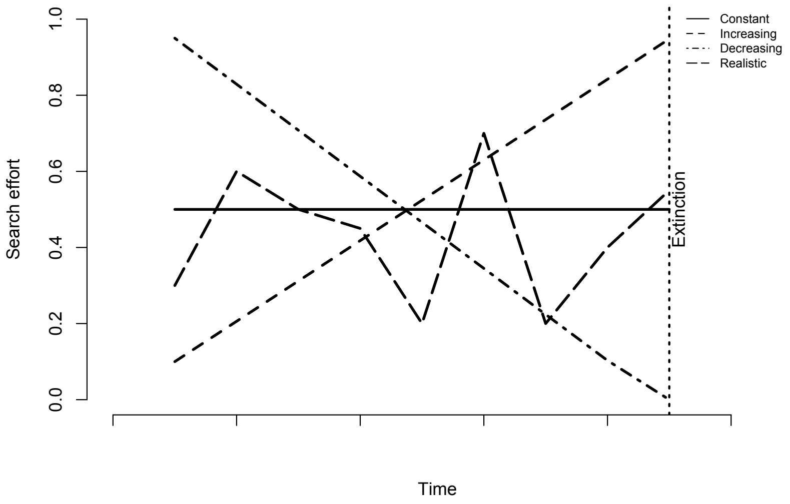

Figure 2.1: Four search regimes were simulated, with the percentage of the habitat searched (search effort) either being held constant, or changing through time according to the regime.

ferred onto a sterile petri dish, weighed, and the number of individuals of each species counted. The medium was then returned to the microcosm. For rare species that were not present in the first sample the process was repeated whilst retaining the original sample until a count was obtained. As numbers became very low, individuals were counted within the entire microcosm and the mass of the medium checked with a balance. Simulations were run on the two species (*Paramecium caudatum* and *Loxocephalus sp.*) where multiple extinctions occurred in 2, 3, and 4 species communities and allowed comparison of both the effect of species richness on predictive accuracy and any interactions between species richness and other variables.

2.3.2 Simulating search effort

REPLICATES of *P.caudatum* and *Loxocephalus sp.* in which extinction was observed over the experimental time period were treated individually, and to each of these records of population abundance through time four different search regimes were applied (Fig. 2.1). These regimes were based in part on the search regimes implemented by Rivadeneira, Hunt and Roy (2009): i) “constant” effort, ii) “decreasing” effort, iii) “increasing” effort and iv) "realistic" search effort. Decreasing search regime was produced by randomly assigning a search effort (between 0 and 0.95) to the first sampling day, and then for each subsequent day search effort decreased by a random fraction (to 0). The increasing search regime was produced exactly as decreasing, but with the random fraction increasing up to 0.95 of the habitat. Thus both increasing and decreasing search regimes covered a variety of rates of change in the fraction of the habitat searched. As suggested by Rivadeneira, Hunt and Roy (2009) a constant search regime, where the fraction of the habitat searched does not change through time, is perhaps the most unrealistic of the four scenarios and should be viewed as a best-case scenario. Both decreasing and increasing search regimes seem plausible as search effort could be either increased to see if a species has gone extinct, or decreased as a species becomes so rare it is assumed to be extinct.

To produce a realistic search effort, change in the area searched through time from a real world scenario is necessary. The Continuous Plankton Recorder project (Sir Alister Hardy Foundation for Ocean Science, SAHFOS) provided this information for plankton sampling along known shipping routes. Using a single data set from route A between the years 1958 and 2009, and then calculating the fraction of the vector of route A sampled per month generated a set of search efforts. So, given the average length of route A in 2009 was 173 nautical miles, and each drag (sample event) was for a distance of 10 nautical miles, the search effort in any given month was calculated (with the assumption of no overlap of sampling events) as the number of drags, multiplied by the length of the drag in nautical miles (10) divided by the average length of the route (173 nautical miles). These monthly search efforts were then treated as the search effort for each sample day. The area covered by sampling generated in this way was high (typically around 50%). A search of the literature provided no accurate estimates of search effort through time whilst assessing the status of rare species and so the search regime generated from the SAHFOS data, whilst not perfect, provides at least some example of a real-world sampling regime.

In order to generate data sets of sighting events under each sampling regime, the original (i.e. complete) data from the microcosms were resampled as follows. Data were converted to total population size within a microcosm. The probability (p) of observing at least one individual at each search event was calculated as:

$$p = 1 - (1 - e)^d \tag{2.1}$$

Where e is the search effort (fraction of the habitat searched) and d is the abundance of the species within the microcosm (both searched and unsearched). This assumes a random distribution of individuals within a habitat, and thus an increasing chance of observation with increasing abundance and search effort. The "observation" of a species was then generated as a random event with a probability of success p. This produced in effect a series of "historic" sighting events, when extinction is known to have occurred. Using these sampling regimes produced a series of sighting events at regular intervals, as sighting events could be as frequent as the recorded abundances. This regularity of search effort is probably unrealistic, as highlighted by sporadic searching both temporally and spatially to assess the extinction of *Lipotes vexillifer* (Turvey *et al.* 2007), the Yangtze River dolphin, and again represents a "best case scenario". A more realistic irregular sampling regime was implemented by running a second simulation, identical to the first, but each sighting event produced was given a 50% probability of being used within the OLE calculation. Simulations were run between 40 and 95 times, thus each population that suffered an extinction event had between 40 and 95 vectors of sighting events produced. Because there was an element of chance in the sighting events, and so in the number of times point estimates of extinction by OLE occurred, a random sample of 500 estimates of extinction from each of the search regimes were used for comparison.

From Solow (2005), Optimal Linear Estimation takes k sighting events and estimates a time of extinction, T_E, using the form:

$$\widehat{T}_E = \sum_{k}^{i=1} w_i t_{n-i+1} \tag{2.2}$$

where the weight vector w, length k, is given by:

$$w = (e^{'}\Lambda^{-1}e)^{-1}\Lambda^{-1}e \tag{2.3}$$

e being a vector of k 1's and Λ is a symmetric k by k matrix with typical element:

$$\Lambda_{ij} = \frac{\Gamma(2\widehat{v}+i)\Gamma(2\widehat{v}+j)}{\Gamma(\widehat{v}+i)\Gamma(j)} j \leq i \tag{2.4}$$

where Γ is the gamma function and v is an estimate of the shape parameter of the Weibull extreme value distribution given by

$$\widehat{v} = \frac{1}{k-1}\sum_{i=1}^{k-2} log\frac{t_n - t_{n-k+1}}{t_n - t_{i+1}} \tag{2.5}$$

t_n being the n times a species is observed over the period of time t. So under the assumption a species is extinct, the upper bound of an approximate $1-\alpha$ confidence interval for T_E is

$$T_E^u = \frac{t_n - c(\alpha)t_{n-k+1}}{1-c(\alpha)} \tag{2.6}$$

where

$$c(\alpha) = (\frac{k}{-log(\alpha)})^{-\widehat{v}} \tag{2.7}$$

Because OLE assumes an extreme Weibull distribution of sightings, it should theoretically only be used with the most recent sighting events (Solow 2005). However there remains some uncertainty as to the optimum number of sighting events as Collen, Purvis and Mace (2010) found that increasing the number of sightings used (tested to a maximum of 18) increases the accuracy of prediction. Consequently all four search regimes had extinction estimates produced with the 5 most temporally recent sighting events (k=5) from a vector of n sighting events, and the total number of sighting events produced by each search regime simulation (k=n).

OLE produces a point estimate of the time of extinction, as well as an upper and lower confidence interval. Previously, accuracy of a prediction has been defined as the true date of extinction falling within the 95% confidence intervals (Rivadeneira, Hunt & Roy 2009). However given that upper confidence intervals can be thousands of years into the future (Collen, Purvis & Mace 2010), this seems unhelpful. Here we regard accuracy as the proximity of the estimated date of extinction to the actual date of extinction, regardless of the upper or lower confidence interval.

All simulations and analyses were carried out using the R package (R Development Core Team 2013). Statistical tests of the difference between treatments were not employed as the numbers of predictions of extinction date was an arbitrary product of both the number of times simulations were run and the stochastic nature of sighting events.

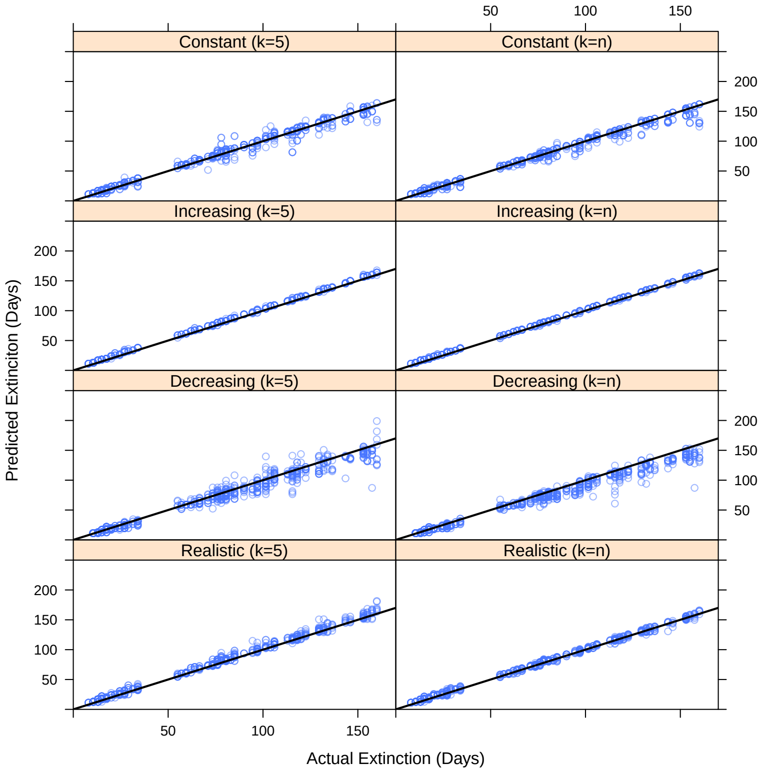

Figure 2.2: Plots are of 500 randomly selected predictions of extinction for each species (*P.caudatum* & *Loxocephalus*) where sampling was regular, i.e. sighting events could potentially occur at every experimental sampling date. Line shows 1:1 (or perfect) prediction. Extinction predictions were run with both k=5 and k=n sighting events. Using all available sighting events produced more accurate and precise results under constant, increasing and realistic search regimes. OLE produced more accurate predictions when k=5 under a decreasing search regime, although precision was reduced. Gaps in the plots are a product of a lack of extinctions between days 34 and 55.

2.4 Results

2.4.1 Observer effects

Regular sampling provided accurate and precise predictions of time to extinction across all search regimes (Fig 2.2). Increasing search effort had the least error (defined as the difference between the actual and predicted date of extinction) associated with predictions (a mean absolute error (overestimation and underestimation combined) of 2.9 days) and the greatest precision (r^2=0.99, p<0.001 for both k=5 and k=n sightings). Decreasing and constant showed relatively large variation in predictive precision (Fig 2.2), although they were still highly correlated (r^2 values higher than 0.97, p<0.001).

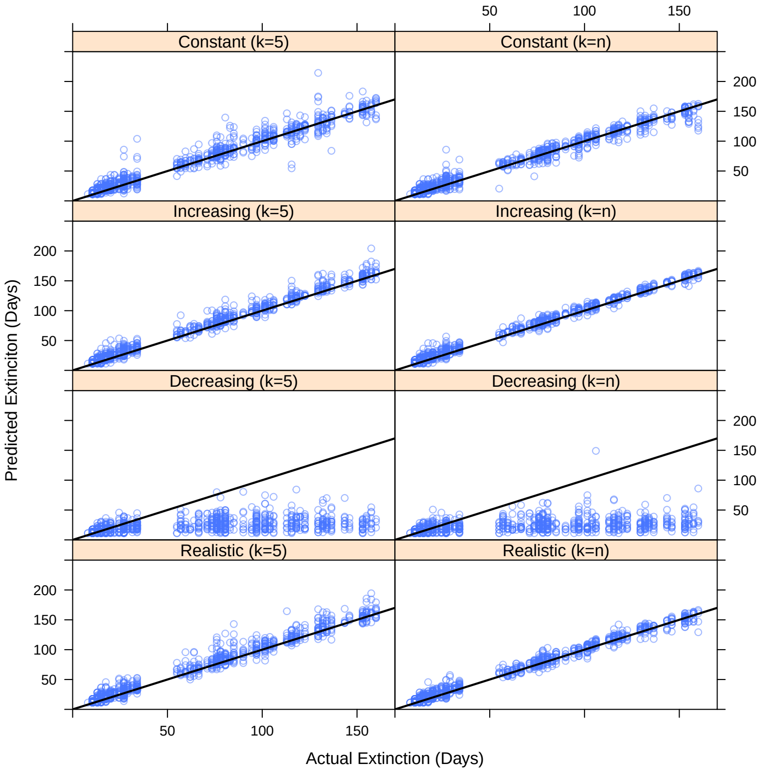

Figure 2.3: Plots are of 500 randomly selected predictions of extinction for each species (*P.caudatum* & *Loxocephalus*) where sampling was irregular, i.e. all sighting events generated by simulations had a 50% chance of being used in the OLE calculation. So when search effort or population densities are high the vector of sighting events produced is not a simply a regular sighting every 2-3 days, but has some stochasticity to mimic probable real-world sampling scenarios. Line shows 1:1 (or perfect) prediction. Extinction predictions were run with both k=5 and k=n. Irregular searching greatly affects the precision of OLE. Decreasing search effort showed a significant loss of both precision and accuracy, with a large increase in underestimation of extinction date. Gaps in the plots are a product of a lack of extinctions between days 34 and 55.

Using k=n minimized mean error under constant, increasing, and realistic search regimes. Under a decreasing search regime mean error was minimized using k=5 sightings, although this decreased the precision of predictions (r^2 0.98 with k=5 and 0.99 with k=n sighting events, $p<0.001$ for both). The difference in predictive error across all four search regimes between k=5 and k=n sightings was less than one day.

Irregular sampling also provided generally accurate predictions under all but a decreasing regime (Fig. 2.3), however the precision of predictions was lower compared to regular sampling. The precision of predictions was again higher using k=n for constant, increasing, and realistic search regimes (Fig.

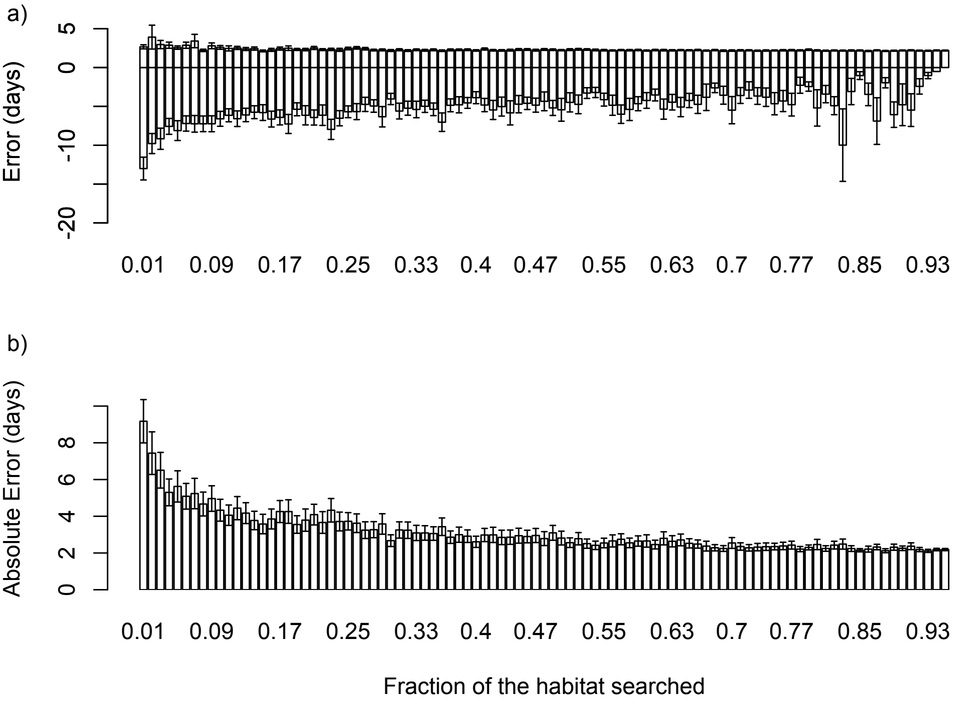

Figure 2.4: Effect of the fraction of the habitat searched on the accuracy of inferred dates of extinction. a) The majority of error in prediction is accounted for by underestimation of the date of extinction. Overestimation shows remarkably little variation as search effort increase above 7%. b) Absolute error shows the increase in overall predictive accuracy as search effort is increased. Above 10% of the habitat sampled there appears to be relatively little increase in predictive precision. All error bars are one SE, missing SE bars indicate means comprised of less than 3 values. Data is from the constant search effort regime with regular searching.

2.3, r^2 values higher than 0.96 and $p<0.001$ for all three regimes). Both increasing and realistic regimes produced estimates of extinction that were prone to overestimation, a pattern that is present with regular sampling although the magnitude of error is small (Fig. 2.2). Irregular sampling with a decreasing search regime produced the greatest error. Predictions of extinction time with a decreasing regime dropped from being generally accurate with regular sampling (mean absolute error of 4.1 days when k=*5*) to providing very poor predictions (mean absolute error of 50.2 days when k=*5*). This error was almost exclusively accounted for by underestimation of the extinction date, and this pattern was more pronounced as the time to extinction increased (Fig 2.3). Precision of estimates with decreasing search effort was also affected by irregular searching ($r^2<0.17$, $p<0.001$ for both k=*5* and $k=n$).

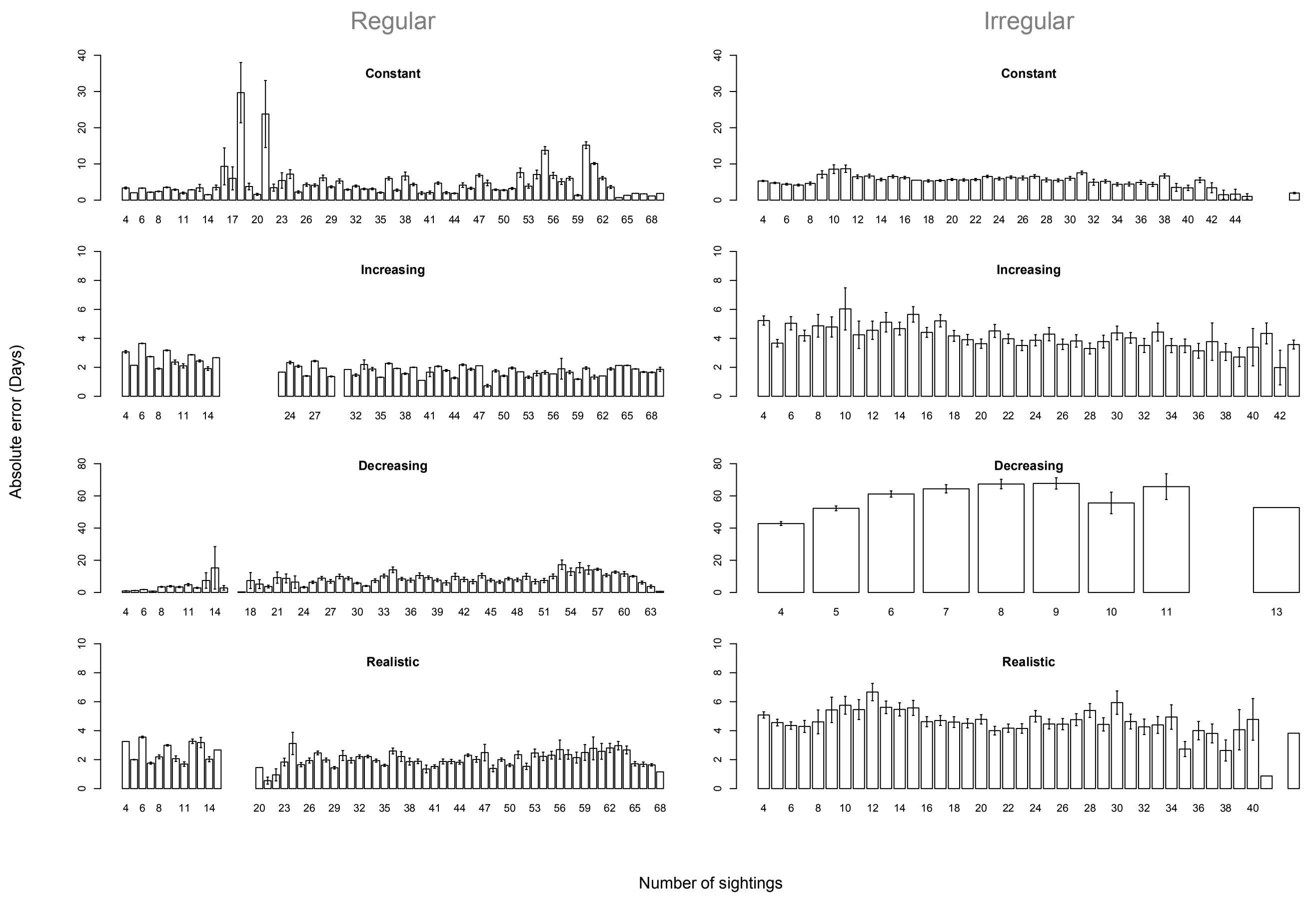

Figure 2.5: Error bars are 1 SE, missing SE bars indicate means comprised of less than 3 values. The number of sighting events used to calculated the extinction date of a species using OLE altered the mean error associated with predictions of time to extinction. Decreasing search regime with irregular sampling appeared to have a slight positive correlation between number of sightings used and error of predictions. This supports the finding that using the last 5 sighting events only for decreasing search regime produced on average less error of predictions. The other three search regimes appear to have no clear relationships between the number of sightings used and the error associated with predictions. Gaps in the plots are both a product of the stochastic nature of sampling and a lack of extinctions between days 34 and 55.

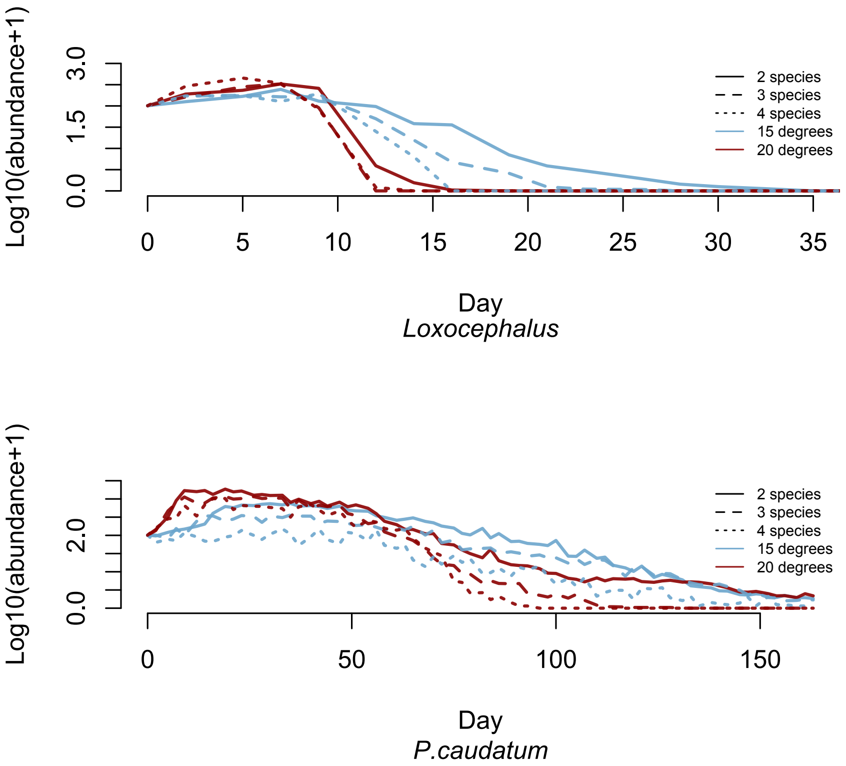

Figure 2.6: Temperature and species richness both negatively correlated with time to extinction, whilst species identity changed the time to extinction by up to an order of magnitude. Higher temperatures and more species rich communities drove both *P.caudatum* and *Loxocephalus* to extinction at faster rates.

The fraction of the habitat searched (held constant through time and with regular sampling) affected the accuracy of predictions, with area of the habitat searched positively correlating with precision (Fig. 2.4). The majority of this error is underestimation (Fig. 2.4a). Overestimation accounts for only a small proportion of the magnitude of the total error, and above 7% search effort there is remarkably little variation in error. Increasing the fraction of the habitat searched from 1 to 4% produced a large decrease in absolute error (an average of 3.9 days, Fig. 2.4b). Similarly an increase from 4% to 10% continued to increase precision, albeit at a lesser rate. Increasing search effort from 10 to 95% produced an increase in precision of only 2.1 days.

The number of sighting events (k) used in the OLE calculation showed no clear relationship to accuracy (Fig 2.5). Increasing and realistic search regimes showed little variation in error as the number of sightings used increased. Con-

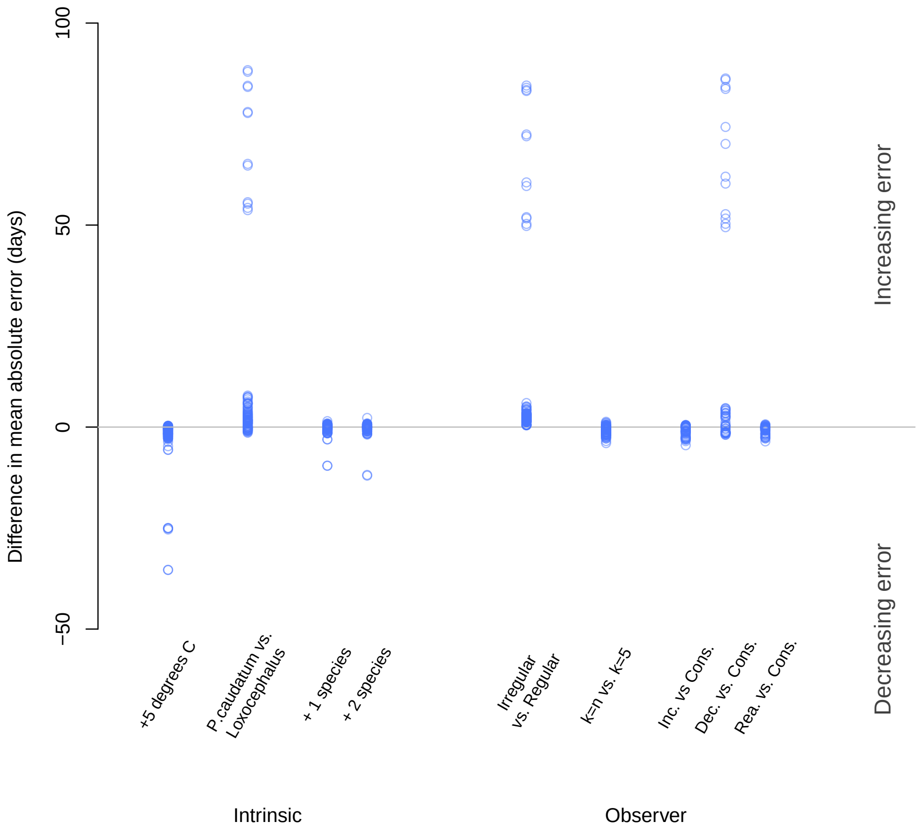

Figure 2.7: Change in error associated with a change in each variable was calculated by subtracting the range of mean errors, grouped by similar intrinsic and observer attributes, from one another. Thus the change in error due to an increase of 5 degrees is the mean error associated with the 20 °C treatment predictions minus the mean error associated with the 15 °C treatment predictions, with all other variables kept constant. Thus all difference in mean error are relative to the reference point, not to the actual date of extinction. Cons. = “Constant” search effort, Inc. = “Increasing” search effort, Dec. = “Decreasing” search effort, Rea. = “Realistic” search effort.

stant search regime peaked in error when k=18, although this can probably be attributed to a low number of estimations of extinction made using 18 sightings (n=16, mean=231), a product of both the stochastic nature of sighting events and a lack of extinctions between days 34 and 53. Decreasing search regime with irregular sampling generated around an order of magnitude more error than regular sampling. With a decreasing regime and regular sampling the number of sightings used shows a weak positive correlation to error, a pattern that becomes more obvious when sampling is irregular.

2.4.2 Intrinsic effects

In this experiment, systems differed in three ways: the number of species present, the identities of those species, and the temperature at which they were kept. These three variables all affected the rate at which species were driven to extinction (Fig. 2.6). However, predictions of extinction produced using OLE appear in general to be unaffected by these variables, with a few notable exceptions (Fig. 2.7). As temperature increased from 15^{o}C to 20 ^{o}C mean error in general decreased, but the majority of this error decreased by an insignificant amount (1-2 days) with a few cases having a much larger reduction in error. Increased species richness had almost no effect on error. The identity of the species was associated with large variation in the error of the predicted date of extinction, with *P.caudatum* almost always having greater error and in a few cases this error was orders of magnitude larger than found in the comparative *Loxocephalus* treatments.

2.4.3 Generation times

In order to better understand the ecological significance of our results we estimated the ecological timescale of our experiments in number of generations. Estimates of maximum intrinsic growth rate (r, Table. 2.1) for the two species (at both 15 and 20 °C) were calculated from the exponential growth phase (identified by visual inspection) of single species communities. Implemented from Stevens (2009) r was the intercept of the linear regression of per capita growth rates at given population densities. Minimum doubling times (T_d, equivalent to generation time at exponential growth (as in Finlay 1977)) were then calculated, by rearranging the equation for exponential growth, as

$$T_d = \frac{ln(2)}{r} \tag{2.8}$$

Minimum generation times were thus calculated as 0.73 days for *P.caudatum* and 0.84 days for *Loxocephalus sp.*.

Table 2.1: Both species identity and temperature affected estimates of maximum r and thus minimum doubling time for *Loxocephalus* and *P.caudatum*. Doubling time of species was used as a proxy to determine the relative time scale of microcosms compared to real world systems.

Species	Temperature °C	r	Doubling time (Days)
Loxocephalus	15	0.83	0.84
	20	0.85	0.81
P.caudatum	15	0.92	0.73
	20	0.95	0.75

2.5 Discussion

Given the problematic nature and history of determining whether a species is extant or extinct (Fisher & Blomberg 2011), and the consequences of getting it wrong (Collar 1998; Jackson 2006), the ability to accurately infer extinction status would be an invaluable tool for conservation. For many species sighting data is all that is available, so if sighting based methods for inferring extinction are accurate then this provides a tool of great utility. For those species where sighting data exist OLE appears, in general, to provide accurate and precise predictions of time to extinction. OLE has, unsurprisingly, proved sensitive to factors both inherent to the system it is applied to, and those arising through the observation process. Under ideal conditions (and assuming an inability to search 100% of a species's habitat) sighting data collected using the highest possible search effort held constant through time with regular, frequent sampling would yield the most accurate and precise predictions of a species's extinction date.

However the major advantage of OLE, and extinction estimators in general, is in their ability to infer extinction under data depauperate conditions. Irregular searching and low search effort can produce large variations in both the accuracy and precision of estimates of extinction. At low search efforts small increases in the search area provide large decreases in error (Fig. 2.4a, b). Search effort correlates positively with the potential to overestimate an extinction event (Fig. 2.4a), and this correlation between search effort and overestimation explains the trends seen in both increasing and decreasing search effort (Fig. 2.1 & 2.2). This makes intuitive sense as searching large proportions of a habitat prior to an extinction event means the chances of observing an individual close to the actual date of extinction increase, and consequently shift the predicted extinction date forward through time (and visa versa).

Overestimation of the extinction date was more common than underestimation (a ratio of ∼3:1), although this is almost certainly a product of low search efforts simply not producing enough sighting events to use in OLE, thus more estimations were made at higher search efforts. That being said where underestimation of the extinction date was present it was generally of greater magnitude than overestimation (means of 6.4 and 3.9 days respectively).

Our results suggest that change in search effort through time, either through increasing or decreasing resource availability or fluctuations brought on by opportunistic sampling, can reduce predictive accuracy, especially if searching is irregular (where patterns observed under a regular sampling appear magnified (Fig. 2.1 & 2.2)). Unfortunately in many situations it may not be apparent whether the search effort has changed (for example where there may be habitat loss, poor records of the area sampled, or areas wrongly identified as suitable habitat). The interaction between changing search effort, search regularity and species identity is one that should be considered carefully, especially if applying OLE or other extinction indices to species whose life histories are particularly long or unknown.

The estimations of extinction with exceptionally high associated error (Fig. 2.7) all share some common features: they are predictions of extinction of the species *P.caudatum* at 15^{o}C under a decreasing search effort with an irregular search pattern. The population dynamics of *P.caudatum* at 15^{o}C (Fig 2.6) are such that for relatively long periods of time population abundances are very low prior to an extinction event: a population of *P.caudatum* survived for 23 days with fewer than 5 individuals, and for 9 days with a single individual. With these dynamics, a decreasing search regime and, importantly, irregular searching, the chances of missing the final few individuals for long periods of time becomes almost a certainty. Where populations fell rapidly to extinction (e.g. *Loxocephalus* in species rich communities at 20^{o}C (Fig 2.6)) predictions produced far less error, even with irregular searching and a decreasing search regime. This supports work by Rivadeneira, Hunt & Roy (2009) who found that model populations with rapid population declines to extinction produced significantly better predictions of time of extinction than those with slower rates of decline, across a range of extinction predictors. It should be noted, however, that because OLE requires a minimum of five sighting events to make a prediction a larger proportion of the population decline is covered by the same number of sighting events for species with rapid rates extinction. This explains some of the overestimation of extinction date seen with a decreasing search regime and irregular searching where extinctions occurred early in the experiment (Fig. 2.2).

There has been some uncertainty as to the optimum number of sightings to use in the OLE calculation (Solow 2005; Collen, Purvis & Mace 2010). Because OLE assumes a Weibull extreme value distribution, large numbers of sightings may violate the asymptotic argument on which the model is based (Solow 2005). Rivadeneira, Hunt and Roy (2009) identified during preliminary analysis that including large numbers of sightings increased the upper bounds of the estimates. However when considering the estimated date of extinction (as opposed to upper confidence intervals as in Rivadeneira, Hunt and Roy (2009)) this was found not to be the case as all regimes other than decreasing showed overall a greater precision when an unlimited number of sightings were used (Fig. 2.1 & 2.2), and there appeared to be no obvious relationship between error and the number of sighting events (Fig. 2.4). Across all search regimes (and under regular or irregular sampling) the difference in mean absolute error between using either k=*5* or $k=n$ was never greater than 3 days. Consequently the use of all available sightings ($k=n$) for real world calculations seems reasonable as for the majority of search scenarios this improved both the precision and accuracy of predictions.

Although on average OLE produces accurate estimations of time of extinction, within this there is both overestimation and underestimation of the extinction date, and when search effort is low and sampling irregular, care should be taken when declaring a species extinct. Previously the confidence intervals (typically set at 95%) produced by OLE and other extinction indices have been used to reduce the chance of erroneously declaring a species extinct (Rivadeneira, Hunt & Roy 2009). However this overcautious approach serves to increase uncertainty, as confidence intervals are typically wide, spanning centuries or millennia (Collen, Purvis & Mace 2010). Within this experiment upper 95% confidence intervals were occasionally over 700 days after the predicted date of extinction (with regular sampling) and over 3000 days after the point estimate of extinction with irregular sampling (Fig 2.8). The use of $k=n$ sighting events reduced the number of extremely high 95% confidence intervals, but only with regular sampling. Within this experiment 95.3% of observed extinctions were encompassed by the upper and lower 95% confidence intervals when sampling was regular, and 82.4% when sampling was irregular. However

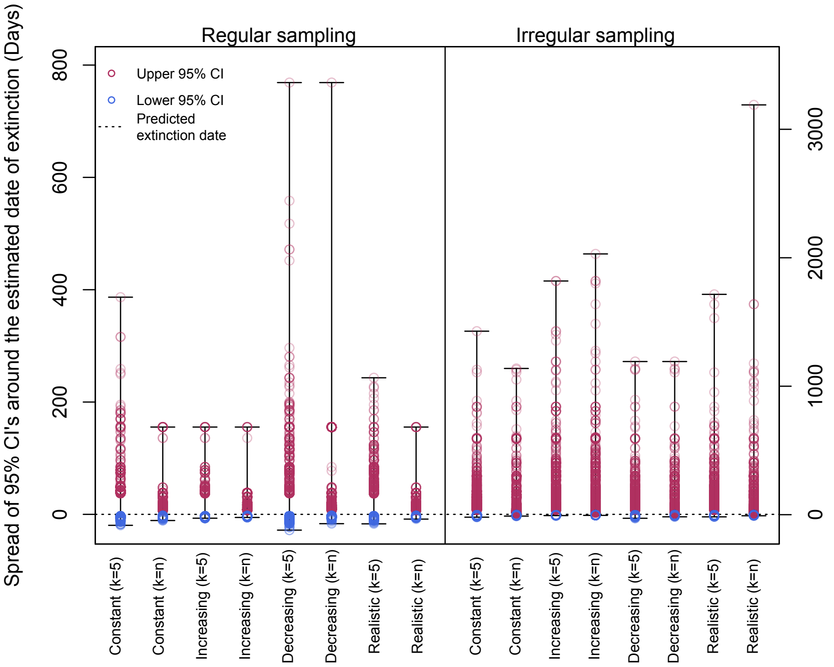

Figure 2.8: Upper and lower 95% confidence intervals (CIs) are often wide, and are dependent on both the number of sighting events used in the OLE calculation and the search regime. Points are a random selection of 500 upper and lower 95% CI estimates for each search regime and each sampling regularity, bars highlight the maximum temporal spacing of CIs around the predicted date of extinction. Using k=5 sightings produce wide confidence intervals more often than when k=n was used. However in many instances there is no difference in the maximum difference from the predicted date of extinction.

an alternative approach is to employ a fixed time period, or “safety-net”, after the point estimate of extinction produced by OLE before declaring a species as extinct. This period should be a balance between the risk of investing time and money on trying to save a species that is extinct and removing funding early and losing a species that could be saved, whilst reducing the uncertainty produce by wide confidence intervals. This is a potentially novel way of applying OLE and using this approach, with a “safety-net” period of 5 days added to point estimates of extinction, 90% of extinction events were encompassed with regular sampling and 73% with irregular. When this “safety-net” is increased to 10 days these figures grow to 95% and 81% respectively.

Estimates of minimum generation times for *P.caudatum* and *Loxocephalus* (between 0.73 and 0.84 days (Table 2.1)) suggest that it is both convenient and appropriate to consider a day within the microcosm system to be roughly analogous to a year in a natural system. These estimates would then be roughly equivalent (0.73-0.84 years) to, say, time of first reproduction of many mammal species (Wootton 1987). If a day can be viewed as roughly analogous to a year (and the species in question has generation times roughly equivalent to those presented here), then imposing a "safety net" of 5 - 10 years, depending on the regularity of historical searching, after the predicted extinction date before a species is declared extinct would reduce any potential "Romeo Error" (declaring a species as extinct when it is still extant, Collar 1998). However this is a rough estimate, not a prescriptive value. Species with significantly different life histories, fecundity, ability to recover from small populations and a myriad other factors, could influence the time period required as a "safety net".

2.6 Conclusions

Optimal Linear Estimation has proved sensitive to both intrinsic and observer controlled factors, however on average it appears to accurately predict the date of an extinction event. Use of the point estimate of extinction generated by OLE, as opposed to the confidence intervals considered previously, provides more sensible estimates of extinction that are more widely applicable to real-world scenarios.

The accuracy of predictions was highly dependent on search effort, search regularity and change in search effort through time. Increasing search effort from 1 to 10% of a species's habitat rapidly reduces the error associated with predictions, whilst searching more than 10% produces a negligible increase in predictive precision. Decreasing search effort through time and irregular searching are liable to produce the greatest predictive error.

Intrinsic properties of the system, such as populations that persist for extended periods of time at very low densities, can magnify error, especially underestimation of the date of extinction. When low search efforts, irregular searching, and species identity interact, error can be orders of magnitude larger than otherwise found. Extensive testing in this model system suggests that to minimise error in real world scenarios using all possible sighting events will eliminate most of the error associated with differing search regimes, and implementing a 5-10 year "safety-net" before declaring a species as extinct will minimise any "Romeo Error". Observer controlled and intrinsic factors can significantly affect predictive accuracy, and should be taken into account when testing extinction predictors in the future.

2.7 Acknowledgments

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* * *

3 sExtinct: an R package for inferring extinction from sighting events

3.1 Abstract

Here, I present a new package for the R statistical computing environment that combines several sighting based methods for inferring historical extinction. The focus of this package is to provide a user-friendly method for comparison between current extinction estimators, and so facilitate the development of future methods for inferring extinction, as well as make current methods widely accessible. I describe the major functions in the package, the different ways they can be used and the data required. I then provide a demonstration of the package, including its graphical outputs and some simple problem solving on an included sample data set.

3.2 Introduction

Over the last 20 years estimating the extinction status of organisms has become a major concern for conservation biologists, with recent examples highlighting the impacts of wrongly declaring a species as extant or extinct (Wilcove 2005; Fitzpatrick *et al.* 2005; Jackson 2006; Fitzpatrick 2006). For many rare species there is little available data, the majority of which consists of “sighting events”, i.e. an individual of that species has been observed as extant at a given point in time. Consequently focus has tended to centre on inferring when/if a species has gone extinct based on the distribution of these historical sighting events. Comparison of the accuracy of these estimators has historically been difficult (Clements *et al.* 2013), however without comparison there can be little improvement of these techniques into the future. In addition, and central to the reason these methods were created in the first place, inferring extinction quantitatively must be accessible to those working in the field of conservation. Currently (to my knowledge) only a single publication has presented the R code for the techniques they implemented (McPherson & Myers 2009) and there is no framework for simply analysing sighting data to test for extinction status.

Given the widespread use of the computing environment R (R Development Core Team 2013), and this obvious shortfall in the availability of methods for inferring extinction, I developed a package designed to be user-friendly to those not familiar with the techniques employed, but flexible enough to be useful for those wanting to extract specific data. The package is called "sExtinct" and can be installed from the Comprehensive R Archive Network (CRAN). Within this package I have, so far, combined six different sighting-based methods for inferring extinction (Robson & Whitlock 1964; Strauss & Sadler 1989; Solow 1993, 2005; Burgman *et al.* 1995; Roberts & Solow 2003). The different methods can be run independently or all together to provide a comparison between the dates of extinction from the different methods.

In the following sections I will describe the implementation of the methods, both individually and together, and how they handle/return data. Following this I will demonstrate the methods and the potential outputs they can generate.

3.3 Description

The sExtinct package is written entirely in the computing language R (R Development Core Team 2013) and takes advantage the R package "lattice" (Sarkar 2008) for visualising extinction probabilities. It currently consists of six methods for inferring extinction (Table 3.1). To simplify data manipulation all the functions for inferring extinction take data in the form of a two column data frame, the first column being the years a species has, or has not, been observed as being extant, and the second column containing the number of sightings that occurred in each year. For all methods except Robson1964() there must be a minimum of 3 times at which sightings have occurred. For Robson1964() only two are required.

If the techniques are being run through the intermediate or high-level functions (Table 3.1) then missing data (i.e. years where no sightings have been recorded) can, but do not have to, be included within this data frame. Some (but not all) of the low level functions require that missing data be included within the data given to the function. Low-level functions, on which the higher-level functions run, are included in their own right for advanced users, but in the vast majority if instances the use of the intermediate or high level functions is appropriate.

Low-level functions (functions with the .fun affix (Table 3.1)) provide the framework on which intermediate and high-level functions work. Details of the arguments of each function change slightly (Table 3.1), and the data required to calculate the probability of persistence and the outputs of each method differ slightly. For example OLE.fun() only requires the dates at which sightings have occurred and produces a point estimate of extinction, but Burgman.fun() requires a data frame containing all the years at which a species has and has not been observed over a given observation period and predicts the probability of persistence of a species at the last year of the observation period. Further details are given in the package help files.

Intermediate-level functions (Table 3.1) use lower level functions to predict if/when a species has gone extinct between the last sighting event and a given year (typically the current year). Arguments for these functions are a standard data set and a value for alpha (for the 1-alpha confidence interval at which a species should be declared extinct, i.e. when the probability of persisting <= alpha the species is assumed to be extinct). In addition some functions also take a test.year (the point in time after the final sighting event which the chance of a species persisting should be calculated up to, typically the current year) and a true/false argument, data.out, which controls whether the full data set of p-values for each time step is returned, or simply the estimated date of extinction (Table 3.1). Those functions where test.year is required will calculate the date of extinction to the nearest whole time step. Using these intermediate level functions circumvents potential data problems (such as missing values), as data are manipulated to be appropriate for the lower-level function being employed. Because of this, the data given to the intermediate level functions are simply a two column data frame of the years at which a species has been observed as being extant, and the number of sighting events in that year (i.e. non-sighting events can, but do not need to, be included).

Table 3.1: The functions contained within the package sExtinct, the arguments that are taken by the functions, a brief description of what the function does, and the publication it is from.

Function name	Function level	Description
Burgman.fun(dd)	Low	Estimates the probability of persistence by the last date in the given sighting record using the method described in Burgman *et al.* (1995)
OLE.fun(dd)	Low	Infers the date of extinction using Optimal Linear Estimation (Roberts and Solow 2003)
Robson1964.fun(dd, alpha, data.out)	Low	Estimates the truncation point (as in Robson and Whitlock 1964)
Solow1993.eq2.fun(dd)	Low	Estimates the probability of persistence by the last date in the given sighting record using the method described in equation 2 of Solow (1993)
Solow2005.eq7.fun(dd)	Low	Estimates the probability of persistence by the last date in the given sighting record using the method described in equation 7 of Solow (2005)
Strauss89(dd, alpha)	Low	Infers time of extinction using the method proposed by Strauss and Sadler (1989)
Burgman(sightingdata, alpha, test.year, data.out)	Intermediate	Tests the probability of a species having gone extinct by a given test.year, using Burgman.fun
OLE(sightingdata, alpha)	Intermediate	Infers the date of extinction using Optimal Linear Estimation (Roberts and Solow 2003)
Robson1964(sightingdata, alpha, data.out)	Intermediate	Calculates the year at which the probability of persistence is 1 to 0.01 in 0.01 steps
Solow1993.eq.2(sightingdata, alpha, test.year, data.out)	Intermediate	Tests the probability of a species having gone extinct by a given test.year, using Solow1993.eq.2.fun
Solow2005.eq7(sightingdata, alpha, test.year, data.out)	Intermediate	Tests the probability of a species having gone extinct by a given test.year, using Solow2005.eq7.fun
Strauss89(sightingdata, alpha, data.out)	Intermediate	Infers time of extinction using the method proposed by Strauss and Sadler (1989)
run.all(sightingdata, alpha, test.year, plot, data.out)	High	Runs all the intermediate level functions

The highest level function, run.all(), combines all the intermediate functions to produce estimates of the date of extinction from all the methods, with the option to plot these and return either a data frame of the estimated chance of persistence at each point in time from each method, or a simplified table of the predicted dates of extinction (rounded to the nearest year). run.all() takes identical arguments, and data, to the intermediate-level functions, with the addition of the true/false plot argument.

3.4 Example

To demonstrate sExtinct, I created a data set of sighting events that is included within the package (as example.data). This data mimics that of many species where there are infrequent and irregular sighting events spread over decades, and where the species hasn't subsequently been observed as being extant. The data frame of sightings is comprised of two columns; the first is the year at which the sightings have (or have not) occurred, the second is a column of the number of sightings that have occurred in that year. Note that the names of columns within the data frame do not matter, only that the first contains the years, and the second the record of sightings. For the sake of brevity, here I will demonstrate the intermediate and high-level functions only, as these are likely to be the most widely applicable.

3.4.1 Intermediate level functions

All intermediate functions take the data frame described above as the initial argument and an alpha value as a second argument. In addition all functions (except OLE()) also take a TRUE/FALSE data.out final argument, and some take an additional test.year (typically set as the current year), which values for probability of persistence are calculated up to (see Table 3.1). So, running the Burgman() function with the included data set:

```
> data(example.data)
> example.data
```

```
  years sightings
1 1907  1
2 1910  1
3 1915  3
4 1916  4
5 1920  3
6 1925  1
7 1930  2
8 1931  1
```

```
> Burgman(example.data, alpha=0.05, test.year=2012, data.out=FALSE)

Estimate:

[1] 1942
```

To return a full list of the probabilities of persistence (column "chance") for each year (yrs) from the last sighting event until the test.year (set here to 1945 to reduce the size of the output data frame):

```
> Burgman(example.data, alpha=0.05, test.year=1945, data.out=TRUE)

   yrs  chance
26 1932 0.38126262
27 1933 0.43633363
28 1934 0.49178012
29 1935 0.54662696
30 1936 0.59996052
31 1937 0.38939500
32 1938 0.24371061
33 1939 0.15138540
34 1940 0.09449704
35 1941 0.05957024
36 1942 0.03798757
37 1943 0.02451162
38 1944 0.01599916
39 1945 0.01055870
```

3.4.2 High level function

The function run.all() provides a simple way to compare all the methods currently encompassed within the package. In addition to the arguments taken by the intermediate level functions, diagnostic plots are called by the argument plot=TRUE (Fig. 3.1). So for a simple comparison:

```
> data(example.data)
> run.all(example.data, alpha=0.05, 2012, data.out=FALSE, plot=TRUE)
    Test            Estimate
  1 OLE             1935
  2 Strauss         1944
  3 Solow1993.eq2   1936
  4 Solow2005.eq7   1941
  5 Robson          1950
  6 Burgman         1942
```

This produces the predicted date of extinction (rounded to the nearest whole time step) given the sighting record for each method. If NA's are produced in this table then the null hypothesis (that the species is still extant) cannot be rejected. The graphical output shows the probability of persistence at each time step where applicable (Fig. 3.1). OLE() produces a point estimate of extinction only, plotted as the vertical dashed line. The given value of alpha is also plotted. As with the intermediate level functions the full set of probabilities of persistence at each year from the last sighting event until the test.year can be produced by using the argument data.out=TRUE.

3.5 Troubleshooting

The graphic output from the plot=TRUE argument in the run.all() function can be used to check whether the methods appear to be working normally and troubleshoot any problems. Commonly NA's may be produced instead of estimates for the date of extinction, typically this is caused by having a value of test.year too close to the last sighting event and increasing this value is likely to solve the problem. However, this is only possible up until the current year, and cannot be extended into the future to "predict" the future time of a species's loss. Issues may also be encountered if

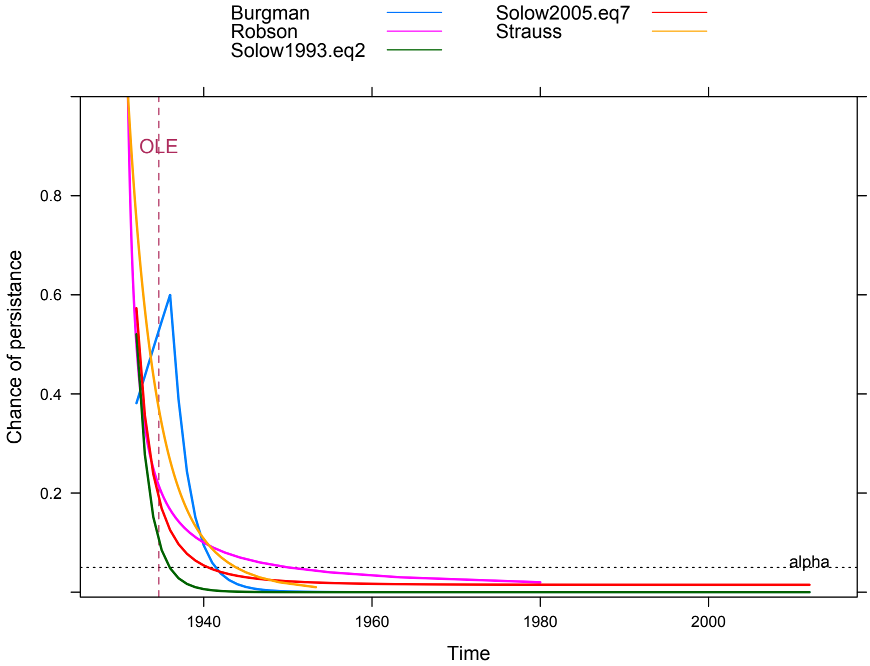

Figure 3.1: Diagnostic plot showing the predicted probability of persistence given the sighting record in the example.data data frame across all methods in the package. OLE produces a point estimate of extinction only (represented as the vertical dashed line), and the alpha level set is marked with the dotted black line.

there are very few sightings, and alpha is relatively large. Here the first year after the last sighting event may have a p-value of persistence less than alpha, and so extinction may be predicted immediately.

The Burgman() function often exhibits an increasing chance of persistence shortly after the last sighting event (Fig. 3.1), as this calculates whether the sightings are randomly distributed. Occasionally the initial estimates may be lower than the value of alpha, however this function has been coded such that the predicted date of extinction is given as the first time the probability returned by the function is <=alpha after any initial increase in the probability of persistence.

The number of sighting events used (both in terms of the number of sightings at each time point and the number of time points where sightings occur) to estimate the date of extinction may, in some cases, invalidate the mathematical assumptions of the methods (Solow 2005). sExtinct does not suppress the number of sighting events used in the calculations, and so care should be taken when analysing sighting histories with many sighting events. A simple solution may be to reduce the number of sighting events at each time point to presence/absence, i.e. the species is either observed or not, although this may affect the accuracy of those methods that can utilise multiple sighting events at each point in time.

3.6 Conclusions

The package sExtinct provides a convenient way of comparing multiple estimators of historical extinction based on sighting only data. The package aims to give a user-friendly interface that allows people unfamiliar with the techniques included to run them with relative ease, however care must still be taken to avoid invalidating any mathematical assumptions made.

3.7 Acknowledgments

This package would not have been possible without code kindly contributed by Ben Collen, Gene Hunt, and Tracy Rout. Additional code was taken from McPherson & Myers (2009). Chih-Wei Tsai checked the translation of MATLAB to R code. Aaron Thierry beta tested the package and manuscript.

3.8 References

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* * *

4 | Historic environmental change may affect our ability to infer extinction status

4.1 Abstract

Correctly classifying a species as extinct or extant is of critical importance if current rates of biodiversity loss are to be accurately quantified. Observing an extinction event is rare, so in many cases extinction status is inferred using methods based on the analysis of records of historic sighting events. The accuracy of such methods is difficult to test. However, recent experiments using microcosm communities suggests that the rate at which a population declines to extinction, potentially driven by varying environmental conditions, may alter our ability to accurately infer extinction status. We tested how the rate of population decline, driven by historic environmental change, altered the accuracy of six commonly used sighting based methods for inferring extinction. We used data from small-scale experimental communities and recorded wild population extirpations, and assessed how the accuracy of the different methods depended on rate of population decline, search effort, and number of sighting events recorded. Although the rate of population decline affected the accuracy of inferred extinction dates, so did the historic population size of the species: faster declines produced more accurate inferred dates of extinction, but only when population sizes were higher. Optimal Linear Estimation (OLE) offered the most reliable and robust estimates, though no single method performed best in all situations, and it may be appropriate to

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The following chapter has been preliminarily accepted for publication in the peer-reviewed journal Conservation Biology use a different method if information regarding historic search efforts is available. Importantly, we show that OLE provided the most accurate estimates of extinction when the number of sighting events used was >10, and future use of this method should take this into account. Data from experimental populations provide added insight into testing techniques to discern wild extirpation events. Care should be taken designing such experiments more closely to mirror the abundance dynamics of populations that suffer real world extirpation events.

4.2 Introduction

Reducing global biodiversity loss in the face of unprecedented population extirpation and species extinction has become a fundamental goal for conservation. However, whilst current extinction rates are thought to be much higher than those recorded in the fossil record (Barnosky *et al.* 2011), quantifying the exact rate of species loss, despite much invested effort, remains problematic (Fisher & Blomberg 2011; Clements *et al.* 2013). This is, in part, due to the difficulty of observing extinction, i.e. the absence of something that is otherwise rarely seen, and this difficulty has given rise to many techniques that attempt to allow historic extinction events to be inferred, rather than observed directly (Burgman *et al.* 1995; McCarthy 1998; Roberts & Solow 2003; Solow 2005). Given the often limited information available on many species, such methods have often concentrated on inferring extinction based on historic sighting events data (e.g. Solow 1993, 2005; Roberts & Solow 2003; Solow & Roberts 2003; McPherson & Myers 2009). Recent work has suggested that such quantitative methods could be used to inform decisions on whether to classify species as extinct (Collen, Purvis & Mace 2010), however the accuracy of these methods remains difficult to test. Traditionally such tests have been tackled with either data from wild populations that may have suffered local extinction events (e.g. Collen, Purvis & Mace 2010), or with data from simulated populations (e.g. Rivadeneira, Hunt & Roy 2009). Recently, we have used experimental microcosm communities to provide detailed abundance time series data for species where the date of extinction can be accurately observed (Clements *et al.* 2013). Such an approach allows one to test the accuracy of estimates because the actual date of extinction is precisely known, something that is rarely possible with wild populations.

The rate at which a population declines to extinction can play an important role in determining how accurately a method for inferring extinction performs (Rivadeneira, Hunt & Roy 2009; Clements *et al.* 2013). Where species persist at low density for a lengthy period of time (and thus are rarely observed), estimates are worse than when the species falls rapidly to extinction. Thus, historic pressures on a species (be those abiotic, such as temperature change or habitat loss, or biotic, such as an invasive species or disease) that increase the rate at which a species declines may alter our ability to judge accurately whether the species has in fact been lost, and over what time frame that may have occurred.

Data that have been collected on wild populations have shown that both the identity of the species (and thus life history) as well as the nature of the threat can alter the rate of population decline (Weimerskirch & Jouventin 1987; Laurance *et al.* 1996; although see Di Fonzo *et al.* 2013). For example, Laurence *et al.* (1996) showed distinct differences in the rates of rapid disease driven population declines of four species of rain forest dwelling frogs, whilst Weimerskirch & Jouventin (1987) recorded differences in the rates of population decline of the Wandering Albatross, *Diomedea exulans*, between islands, probably as a result of each island's location in relation to fishing areas. Given the high rates of environmental change over the last 100 years (Crowley 2000) the potential for factors that govern the rates of a species's decline to alter our ability to infer whether a species is extinct is of concern, and quantifying this effect is an issue that may affect our current understanding of the scale of biodiversity loss.

Here, we utilise small-scale experimental communities to test whether there is a negative correlation between rate of population decline and the magnitude of the error of estimates of time of extinction produced using six commonly applied methods. We then apply the same techniques to eight historic wild population extirpations. We use various rates of environmental change to alter the dynamics and extinction times of the experimental communities, whilst the wild population data are chosen to include a variety of rates of population decline driven by a number of different processes (including habitat loss, disease and extreme weather events). Given time series of the abundance of these experimental and wild populations, we generate sets of sighting events using three search regimes (Rivadeneira, Hunt & Roy 2009; Clements *et al.* 2013). We then examine the effect of the rate of population decline, as well as the effect of search regime and the number of sighting events the calculations use, on the accuracy of estimations of the date of extinction, and compare the robustness of the six techniques. Finally, we make recommendations about the use of such techniques in real-world scenarios.

4.3 Methods

4.3.1 Experimental data

Experimental data were gathered on the abundances of replicate populations of the bactiverous ciliate *Loxocephalus sp.*, which were subjected to various rates of temperature change through time (Fig 4.1a). These different rates of directional temperature change produced varying rates of population decline, and thus times of extinction (Fig 4.1b). Detailed explanations of the experimental set up, temperature treatments, and sampling methodology are covered in Chapter 5 and Appendix 8.1.1.

4.3.2 Wild population data

Data on population dynamics followed by extirpation events were collected from three sources: a literature search (using Google Scholar with search terms such as “extirpation”, “population extinction”, and “extinction dynamics”), the Living Planet Database (Collen *et al.* 2009), and Fagan and Holmes (2006). From these datasets we selected eight time series (Parr 1992; Burrows *et al.* 1995; Laurance *et al.* 1996; Fagan & Holmes 2006): one mammal (African Wild Dog - *Lycaon pictus*), four birds (Hawaiian Crow - *Corvus hawaiiensis*, Corncrake - *Crex crex*, Whooping Crane - *Grus americana*, European Golden Plover - *Pluvialis apricaria*), and three amphibians (Waterfall Frog - *Litoria nannotis*, Common Mist Frog - *Litoria rheocola*, Sharp Snouted Day Frog - *Taudactylus acutirostris*), each with at least seven recorded population abundances prior to a recorded extirpation event (a recorded population count of 0). These time series covered a range of rates of population decline from slow to fast (estimated by fitting linear regressions to the abundance data, Fig. 4.2), hypothesised to be caused by a variety of factors including extreme weather events, disease, habitat loss and degradation, and invasive species. These rates of decline ranged from the very rapid (e.g. Common Mist Frog, *Litoria rheocola*) where approximately 40%

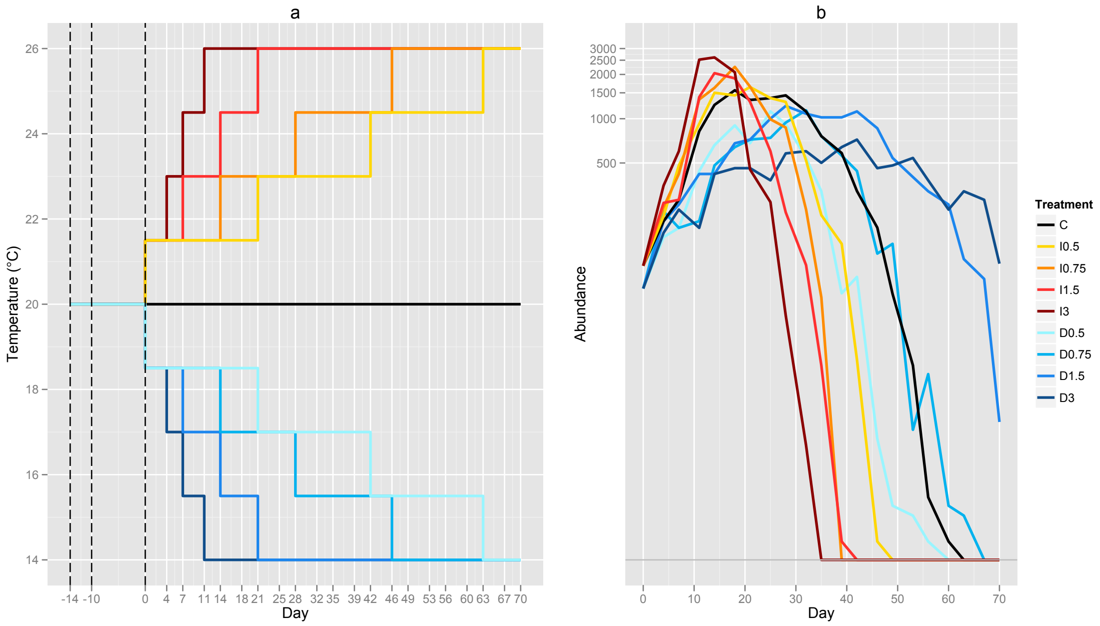

Figure 4.1: (a) Microcosms were heated or cooled at varying rates by moving them between incubators of different temperatures, giving a series of treatments where the rate of temperature change varied. "D" indicates a treatment where temperatures decreased over time, "I" where temperature increased, and "C" where the temperature was constant. Rate of change is printed next to the direction of change, and is measured in degrees per week (so D0.75 is a treatment where temperature decreased by 0.75 °C per week). The medium was initially incubated at 20 °C, with two bacteria species (*Serratia marcens* and *Bacillus cereus*) added at day -14, the protozoa added at day-10, and the media split into microcosms at day 0. (b) Microcosms were sampled through time for abundance data, plotted are the means of replicate populations (error bars not included to improve clarity). Different temperature treatments produced different rates of population decline, with warmed treatments having much faster rates of decline than cooled.

of the initial population was lost per year, to the relatively slow (e.g. Corncrake, *Crex crex*), where the population declined by roughly 16% of the initial population per year.

4.3.3 Creating sighting events

ABUNDANCE data from replicates of *Loxocephalus* where extinction was observed (all populations except those in the treatments D1.5 and D3, where no extinctions were recorded), and wild populations, were converted into sighting data based on the method proposed by Clements *et al.* (2013, see below). To these records of abundance through time three simulated search regimes were applied: (i) constant, (ii) increasing, and (iii) decreasing. The "constant" search regime was simulated with search efforts (the fraction of

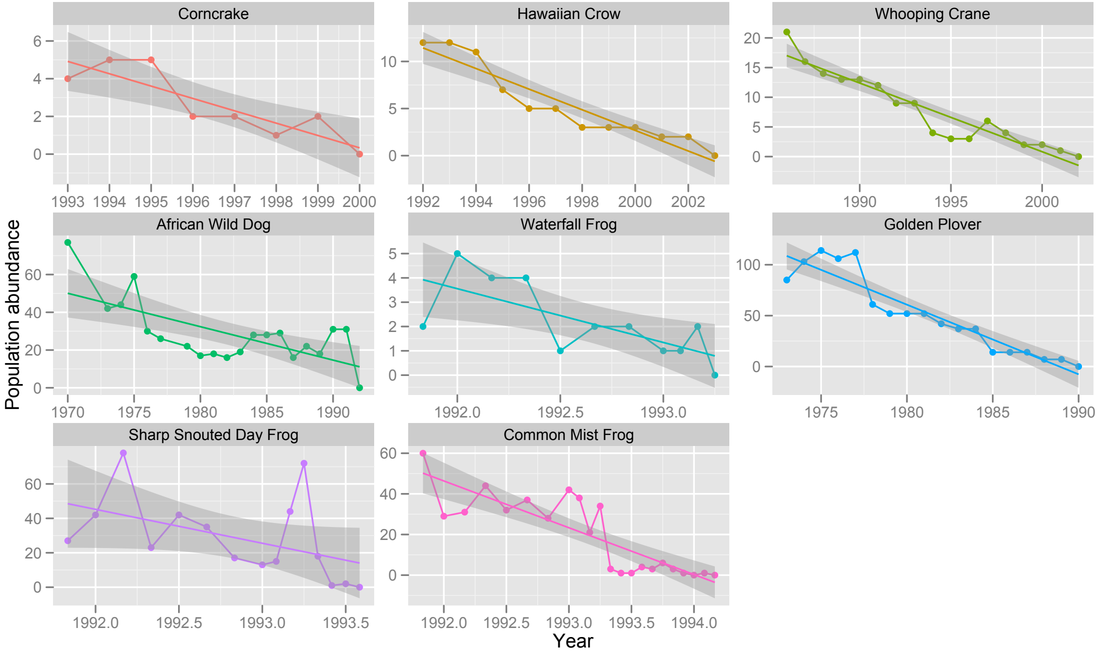

Figure 4.2: Population dynamics prior to extinction of eight species, collected from the literature. Linear regressions give an indication of each population's rate of decline, shaded areas are +/- one standard error. Species are ordered by the rate of population decline, from slowest (top left) to fastest (bottom right). Slopes are as follows: Common Mist Frog = -23.04, Sharp Snouted Day Frog = -19.70, Golden Plover = -6.84, Waterfall Frog = -2.21, African Wild Dog = -1.77, Whooping Crane = -1.16, Hawaiian Crow = -1.09, Corncrake = -0.65.

the habitat searched) of 0.01 to 0.95, in 0.01 steps, held constant through time. The "increasing" search regime had a randomly assigned initial search effort, and then increased by a random fraction at each time step, until the search effort reached 0.95 after which it remained constant. "Decreasing" mirrored the increasing search effort, but the fraction of the habitat decreased through time. These simulated search efforts at each point in time were then used to generate series of sighting events. Multiplying the search effort (the fraction of habitat searched) by the total number of individuals in the entire habitat gave the expected number of individuals observed. The actual number observed was drawn from a Poisson distribution with mean set to this expectation.

As in Clements *et al.* (2013), these sampling regimes produced regular sighting events when search effort or abundance was high. However in reality this is probably unrealistic, as sampling of wild populations is often sporadic. Thus, two search "regularities" were simulated: "regular" sampling (as above), and "irregular" sampling. Irregular sampling was implemented identically to the regular sampling, but with every time point where observations occurred having a 50% probability of being used. All analyses present results that include data from both regular and irregular sampling, with the results of the effect of regularity of sampling on the accuracy of extinction estimates presented in Appendix 8.1.2.

Hereafter, we refer to the times at which sightings were recorded as "sighting events" (these are days in the experimental system, and months or years in the wild population data). At each of these points in time there are a number of observations generated, which depend on the abundance of the population and the amount of the habitat searched: these are referred to as "sightings". If there were fewer than four sighting events then estimates were not made.

The sighting records derived above were used to test the six sighting-based methods for inferring historical extinction currently included in the R package "sExtinct" (Appendix 8.1.3, Chapter 3). These methods do not explicitly take into account the search effort that generated a sighting event (although methods that do this do exist, e.g. Thompson *et al.* 2013). Thus, given that the methods here will be applied where search efforts are inherently unknown, it is especially important to gauge their performance under various search efforts and drivers of predictive error. The methods are referred to by simplifications of the function names in the R package, and are as follows: (i) Burgman (Burgman *et al.* 1995), (ii) OLE (Roberts & Solow 2003; Solow 2005), (iii) Robson (Robson & Whitlock 1964), (iv) Solow1993.eq2 (Solow 1993), (v) Solow2005.eq7 (Solow 2005), and (vi) Strauss (Strauss & Sadler 1989). Very high numbers of sighting events caused the Burgman technique to fail, and so the number of sightings was converted to presence/absence data (i.e. an individual had been observed or not at that time point) for use with this method.

Of the methods included in the sExtinct package, three (Burgman, Solow 1993.eq2, and Solow2005.eq7) calculate the probability that a species has gone extinct at a given point in time. For these methods, the package tests the probability of extinction iteratively at each time point after the last sighting event, up until a given date (the "test.year", see "sExtinct" help files). The date of extinction is then calculated as the date at which the probability of a species persisting falls below the alpha value. Typically, for real world data the test.year will be set to the current year (i.e. what is the probability that a species is extinct). Preliminary testing (not presented) suggested that the maximum extinction date for the microcosm system would not fall above day 300, and for the real-world extirpations not above the year 2200, so we set the test.year conservatively at day 400 for the experimental data and 2300 for the wild population data. For those methods that simply produce a point estimate of the date of extinction from a sighting record (OLE, Robson, Strauss), only estimates that were less than or equal to day 400 were included in the analysis. After the simulations had been run, we were able to show that the maximum estimated date of extinction for the microcosm data was day 225, and year 2061 for the real-world data, both well under the time up to which extinctions were tested.

Simulated samplings were run on the experimental and wild population data enough times to provide 500 extinction estimates for each combination of search regime and search regularity. For the experimental data the simulations were run 950 times for each individual experimental population. This number was chosen because the constant search regime had a fixed number of search efforts (95, see above), and this was then repeated 10 times to generate a high number of extinction estimates. This was then mirrored in the increasing and decreasing regimes. In total, across the replicate populations, search regimes, search regularities, and number of sighting events, this produced 631,452 simulations where at least four sighting events were produced (and thus an extinction estimate could be made). Given the generally low population sizes and short time series of the wild population data, all simulations were repeated four times as often as the experimental simulations to produce a sufficient number of occasions where four sighting events occurred, producing a total of 746,148 sets of more than four sighting events.

To assess the accuracy of each method, error was calculated as the difference between the inferred date of extinction and the observed date of extinction. Because the number and temporal distribution of sighting events were determined by the search regimes, all analysis were carried out on a subset of the data; 500 randomly selected extinction estimates from each search regime for each of the six methods for inferring extinction. The wild population data covered a range of population decline rates, and a range of time spans over which those declines were monitored. Because of these different observation periods, error of inferred dates of extinction was normalised by dividing it by the minimum time between observations (for most species this was 1 year, but could be as little as four weeks (e.g. for the Common Mist Frog).

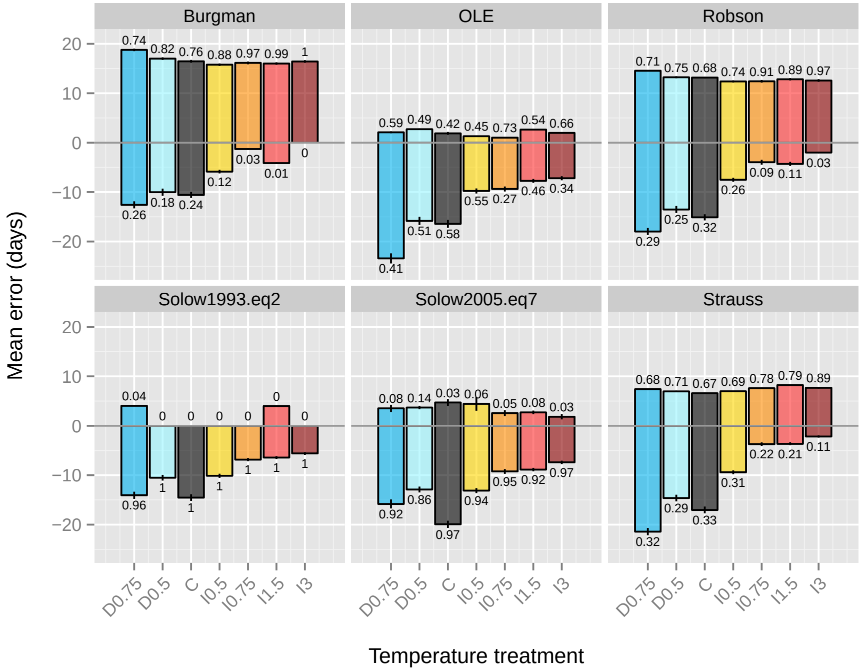

Figure 4.3: Mean error of predictions made by each method across the different temperature treatments. Treatments are ranked from fastest decreasing on the left, to fastest increasing on the right. Error is the difference between the predicted date of extinction and the observed date of extinction, calculated from of a random subsample of 500 data points from each combination of search regime and search regularity. Bars represent +/- 1 s.e. and printed above or below each bar is the proportion of estimates that were overestimates or underestimates of the date of extinction.

All simulations were carried out using the R statistical software (R Development Core Team 2013). In the main we assess the accuracy of estimates in terms of relative error (the distance from the inferred date of extinction to the observed date of extinction, split into overestimation and underestimation of the extinction date). We used the frequency and magnitude of overestimation and underestimation to assess the overall robustness of each method.

4.4 Results - experimental data

4.4.1 Effects of environmental change

THE rate and direction of temperature change altered the rate at which populations declined (Fig 4.1b); warmer treatments produced faster rates of extinction, and cooler treatments slower. These environmentally driven rates of decline affected the accuracy of estimates, with mean error

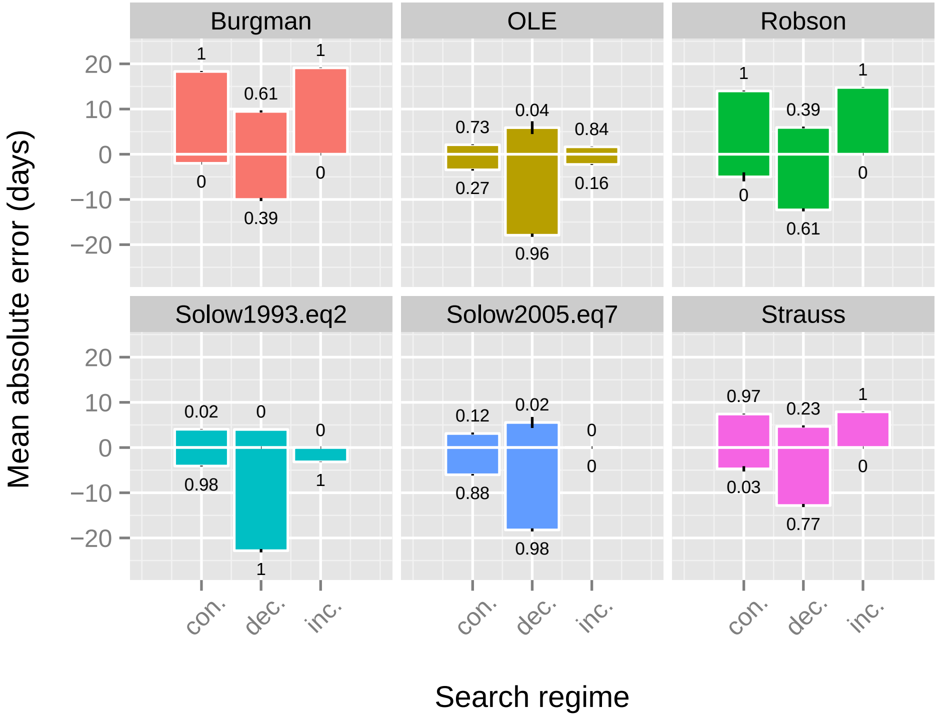

Figure 4.4: Mean error of predictions made by each method under the different simulated search regimes. Error is the difference between the predicted date of extinction and the observed date of extinction, calculated from of a random subsample of 500 data points from each combination of search regime and search regularity across the seven temperature treatments (Fig. 4.3). Effect of search regularities are presented in Appendix 8.1.2. Bars represent +/- 1 s.e. and printed above or below each bar is the proportion of estimates that were overestimates or underestimates of the date of extinction. “con” = constant, “dec” = decreasing, “inc” = increasing.

of estimates tending to be higher in cooler treatments, and lower in warmer treatments (Fig 4.3). In general this effect was most noticeable in the change in the accuracy of underestimates of extinction, with all methods showing a clear decrease in the mean error of underestimates at warmer temperatures.

The proportion of underestimates to overestimates of the extinction date was also affected by the treatments, with, in general, warmer treatments having a greater proportion of overestimates than cooler treatments, although this was not true for Solow1993.eq2 and Solow2005.eq7.

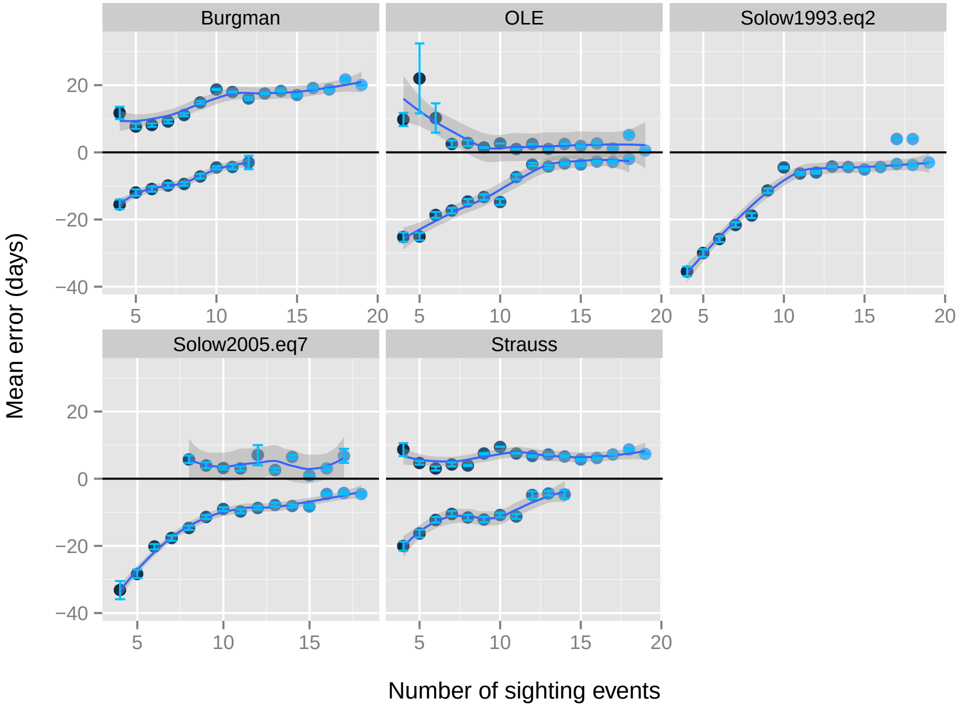

Figure 4.5: Mean error of predictions depending on the number of sighting events (i.e. dates at which sightings occurred) used to calculate extinction across all search regimes and experimental treatments. Error is the difference between the predicted date of extinction and the observed date of extinction, calculated from of a random subsample of 500 data points from each combination of search regime and search regularity across the seven temperature treatments (Fig. 4.3). Error bars are +/- 1 s.e., trends are shown by a LOESS smoothing, shaded areas represent +/- 1 s.e of the LOESS. The effect of the number of sightings on the Robson method is not presented, as the techniques estimates extinction from the distribution of the last 2 sighting events only.

4.4.2 Effects of search regime

THE effects of search regime and number of sighting events used (below) were calculated across data from the seven temperature treatments where extinction occurred. Search regime dramatically altered the accuracy of estimates (Fig 4.4). For half of the methods (OLE, Solow1993.eq2, Strauss) error was minimised when the search regime was either constant or increasing, and the greatest error was generated when the search regime was decreasing (Fig 4.4). For OLE and Solow1993.eq2 the vast majority of the error generated by decreasing search effort was underestimates of the date of extinction. Solow2005.eq7 produced no estimates of extinction when search efforts were increasing.

Burgman, Robson, and Strauss all showed similar patterns of error, with the greatest magnitude of overestimates occurring when the search regime was either constant or increasing (this error was typically much greater than OLE, Solow1993.eq2 or Solow2005.eq7), and the greatest magnitude of underestimates occurring when search effort was decreasing.

4.4.3 Effects of number of sightings used

The number of sighting events (time points at which sightings were recorded) used to infer extinction altered the accuracy of all of the methods tested (Fig 4.5). In general the more sighting events used, the lower the mean error, this was especially true for underestimates of the date of extinction, which, across all methods, increased in accuracy as the number of sighting events used increased. In general, the greatest accuracy of estimates was achieved when the number of sighting events was greater than 10, and this was especially noticeable with OLE, Solow1993.eq2, and Solow2005.eq7. The Robson method was excluded from this analysis, as it uses only the last two recorded sighting events to estimate extinction.

4.4.4 Robustness of methods

The method used had a large impact on the accuracy of estimates (Fig 4.6a). Mean absolute error (mean error normalized to positive values) was calculated for each method across all search regimes, and temperature treatments, to give an indication of each method's applicability to real-world data (where information of search effort and rates of extinction are usually unknown). OLE produced the lowest mean error (7.9 days), with Solow2005.eq7 also having relatively low error (9.1 days). All other methods produced mean errors >10.4 days, with the greatest mean error associated with estimates made using Burgman (19.1 days). All methods inferred extinction to have occurred between day 0 and day 400 (the last observed extinction date was day 70) in a high proportion of simulations, except Solow2005.eq7 where extinction was inferred to have occurred in only 1/4 of the simulations (Fig 4.6a). OLE, Solow1993.eq2, and Solow2005.eq7 all produced less mean error than when a random method was selected for each inference of extinction, but more mean error than when the method that produced the lowest error for each inference of extinction was selected (Fig 4.6a).

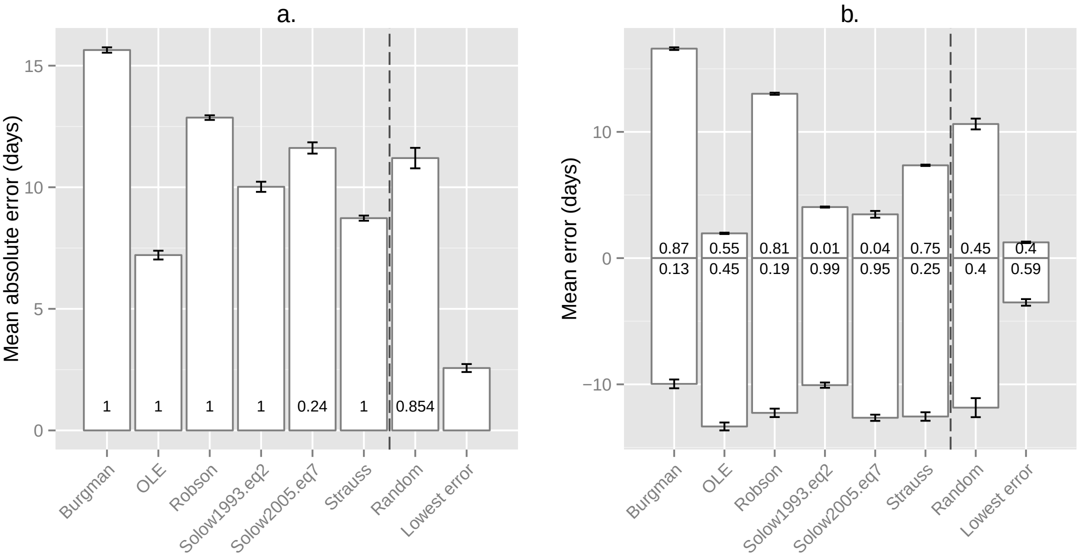

Figure 4.6: a) Mean absolute error and b) mean relative error of predictions made by each method across all search regimes, temperature treatments, and number of sighting events, bars show +/- 1 s.e. Error is the difference between the predicted date of extinction and the observed date of extinction, calculated from of a random subsample of 500 data points from each combination of search regime and search regularity. The mean error if a random method is chosen for each inference of extinction, or if the method with the lowest error is always chosen, are also plotted. Printed in each bar of a) is the fraction of the simulations where there were 4 or more sighting events produced for which each method produced an extinction estimate that fell between 0 and 400 days (note that the last potential extinction event in the experimental treatment was day 70, and that the fraction of simulations that produced estimates is not printed for the“lowest error” as selecting the method with the lowest error inherently means that an estimate has been made), and in b) the proportion of those estimates that fell after the observed date of extinction, and before the observed date of extinction.

When positive and negative errors are plotted separately, instead of being normalised to positive values, it becomes clear that most methods are prone to either overestimation or underestimation of the date of extinction (Fig 4.6b). In some cases this bias is dramatic: Solow1993.eq2 and Solow2005.eq7 underestimate the date of extinction 99% and 95% of the time respectively, whilst Strauss, Robson, and Burgman all overestimate extinction more than 79% of the time (Fig 4.6b). Only OLE shows little bias in the frequency of overestimation to underestimation. The magnitude of this overestimation or underestimation is highly dependent on the method. In most cases, however, the magnitude of error is consistently weighted to either underestimation or overestimation, with the exception of Robson, where the magnitude of the error is

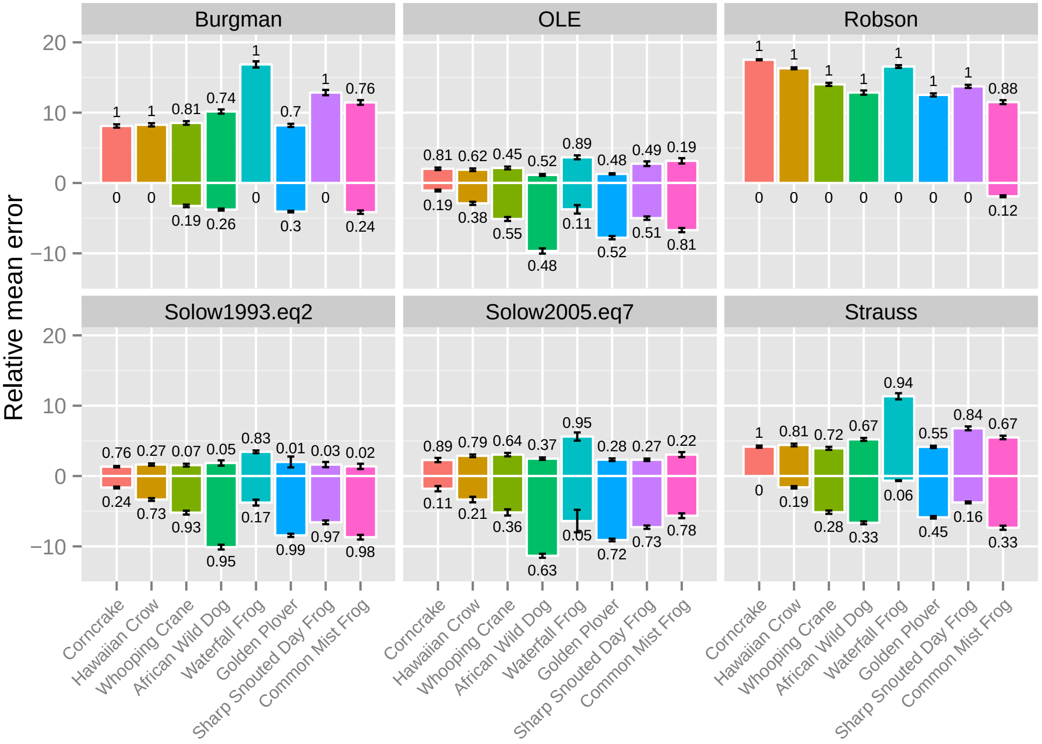

Figure 4.7: Effect of species identity on accuracy of inferred dates of extinction across all search regimes and numbers of sighting events. Error is the difference between the predicted date of extinction and the actual date of extinction of a random sample of 500 data points from each search regime, for each method. Error is normalised across the different observation periods by dividing the error in estimates of the date of extinction by the minimum time between recorded abundances during the observation period of each species. Species are ordered by the rate of population decline, from the slowest (on the left) to the fastest (on the right), with the expectation being that slower declining populations are likely to have greater mean error than faster declining. Bars represent +/- 1 s.e. and printed above or below each bar is the proportion of estimates that were overestimates or underestimates of the date of extinction.

roughly equal (Fig 4.6b). In many instances the difference in the magnitude of the mean error is large, for example OLE tends to have greater error in the underestimates, rather than overestimates, of the date of extinction.

4.5 Results - wild population data

4.5.1 Effects of rate of population decline

THE decline dynamics of the wild populations altered the accuracy of inferred dates of extinction, but the relationship between the rate of decline and accuracy was dependent on the method used (Fig 4.7). A general pattern of decreased accuracy with faster rate of population decline is apparent in all methods except Robson, which showed a decrease in mean error as populations declined at faster rates (Fig 4.7). For OLE, Solow1993.eq2, and Solow2005.eq7 this pattern was driven in the main by an increase in the magnitude of the error in underestimates of extinction, whilst for Burgman the opposite is true (Fig 4.7). Strauss showed an increase in the magnitude of the error associated with both overestimates and underestimates of extinction as populations declined more rapidly. Interestingly OLE, Solow1993.eq2, Solow2005.eq7, and Strauss all show very similar patterns of error across the different species.

Some species had consistently large error associated with their inferred dates of extinction across the majority of the methods tested (notably the Waterfall Frog, which tended to have an inferred extinction date after the actual date of extinction, Fig 4.7). No species had consistently low error in the estimated date of extinction, although the Corncrake and Hawaiian Crow had low error in all estimates save those made by Robson (Fig 4.7).

4.6 Discussion

We show that the rate at which a population has declined may influence the accuracy with which we can infer when that population has gone extinct. Previously it has been suggested that more rapid rates of decline may facilitate accurate inference of extinction (Rivadeneira, Hunt & Roy 2009; Clements *et al.* 2013), and this is indeed seen with some inference methods using data generated from microcosm communities. However, when using data from wild populations the opposite is often observed, with species that decline slowly typically having less error associated with inferred dates of extinction. In line with previous studies (Rivadeneira, Hunt & Roy 2009; Clements *et al.* 2013), we find that the search regime can strongly influence the accuracy of estimates, but that the most important driver of predictive error appears to be the inference method used, and that in general OLE is the most accurate and potentially most widely applicable of the methods tested.

The rate at which a population declines to extinction may vary based on generation time and reproductive output, as well as the rate of biotic or abiotic environmental change (Fig. 4.1b, 4.2). We show that, whilst different rates of population decline can alter the accuracy of estimates, the nature of this effect is not necessarily consistent across different sources of data (Fig 4.3, 4.7). Data from experimental populations produce results similar to those previously observed (Clements *et al.* 2013); more rapid rates of population decline, driven by environmental change, lead to more accurate estimates of extinction time. However, this was dependent on the identity of the method used, probably due to the different assumptions underlying each of the methods (Appendix 8.1.3), and consequently how each method predicts the probability of extinction to change through time (Appendix 8.1.4). For example, Solow2005.eq2 does not predict extinction where search efforts have increased over time, probably because the method assumes the pre-extinction sighting rate decreases, an assumption clearly broken when search effort increases over time. Burgman on the other hand consistently overestimates extinction when search efforts are increasing or constant; however, this is likely to be in part due to the need to reduce high numbers of sightings to presence and absence data. When there are low numbers of multiple sightings at each time point this method may perform better than is suggested by the results here.

It seems unlikely that the results presented here will be particular only to extinctions driven by directional environmental change (as in the microcosms), as previous work using data from modelled populations has shown similar findings (Rivadeneira, Hunt & Roy 2009). Data from wild population extirpations, however, often show an opposing pattern, with increasing error of estimates when the rate of population decline was rapid (Fig. 4.3, 4.7). The differing results generated using microcosm data and those from real world population extirpations may appear conflicting. This could be driven by the difference between the drivers of extinction (directional environmental change in the experimental set up and a variety of pressures in the wild population data), but is more likely to be driven by an interaction between the way sighting events are produced, and the (generally) lower abundances of the wild populations over short observation periods (Fig. 4.2, Appendix 8.1.5). When population abundances are low, and observation periods are short (e.g. the Corncrake, Fig. 4.2), there are only a limited number of possible times at which sighting events can be produced. This means that, unlike the microcosm data, there is limited time over which wild populations can produce temporally sporadic sightings. Sighting events are further reduced by decreasing search efforts, irregular sampling, and because some methods require at least four sighting events to infer extinction. Given that widely temporally spaced sighting events tend to produce estimates long after a population has been observed to go extinct, there are fewer opportunities for poor estimates of extinction to be produced. Consequently, when there are a small number of sighting events that are closely clustered the inferred date of extinction cannot fall far from the observed extinction event. This highlights a problem found in many records of contemporary wild population extirpations: they are both spatially and temporally limited. However, real historic sighting events may cover relatively long periods of time, with potentially a relatively high number of sighting events, a case in point being the most recent sightings of the Dodo (*Raphus cucullatus*): 1598, 1601, 1602, 1607, 1611, 1628, 1628, 1631, 1638, 1662 (Roberts & Solow 2003). Consequently, we suggest that data from microcosm experiments may in fact be far more suitable for testing methods of inferring extinction, not only because the date of extinction can be accurately gauged (Clements *et al.* 2013), but because sighting records more akin to those found historically can be produced than are feasible using short abundance data sets from wild populations. Sighting records produced using microcosm data must then be compared to those typically found in real-world scenarios to see whether such sighting records are appropriate. When designing future microcosm-based experiments the conditions should be such that lower population abundances through time are produced to more accurately reflect wild population declines, which can be achieved by using lower temperatures, lower nutrient levels, or smaller habitats.

The rate and form with which a population declines, and historic search efforts, are both significant drivers of the temporal distribution of sighting events, and, consequently, both are important factors in determining the accuracy of inferred dates of extinction (Rivadeneira, Hunt & Roy 2009; Collen, Purvis & Mace 2010; Clements *et al.* 2013). To illustrate this conceptually, imagine a situation where a population declines slowly to extinction, but search efforts slowly increase, potentially due to increasing concern for that species; a constant frequency of sighting events could result, whilst masking the decline of a population up until a seemingly abrupt extinction event. Conversely, populations that crash from high abundances to extinction over a very short time period (e.g. *Euphydryas editha*, Thomas *et al.* 1996) may have high numbers of sighting events prior to extinction. However, sighting records are typically produced by sporadic chance observations of a species, often as a byproduct of some other endeavor, rather than systematic searches for an endangered species (Roberts & Solow 2003). Gauging historic search efforts is therefore likely to be difficult. In real world terms, this means that appropriate choice of which method to apply, and the number of sighting events to use, are likely to be the two main ways in which error can be minimized. Identifying techniques that provide robust, accurate estimates over a variety of different potential drivers of error is thus of critical importance.

We find the method that produces the lowest mean error among our tests is OLE (Fig. 4.4, Appendix 8.1.6), and that it also exhibits relatively little bias towards either overestimation or underestimation of the date of extinction. In addition, and unlike some other methods (notably Solow2005.eq2), OLE infers extinction to have occurred in a high proportion of the simulations (Fig. 4.6a, Appendix 8.1.6). This means that for many real-world situations, where historic search efforts and rates of population decline remain unknown, OLE should be regarded as the most reliable of the six methods tested here. Of particular importance to the real-world application of this method is our finding that using OLE with 10 or more sighting events typically produces the most accurate estimates of extinction. This contradicts the widely held belief that OLE should be used with the five most recent sighting events only (Solow 2005), and consequently we recommend a change in how this method is used in the future.

In situations where search effort has decreased through time OLE, and in fact the majority of methods tested here, does poorly (Fig. 4.4). This is probably a function of infrequent sighting events that are not representative of actual population declines (sighting frequency declines rapidly, driven by search effort rather than population decline). Irregular sampling often exacerbates this pattern, although the size of this effect is rather small and in general the methods tested are robust to the regularity of sampling, an encouraging finding when applying such methods to real-world data (Appendix 8.1.2). The Robson and Strauss methods do better than the other four methods tested when search effort is decreasing (Fig. 4.4), almost certainly a function of their tendency to overestimate the date of extinction in most other circumstances (Fig. 4.6b), a fact that makes them less appropriate for use where search efforts are constant or increasing. If there were an indication that the search effort through time that produced a series of historic sighting events had declined, but the exact search effort had not been recorded, then choosing either

Robson or Strauss as an alternative to OLE would be appropriate. However, if no such information is available then OLE should be used, as it is relatively robust to search effort and overall produces accurate estimates of extinction.

Where a more detailed knowledge of sampling intensity over time is known, other methods may be applicable. For example, search effort through time may be explicitly accounted for in the method proposed by Thompson *et al.* (2013). However, the availability of information on historic search efforts is often lacking - a function of the often stochastic nature of sighting events - and potential solutions for effectively selecting extinction estimators in the absence of this information have been suggested (Vogel *et al.* 2009). For example, the use of L-moments to assess how well the assumptions of each method are met by the underlying distribution of historic sighting events could be implemented (Vogel *et al.* 2009). Testing the L-moment approach using experimental data with known extinction dates, and varying rates of species decline, could form an interesting future direction for the selection of such sighting based methods of extinction.

4.7 Conclusions

In conclusion, to gauge accurately the current rate of biodiversity loss we must be able to reliably classify a species as either extinct or extant. However, many factors may influence our ability to infer extinction status correctly, not least the choice of inference method. In an ideal situation, methods could be selected based on their strengths. Unfortunately, this is probably an unrealistic scenario given the often poor knowledge of important factors that relate to these strengths, such as search effort. Consequently, methods should be applied that are robust to a variety of drivers of uncertainty. This work shows that in the majority of cases OLE (Roberts & Solow 2003; Solow 2005) provides the most accurate estimates of the time of extinction of experimental and wild populations. Importantly, and contrary to previous work (Solow 2005), we show that the accuracy of OLE improves as the number of sighting events used increases, and that ideally one should infer extinction using this technique with a minimum of ten sighting records. Using a robust technique such as OLE will allow more accurate inference of the current extinction status of species than would be possible if one were to pick one of the six methods tested here without any prior knowledge. However, in certain circumstances (especially when historic search efforts have been decreasing) inferred dates of extinction should be treated with care. If there was an indication that this had occurred, using either Robson (Robson & Whitlock 1964) or Strauss (Strauss & Sadler 1989) instead could be appropriate. Where greater information on search efforts is available, techniques that explicitly account for search intensity should be considered (e.g. Thompson *et al.* 2013).

4.8 Acknowledgements

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5 The effects of directional environmental change on extinction dynamics in experimental microbial communities are predicted by a simple model

5.1 Abstract

GLOBAL temperatures are expected to rise between 1.1 and 6.4 °C over the next 100 years, although the exact rate will depend on future greenhouse emissions, and will vary spatially. Temperature can alter an individual's metabolic rate, and consequently birth and death rates. In declining populations, these alterations may manifest as changes in the rate of that population's decline, and subsequently the timing of extinction events. Predicting how temperature change can alter the timing of such events could be of considerable use. We use a small-scale experimental system to investigate how the rate of temperature change can alter a population's time to extinction, and whether it is possible to predict this event using a simple phenomenological model that incorporates information about population dynamics at a constant temperature, published scaling of metabolic rates, and temperature. In addition, we examine (i) the relative importance of the direct effects of temperature on metabolic rate, and the indirect effects (via temperature driven changes in body size), on predictive accuracy of the model (accuracy defined as the proximity of the predicted date of extinction to the mean observed date of extinction), (ii) the combinations of model param-

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eters that maximise accuracy of predictions, and (iii) whether substituting temperature change through time with mean temperature produces accurate predictions. We find that extinction occurs earlier in environments that warm faster, and this can be accurately predicted. Increasing the number of parameters that were temperature-dependent increased the model's accuracy, as did scaling these temperature-dependent parameters with either the direct effects of temperature alone, or with the direct and indirect effects. Using mean temperature through time instead of actual temperature produces less accurate predictions of extinction. These results suggest that simple phenomenological models, incorporating metabolic theory, may be useful in understanding how environmental change can alter a population's rate of extinction.

5.2 Introduction

GLOBAL climate change is forecast to alter environmental conditions significantly over the next 100 years (IPCC 2007). Current predictions suggest that global temperatures will rise between 1.1 and 6.4 °C over this time period, although the exact magnitude, and therefore rate, of temperature change will depend both on location and future greenhouse gas emissions (IPCC 2007). This heterogeneity in the rate of temperature change means that some areas (e.g. the polar regions) are expected to experience rates of warming up to twice the global average (Koenigk *et al.* 2007), with potentially profound impacts on the species that reside there (Thomas *et al.* 2004). Temperature can have complex effects at the individual level - for example increasing temperature increases metabolic rate, meaning that an individual will use resources at a faster rate, senesce faster, and ultimately die sooner (Van Voorhies & Ward 1999; Brown *et al.* 2004) - with potential consequences for the persistence of populations. Understanding how varying rates of temperature change may alter a population's dynamics is therefore essential.

Recent developments in theory have produced an apparently accurate, and convenient, way of incorporating the effects of temperature change into mathematical models (Brown *et al.* 2004). An organism's metabolic rate, and dependent functions such as growth and mortality rates, have been shown to scale with its body size and temperature, via the Arrhenius equation (Gillooly *et al.* 2001; Brown *et al.* 2004). Thus, the metabolic rate of an individual (I)

scales with temperature (T, in Kelvin) and body size (M), as

$$I = i_0 M^{3/4} e^{-E/kT} \tag{5.1}$$

where i_0 is the normalization constant, independent of body size and temperature, E is the activation energy (Ernest *et al.* 2003), and k is the Boltzmann constant (Boltzmann 1872). Such scaling relationships hold over a large number of taxa, life histories, and trophic positions (Brown *et al.* 2004, although see DeLong *et al.* 2010), and this has led to this method's widespread application (e.g. Berlow *et al.* 2009; Reich *et al.* 2006; Woodward *et al.* 2005), though there is also evidence suggesting that there is significant variation in the rate of this scaling (i.e. the activation energy) both within and across species (e.g. Chown *et al.* 2007; Glazier 2010; White, Cassey & Blackburn 2007). Furthermore, temperature has been shown to alter an individual's body size (e.g. Atkinson 1994, Atkinson *et al.* 2003), meaning temperature can both directly alter metabolic rate, and indirectly alter it through the temperature dependence of body size.

From this scaling of metabolic rate (eq. 5.1), the subsequent change in birth, growth, feeding, and mortality rates, as well as other metabolic dependent functions, can similarly be scaled via the same power function (Brown *et al.* 2004). Thus a parameter, P, at temperature t_2 is scaled from the parameter at t_1 as

$$P_{t_2} = i_0 P_{t_1} M^{3/4} e^{-E/kT} \tag{5.2}$$

Temperature influences a wide range of biological processes, but it may be possible to simplify this complexity by incorporating the effects of temperature change into phenomenological models. For many species phenomenological models, such as the Lotka-Volterra equations (Lotka 1920; Volterra 1928), provide a way of describing a population's dynamics through time. Their advantage lies in their simplicity, and consequently the relatively small amount of information required to parameterise them. Knowing whether it is possible to use such simplistic models to accurately predict how a changing environment may alter population dynamics, instead of requiring more complex mechanistic models (that as a result of their complexity require far more information to parameterise), is of some importance.

Ideally, one would tackle such a question using data produced by manipulating natural communities. However, the complexity of such systems, where populations interact within a network of other species, in heterogeneous habitats, means collecting data and isolating replicate populations, whilst eliminating confounding factors, is often unfeasible (Griffen & Drake 2008). Progress can be made, however, by utilising small-scale experimental systems, which by their nature are highly tractable, and allow abiotic factors such as temperature to be precisely manipulated (Griffen & Drake 2008). Whilst such experiments are clearly simplifications of real world systems (and have their critics, e.g. Carpenter (1996)), the ability to replicate extinction events (which are often undesirable in wild populations) has meant that such an experimental approach has often been used as a proving ground for theory (Lawler 1998). Furthermore, if one cannot understand dynamics in such simple systems, then one is unlikely to be able to do so in highly complex natural ones. One of the simplifications often involved in such experimental communities is that they are closed, i.e., there is no immigration or emigration, and/or no inflow or outflow of resources (e.g. Godoy & Costa 2005; Drake & Griffen 2010). Whilst such closed systems may appear unrealistic, naturally occurring populations range from being quite closed to very open (e.g. Polisini *et al.* 1970; Hanski & Singer 2001; Mora & Sale 2002), and indeed it is often the scale of study that determines how open or closed a system is (Camus & de Ciencias 2002). Experiments with closed systems are obviously relevant for more closed populations, such as those on isolated islands, isolated habitat patches, or isolated lakes. However, understanding closed system dynamics can provide important information on within patch dynamics required to understand and model populations experiencing immigration, emigration, and resource flows (e.g. Hanski 1998; Logue *et al.* 2011).

We use closed experimental microcosms to investigate the empirical effect of the rate of temperature change on a protozoan population's time of extinction, and whether these extinction events can be predicted using a simple phenomenological model that incorporates metabolic theory. To address this we fitted a phenomenological model that included intrinsic growth (r), carrying capacity (K), and an exponential decay in the population size (λ) to the dynamics of an experimental population where temperature remained constant. Then, using information on rates of temperature change through time, we scaled the model parameters to predict when a population would go extinct in the treatments where temperature had been manipulated. Using this method, we then addressed the following objectives: (i) to accurately (defined as the proximity of the predicted date of extinction to the observed date of extinction) predict extinction events where temperature change through time had occurred, (ii) to examine if predictions were more accurate if the actual temperature change over time was modelled, rather than the mean temperature, and (iii) to examine the relative importance, on the predictive accuracy of the model, of scaling parameters either directly, or indirectly (via published temperature driven changes in body size), with temperature.

5.3 Methods

5.3.1 Experimental setup

Experimental microcosms consisted of lidded petri dishes (diameter 100mm, height 25mm) containing 50ml of medium. Medium consisted of Chalkley's solution (Thompson *et al.* 1988), which provided essential salts, and 0.05g/L of protist pellet (Carolina Biological Supply, Burlington, NC) which provided organic nutrients. The medium was inoculated on day -14 with the bacteria *Bacillus cereus* and *Serratia marcescens* and incubated at 20 °C. On day -10, 200 individuals of the bactiverous protist *Loxocephalus sp.* (a long-term laboratory culture, originally obtained from the Culture Collection of Algae and Protozoa) were added to each litre of medium, incubated at 20 °C, and left to reach high densities. On day 0, the first day of the experiment, the medium was homogenised and 50ml added to each of 27 petri-dishes (three replicate populations of nine temperature treatments).

The nine treatments comprised of one (termed treatment C) that was incubated at 20 °C for the duration of the experiment (70 days) and eight treatments that were either heated or cooled. All heated treatments started at 20 °C and finished at 26 °C, and all cooled treatments started at 20 °C and finished at 14 °C, however the rate at which microcosms reached these final temperatures differed between treatments (Fig. 5.1). This was achieved by moving the microcosms between nine incubators set at 1.5 °C increments from 14 °C to 26 °C. Thus, the eight rates of cooling and heating were: i) increasing by 3 °C per week (I3), ii) decreasing 3 °C per week (D3), iii) increasing 1.5 °C per week (I1.5), iv) decreasing 1.5 °C per week (D1.5), v) increasing 1.5 °C every 2 weeks (I0.75), vi) decreasing 1.5 °C every 2 weeks (D0.75), vii) increasing 1.5 °C every 3 weeks (I0.5), viii) decreasing 1.5 °C every 3 weeks (D0.5, Fig.

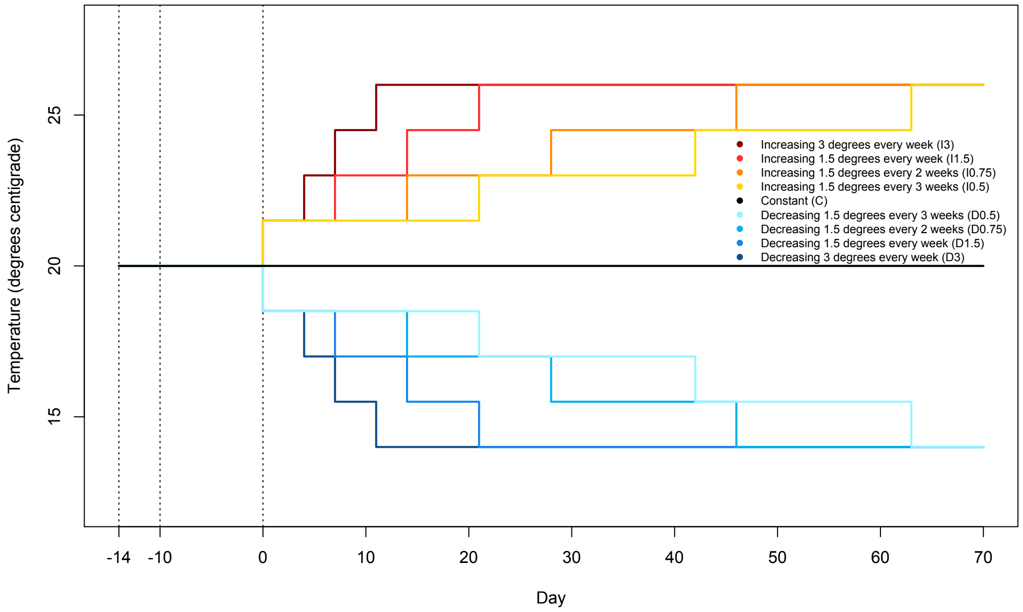

Figure 5.1: Microcosms were heated or cooled at varying rates by moving them between incubators of different temperatures, giving a series of treatments where the rate of temperature change varied. All media was initially incubated at 20 °C.The two bacteria species (*Serratia marcescens* and *Bacillus cereus*) were then added at day-14, *Loxocephalus sp.* were added at day-10, and the media split into microcosms at day 0.

5.1).

5.3.2 Sampling to estimate abundance

Sampling to estimate population abundances was based on that of Lawler and Morin (1993) and was the same as that of Clements *et al.* (2013). Population abundances were estimated twice a week for 70 days. To estimate population abundances the microcosm medium was mixed by repeat pipetting, and a known volume extracted using a Gilson pipette. The individuals of *Loxocephalus sp.* in this known volume were then counted using a stereoscopic microscope, and from this the total population size in the microcosm was estimated. Medium was replaced into the microcosms after counting. When populations became very low, individuals were counted in the whole microcosm under the microscope. A species was recorded as extinct when no individuals were observed after 5 minutes of searching on two consecutive sampling days. Evaporative loss was replaced with distilled water prior to each sampling event.

5.3.3 Phenomenological model

To the mean population abundances of treatment C (incubated at a constant 20 °C) we fitted, using a maximum likelihood approach, a deterministic, four-parameter phenomenological model that incorporates logistic growth followed by exponential decay. This model was chosen because it requires relatively little information to parameterise (only the population size through time, and not, for example, resource abundances). Mean abundances for each treatment were used, as individual replicate populations were highly variable and either produced inappropriate parameter estimates or failed to find a set of parameters that produced a maximum likelihood for the model. Thus we attempted to predict extinction of the "best case scenario", where there is little population variability. Initial population growth was modelled as

$$\frac{dn}{dt} = r \cdot n(1 - \frac{n}{K}) \tag{5.3}$$

Where r is the intrinsic growth rate and K is the carrying capacity. After initial logistic growth an exponential decay in the carrying capacity, K, caused a decline in abundance that drives model populations to extinction. This decline in the model carrying capacity mirrored the observed decline in carrying capacity within the experimental microcosms, which was driven by a decline in the availability of the protist resource (bacteria), in response to the closed and nutrient limited nature of the microcosms. The time at which exponential decay occurred was determined by the parameter alpha, such that when

$$n \geq \alpha \cdot K \tag{5.4}$$

the growth model became

$$\frac{dn}{dt} = r \cdot n(1 - \frac{n}{-\lambda K}) \tag{5.5}$$

where λ is the decay constant.

5.3.4 Scaling parameters with metabolic theory

Estimated parameter values for the constant temperature treatment (C) were then scaled with metabolic theory (eq. 5.2). Temperature has been shown to alter not only an individual's metabolic rate, but also body size (in protozoa body size decreases linearly with temperature by ~2.5% per +1 °C (Atkinson *et al.* 2003)). We assessed the relative importance of including direct and indirect effects of temperature, by predicting population extinction when parameters were scaled in three ways: (i) with temperature only, (ii) with predicted change in body size only, and (iii) with both temperature and body size. Thus in the first case model parameters were determined by the direct effects of temperature as

$$P_t = P_C e^{-\frac{E}{kT}} \tag{5.6}$$

where E is the activation energy, k is the Boltzmann constant, T is the temperature (in Kelvin), and P_C is the estimated value of the parameter from the constant temperature treatment.

In the second instance we scaled the model parameters with body size, which was determined by temperature, so the magnitude of the parameter at a given temperature (P_t) was

$$P_t = P_C M^{3/4} \tag{5.7}$$

where P_C is the parameter in the constant treatment and

$$M = M_{C_t} + ((t - C_t) \cdot 0.025) \tag{5.8}$$

where t is the temperature of the treatment, C_t is the temperature of treatment C (20 °C) and M_{Ct} is the mass at the temperature of the constant treatment.

Thirdly we scaled the parameters with both the predicted change in body size and temperature scaling as in Brown *et al.* (2004)

$$P_t = P_C M^{3/4} e^{-\frac{E}{kT}} \tag{5.9}$$

We assume the parameters r (the intrinsic growth rate), λ (the decay constant) and K (the carrying capacity) will increase with increasing temperature, due to an increase in the protist's bacterial prey densities at higher temperatures, and a subsequent increase in the rate of consumption of organic nutrients (Membré *et al.* 2005; Vasseur & McCann 2005). Thus as temperature increases the growth rate of the protists will increase, as will the carrying capacity and the speed at which these populations decline after the growth period. We

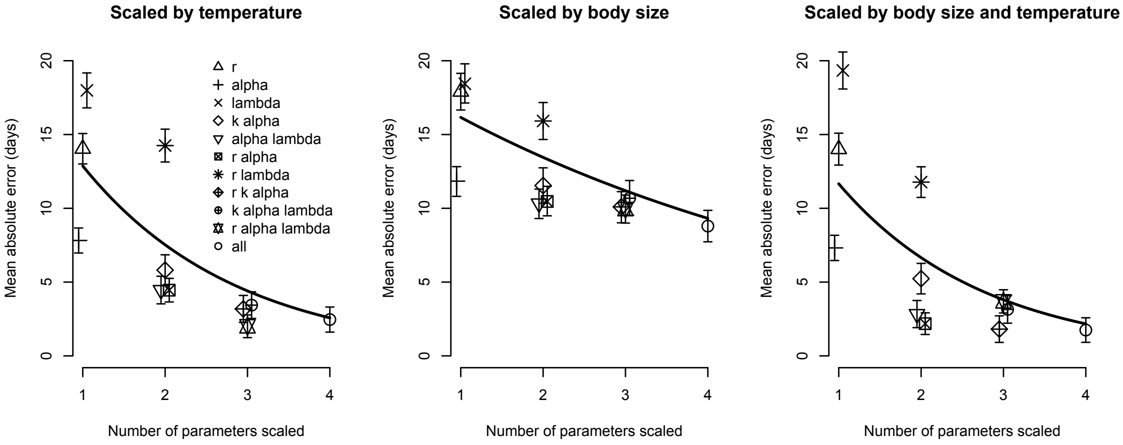

Figure 5.2: Mean error of predictions (+/- 1 s.e.) was calculated as the absolute difference (in days) between the predicted date of extinction given by the model and the mean extinction date observed in the experiment, for a random subset of 500 predictions. Data were only included where predictions of extinction were made for all of the seven experimental treatments where extinction was observed (I3, I1.5, I0.75, I0.5, C, D0.5, D0.75). Increasing the number of parameters scaled in general decreased the error associated with extinction predictions. The lowest error was found where all four model parameters were scaled with both body size and temperature.

assume that α, the fraction of the carrying capacity at which exponential decay occurred, decreased with temperature as higher bacterial densities used the available chemical energy at a faster rate, and thus populations began to decline earlier at higher temperatures. Across species, body size is predicted to negatively correlate with r (Blueweiss *et al.* 1978) and K (Damuth 1981). We assumed that this pattern held between treatments, and that increased body size would decrease λ, as individuals senesce more slowly (Van Voorhies & Ward 1999), but increase α, as larger individuals require more nutrients.

We investigated how these three scaling methods (i. temperature only, ii. body size only, iii. both temperature and body size) affected prediction accuracy when they were applied to all possible combinations of parameters. I.e., each model parameter was scaled individually (whilst holding the other three parameters constant), in all possible combinations of pairs (whilst holding the other two parameters constant) and triples (whilst holding the other one constant), and all four simultaneously (Fig. 5.2).

In order to include uncertainty in the model predictions we drew parameter values from a normal distribution with a mean of the predicted parameter value at the constant treatment, and the standard deviation as the predicted error for each parameter. Then, for every combination of parameters (four individually, six in pairs, four in triplicate, and one where all four are scaled simultaneously, i.e. 15 in total for each scaling method) we ran the simulations 200 times, for 140 time steps (equivalent to 140 days), to produce predicted dates of population extinction for each temperature, each combination of parameters, and each scaling method. Preliminary testing (not reported) showed that 200 simulations were sufficient to produce robust estimates of the mean error of predictions, with no change in the mean error if simulations were run more than 200 times. In addition, we draw the activation energy (E) from a normal distribution, with a mean of 0.652 and a standard deviation of 0.061 (Hansen *et al.* 1997; Vasseur & McCann 2005), as the rate of this scaling varies within species (Brown *et al.* 2004).

5.3.5 Assessing the accuracy of predictions

To investigate how well the dynamics produced by the model could match the observed data we compared the output from the model where all four parameters were scaled with both body size and temperature (this represents the most realistic scenario as protists are known to alter body size with temperature, and both body size and temperature are known to alter metabolic rate) to the observed population dynamics. We assessed the fit of the model dynamics using r-squared values rather than a model comparison method such as AIC, as here we fit a single model to the mean data of one temperature treatment, and then use that model to attempt to predict when the other treatment populations will go extinct.

The error of the model in predicting future extinction events was gauged by comparing the predicted date of extinction to the mean date of extinction of the replicate populations of each temperature treatment, calculated as the first day at which the mean abundance of the replicates fell below one individual. Mean extinction date was used as the model was fitted to the mean abundance of the constant treatment.

Mean error (the difference between the predicted and mean observed date of extinction) for each combination of scaled parameters was calculated from 500 randomly selected predictions across all the temperature treatments and only where predictions were made for all experimental treatments where extinction was observed (i.e. I3, I1.5, I0.75, I0.5, C, D0.5, D0.75). A random sub-sample

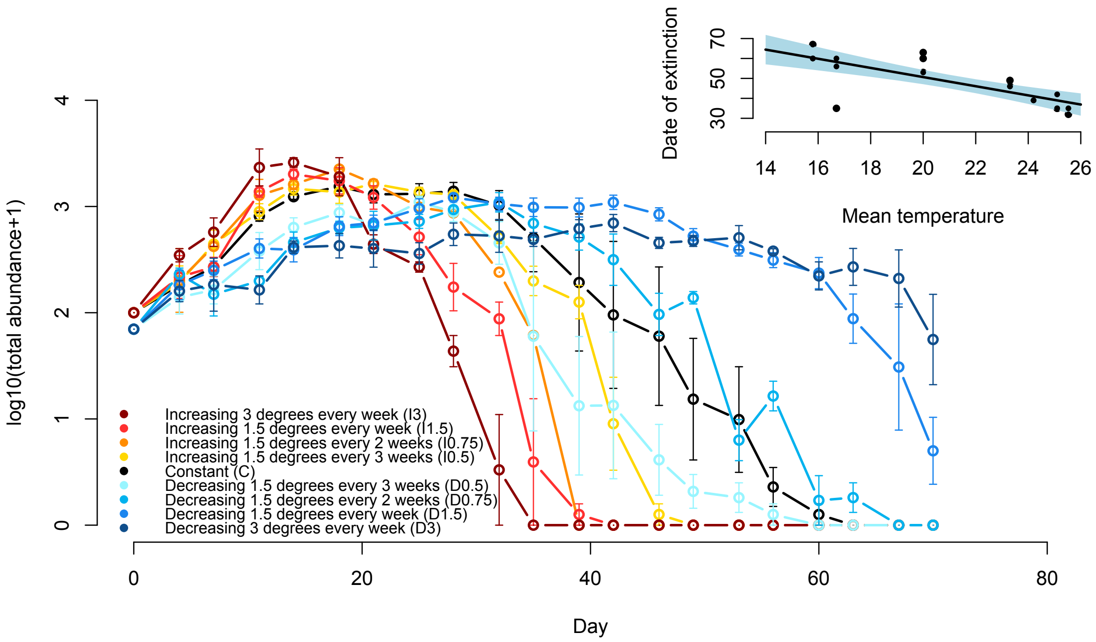

Figure 5.3: Rate of temperature change altered the speed at which replicate populations of *Loxocephalus* went extinct, and also the rate at which populations grew and the maximum population sizes that they reached. Bars are one SE. Inset, mean temperature negatively correlated with extinction date (linear regression fitted to the extinction dates of all replicate populations where extinction occurred (black dots)). Blue area highlights 95% CI. Mean temperature negatively correlated with extinction date (linear regression, coef=-2.3, s.e.=0.46, t=4.93, p<0.001).

of estimates was used as there was an imbalance in the number of estimates produced by each method.

5.4 Results

5.4.1 Effects of temperature treatment

EXTINCTIONS occurred in 19 of the 25 populations, and all but two treatments had extinctions in all the replicate populations (all populations of D1.5 and D3 were extant at day 70). One replicate of each of the treatments I0.75 and I3 was contaminated, and so were excluded from the analysis. Where extinctions did occur, mean temperature negatively correlated with mean extinction date (linear regression, coef=-2.3, s.e.=0.46, t=4.93, p<0.001, Fig. 5.3), and the date of extinction differed significantly between treatments (ANOVA, d.f.=6, F=7.77, p<0.01, Fig. 5.3).

5.4.2 Model predictions - scaling parameters with temperature and body size

When model parameters were scaled with the direct effect of temperature only (i.e. no indirect effects via body size, eq. 5.6) there was relatively low mean error (error calculated as the difference, in days, between the predicted and mean observed date of extinction), although this was highly dependent on which combination of parameters were scaled (Fig. 5.2). There was a significant negative correlation between the number of parameters scaled with temperature and the mean error of the predicted dates of extinction generated by the model (log linear regression, log-coef=-0.51, log-s.e.=0.14, t=3.63, p<0.05, Fig. 5.2). The lowest mean error occurred when the parameters r, α and λ were scaled with temperature (mean error of 2.0 days with a standard error of 0.77).

When parameters were scaled with the indirect effects of temperature, via changes in body size (i.e. no direct effects of temperature, eq. 5.6), there was a relatively large error associated with the predicted date of extinction (Fig. 5.2). Scaling greater numbers of parameters with body size had less of an impact on the mean error, although there was still a significant negative correlation between the number scaled and the mean error (log linear regression, log-coef=-0.18, log-s.e.=0.05, t=3.57, p<0.01, Fig. 5.2). The lowest mean error was produced when all four model parameters were scaled (mean error 8.80 days with a standard error of 1.07).

When model parameters were dependent on direct and indirect effects of temperature (eq. 5.9), prediction error was very similar to when parameters depended on only the direct effect of temperature (Fig. 5.3). Again, there was a strong negative correlation between the number of parameters scaled and the mean error (log linear regression, log-coef=-0.56, log-s.e.=0.15, t=3.73, p<0.05, Fig. 5.2). As when parameters were scaled with body size only, the lowest mean error was produced when all four parameters were scaled (mean error 1.74 days with a standard error of 0.83). This error was not significantly different in magnitude to the error produced by the combinations r-K-α (scaled both directly and indirectly, t-test, d.f.=995.1, t=0.63, p>0.05), r-α-λ (scaled only directly, t-test, d.f.=996.5, t=1.22, p>0.05), or when all four parameters were scaled with only the direct effects of temperature (t-test, d.f.=996.6, t=1.02, p>0.05).

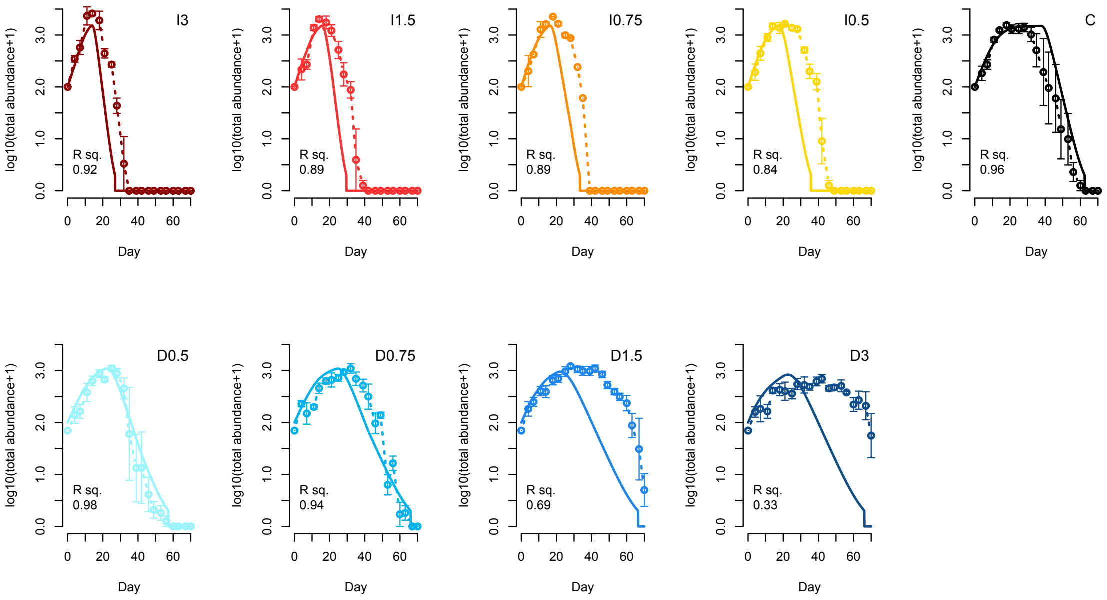

Figure 5.4: Predicted abundances when all four parameters were scaled with both predicted change in body size and actual temperature (solid lines) are plotted against the experimental data (open circles). The model prediction for treatment "C" is based on the parameters produced from the maximum likelihood estimation, all other predictions are produced by scaling the model parameters with mean temperature. Model fits were high (r-squared values 0.69-0.98) for all treatments except D3. The model tended to systematically predict extinction before it had occurred in increasing temperature treatments, and also in the two decreasing treatments where extinction was not observed (D1.5, D3). Bars are one SE.

5.4.3 Model fits to observed data

The fit of the model was generally good (for all but one of the temperature treatments the r-squared value was greater than 0.69, with the maximum r-squared value being 0.98 for the treatment D0.5, Fig. 5.4). In general the model predicted the date of extinction to be earlier than the mean observed extinction date in the experimental communities (Fig. 5.4), but most of the time this error was still within a few days of the mean date of extinction.

In addition to comparing the model fits (r-squared values) where the parameters were scaled with predicted change in body size and the actual temperature at each time step, we compared the fits of the model where mean temperature within each treatment was used to scale the parameters (Fig. 5.5). Here every fit is poorer than when temperature at each time step is

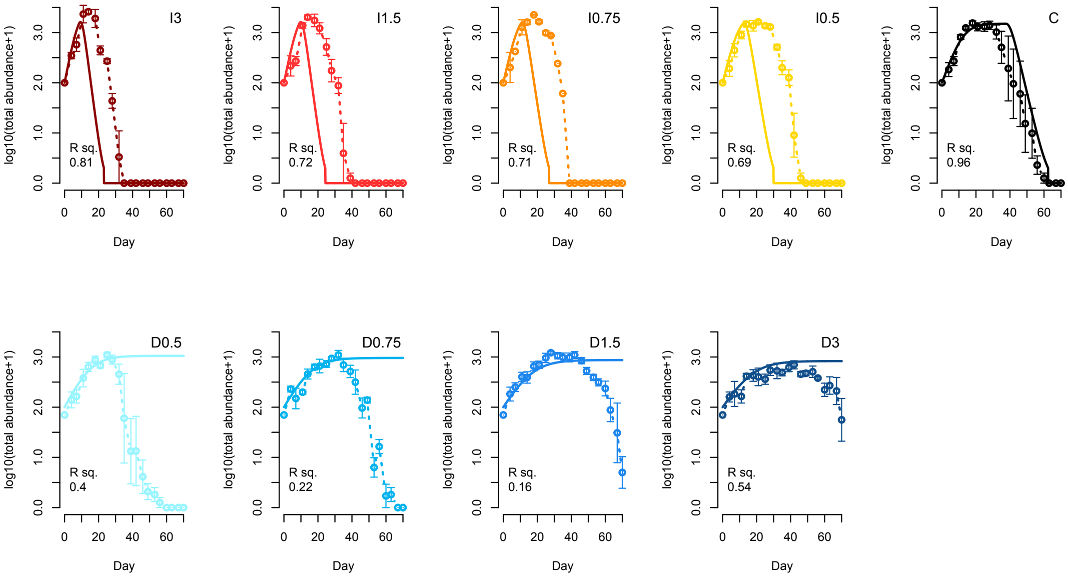

Figure 5.5: Predicted abundances when all four parameters were scaled with both predicted change in body size and mean temperature of each treatment (solid lines) are plotted against the experimental data (open circles). The model prediction for treatment "C" is based on the parameters produced from the maximum likelihood estimation, all other predictions are produced by scaling the model parameters with mean temperature. Model fits were high where temperature increased through time (I3-I0.5), but poor where temperature decreased, especially in comparison to predictions made where parameters were scaled with the actual temperature at any given time point (Fig. 5.4). Bars are one SE.

used (Fig. 5.4, 5.5). Predictions for the abundances of the four increasing temperature treatments still followed the patterns observed in the experimental data, and, as with the fits made using actual temperature change through time, predicted dates of extinction were earlier than the mean observed dates (Fig. 5.4, 5.5). In treatments with decreasing temperatures the model fits were poor, with populations predicted to persist past day 70 (the last day of the experiment).

5.5 Discussion

We show that in a closed, nutrient limited system, the greater the mean temperature (which depends on the rate of temperature change through time), the earlier a population goes extinct. Using a simple phenomenological model, and scaling the parameters with temperature and predicted changes in protist body size, we were able, with reasonable accuracy (R-squared values in most cases >0.84, and mean error of <2 days), to predict the mean date of extinction of a population in a changing environment, from information on the population trends in a constant environment. The accuracy of these predictions was dependent not only on which combinations of parameters are scaled, but also the method by which the parameters are scaled and the rate and direction of temperature change, with the most accurate estimates produced when all four parameters are scaled with both the direct and indirect effects of temperature.

The simple four-parameter phenomenological model provides a good fit to the population dynamics of the constant treatment (C, Fig. 5.4), and, using metabolic theory, we were able to accurately predict the date of extinction in most temperature treatments (Fig. 5.4). However, the accuracy of predictions was dependent upon which parameters of the model were scaled with temperature, and what scaling method was used. In general, across all the scaling methods, increasing the number of parameters scaled produced the most accurate estimates of time of extinction (Fig. 5.2). This is somewhat unsurprising, as temperature is known to alter birth rate, death rate, carrying capacity and the rate at which prey are consumed, all of which are directly or indirectly included within the model presented here, and thus scaling all parameters should include all of the temperature-dependent processes that are occurring within the experimental communities. There is, however, some redundancy in the model, as in some instances scaling only three parameters provided as accurate predictions as scaling all four simultaneously (Fig. 5.2). Where all four parameters are scaled with both the direct and indirect effects of temperature the mean error across treatments was around 1.7 days, or approximately two generations of *Loxocephalus sp.* (Clements *et al.* 2013).

There were large differences in accuracy between the different scaling methods, although to some extent this also depended on the number of parameters being scaled (Fig. 5.2). When all four parameters were scaled simultaneously with only the predicted change in body size (indirect temperature effects), the error associated with predictions was high (a mean of 8.8 days). Scaling all four parameters with only the direct effects of temperature produced a pattern of errors very similar to that produced when parameters are scaled with both temperature and assumed change in body size (predicted to be -2.5% per +1 °C (Atkinson *et al.* 2003), body size was not measured in the experiment, Fig. 5.2). Indeed, there was no significant difference between the mean error produced when all four parameters were scaled with temperature alone, or with temperature and body size. This implies that when modelling such population declines using metabolic theory, the inclusion of the direct effects of temperature is far more important than any body-size driven indirect effects.

The fit of the model depended on the rate of temperature change, with the worst fits in the treatments D1.5 and D3 (Fig. 5.4). This was due to the simplicity of the model and the method for implementing the decrease in carrying capacity (K). The timing of the exponential decline of carrying capacity was controlled by the parameter α, the fraction of the carrying capacity at which exponential decay of the carrying capacity begins to occur. However other assumptions made in the model, specifically that carrying capacity decreases as temperature decreases, occasionally affect the implementation of this decrease in K. This was especially problematic with a rapidly decreasing temperature (i.e. D1.5, D3), as carrying capacity also decreased rapidly, and so the exponential decay of K occured sooner. While a more mechanistic model might improve this situation, there is a downside: more prior knowledge is required to parameterise such a model. Given the underlying variation in extinction date inherent within each temperature treatment, and the already relatively high accuracy of the phenomenological model in predicting extinction (where extinction has occurred), it seems unlikely that the benefits of using a more mechanistic model will outweigh the costs, at least within the small-scale closed system presented here.

Whilst mean temperature negatively correlates with mean extinction date, there exists among-replicate variation in extinction date (Fig. 5.6). In most cases this variation is small, but this is not always the case. Where the model was bootstrapped to include the error associated with parameter estimations, all the extinction events fell within the 95% confidence intervals, and in fact the vast majority fell very close to, or exactly on, the mean predicted date of extinction (Fig. 5.6).

Using metabolic theory to incorporate temperature change into theoretical models has, in part given its convenience and simplicity, been widely applied (Woodward *et al.* 2005; Reich *et al.* 2006; Berlow *et al.* 2009), although variation in the how metabolic rate scales both within and across species suggests that it may not be applicable in all cases (Chown *et al.* 2007; White *et al.* 2007; Glazier 2010). We find that when the body size and temperature components of metabolic theory are both used to scale parameters, model fits

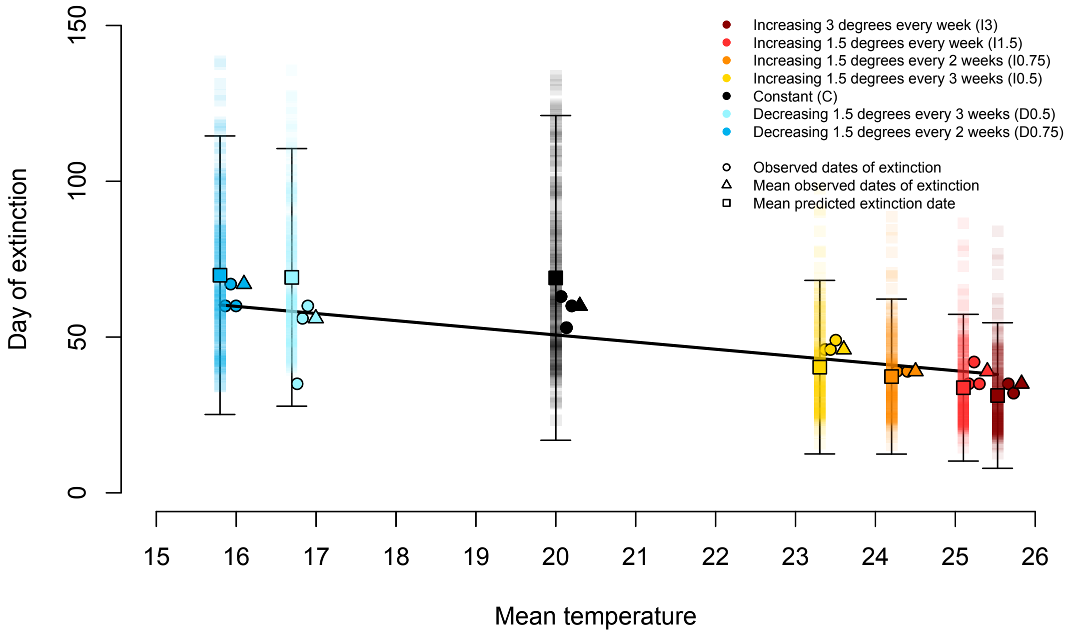

Figure 5.6: Whilst mean abundance was used to parameterize the phenomenological model, and to gauge the accuracy of model predictions, there was some variance amongst replicates. Mean predicted date of extinction in most cases falls very close to the majority of recorded extinction events. Bars show the 95% confidence intervals of model predictions. All extinction events fell within the 95% CI's, although the confidence intervals were often large (over 100 days for treatment C). Points within a temperature treatment are horizontally displaced for clarity.

to observed data are good (Fig. 5.4), and that in this instance using metabolic theory to scale parameters is reasonable.

The simplicity of the model, and consequently the assumptions made by it, may limit its applicability in real world scenarios. Effectively the model assumes that a species suffers an exponential decline in its population size from carrying capacity until extinction (in this case driven by a closed system with a degrading habitat and limited resources). This assumption may well be valid in some circumstances in the natural world (exponential declines in habitat size have previously been observed, and have led to the declines of resident species (Short & Burdick 1996; Hughes *et al.* 2002)), but caution should be exercised as sudden population crashes (Lande 1993), rescue effects (Brown & Kodric-Brown 1977), or habitat restoration (Waltz & Covington 2004) may invalidate such an assumption. Temperature driven extinction has been proposed through more complex dynamics, such as increased demographic stochasticity, and this may be an additional factor that leads to population loss (Vasseur & McCann 2005). However, the data presented here is unsuitable for testing such hypotheses due to the nutrient limitations of the system, and consequent lack of potentially destabilising oscillations.

The work presented here does, however, provide a conceptual starting point for understanding the effects of the rate of temperature change in more complex systems, and adds to work that has previously used simplistic systems as a way of gaining an understanding of complex dynamics, especially those processes that govern the extinction of populations (see Griffen & Drake 2008). This work contributes to this body of knowledge by showing how simplistic models can be used to forecast extinction in relatively simple systems, and provides a basis for theoretical exploration of these problems in more complex systems. Whilst the closed system we present here may appear unrealistic, in reality resource availability in a habitat can be very low, particularly in declining "sink" populations, with an insignificant flux of resources (e.g. Polisini *et al.* 1970). In such scenarios, where the influx of nutrients is much lower than that required to sustain a viable population, extinction will occur. The work presented here suggests that in such a scenario extinction will happen much more rapidly where mean temperature is high, and that it may be possible to infer how such a system would then behave under varying rates of temperature change, using simple phenomenological modelling. Where resource flux is high the use of a more mechanistic model that takes into account the possibility of an increase in the abundances of prey may provide a more helpful starting point. Evaluating the relative merit of phenomenological and mechanistic models to predict extinction in a more open system would provide an interesting next step to the work presented here.

5.6 Conclusions

In conclusion, temperature is known to alter many individual and population level processes, including metabolic rate, which in turn alters the rate of senescence and time of death of an individual (Van Voorhies & Ward 1999; Brown *et al.* 2004). Predicted future changes in temperature could thus alter not only the persistence of individuals, but of populations and potentially of a species. Predicting population declines in the light of current global change is necessary, but for many species the detailed knowledge required to parameterise a complex, mechanistic model will be unavailable, or be too costly or time consuming to obtain. Fitting a simple model of logistic growth with an exponential decline allows accurate predictions of population extinction under various rates of temperature change in a model system. However, rapid rates of cooling invalidate assumptions made by the model. Using mean temperature over a given time period (rather than actual temperature change) significantly reduces the accuracy of the predictions made by the model, and should be avoided. The results presented here suggest that using such simplistic models, and incorporating the actual rates of temperature change, to predict the effect of future climatic change has some merit, and that scaling parameters with metabolic theory is, in this instance, appropriate.

5.7 Acknowledgements

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6 Interactions between assembly order and environmental change can alter both short and long-term community composition

6.1 Abstract

Both the order in which species arrive in a community, and environmental conditions such as temperature, are known to affect community structure. Little is known, however, about the potential for, and occurrence of, interactions between assembly history and the environment. Of particular interest may be the interaction between temperature and community assembly dynamics, especially in the light of predicted global climatic change and the fundamental processes that are governed, through metabolic rate, by an individual's environmental temperature. We present, to our knowledge, the first experimental exploration of how the influence of assembly history, temperature, and the interaction between the two, alters the structure of communities of competitors, using small-scale protist microcosm communities where temperature and assembly order were manipulated factorially. In our experiment, the most important driver of long-term abundance was temperature, but long-lasting assembly order effects influenced the relationship between temperature and abundance. Any advantage of early colonisation proved to be short lived, and there was rarely any long-term advantage to colonising a habitat before other species. The results presented here suggest that environmental conditions shape community composition, but that occasionally temperature could interact with the stochastic nature of community assembly to significantly al-

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The following chapter has been published in the peer-reviewed journal Ecology and Evolution ter future community composition, especially where temperature change has been large. This could have important implications for the dynamics of both rare and invasive species.

6.2 Introduction

Species' abundances and distributions are predicted to change substantially under anthropogenically driven climate change (Condit *et al.* 1996, Iverson & Prasad 1998, Perry *et al.* 2005). Current predictions suggest that global temperatures are set to rise between 1.1 and 6.4 °C over the next 100 years (IPCC 2007), with potentially profound impacts on ecosystems and communities worldwide (Kasischke *et al.* 1995, Thomas *et al.* 2004, Pandolfi *et al.* 2011). Temperature can directly determine which species survive in a habitat (Ferguson 1958, Southward 1958), but can also alter individual, population, and community scale processes, which in turn can have complex cascading effects (Kratina *et al.* 2012). For example, as the metabolic requirements of an organism increase with increasing temperature, resource competition will intensify, so higher temperatures may result in greater interspecific interaction strengths (Gresens *et al.* 1982, Sanford 1999, 2002, Englund *et al.* 2011). Jiang & Morin (2004) showed that a temperature difference of just 2 °C reversed competition between two ciliate protozoa; initial rapid competitive exclusion was replaced by co-existence. This change in community structure could in turn impact food web stability (Rall *et al.* 2009), and may have the potential to alter ecosystem function. For example a shift in community composition caused by temperature change has been shown to alter cyanobacterial diversity, with, in some instances, a shift to toxin-producing species (Kleinteich *et al.* 2012).

Another way in which community composition can be altered is through assembly order effects, where the order in which species colonise a habitat can influence the competitive ability or abundance of a species (Shorrocks & Bingley 1994, Almany 2003, Louette & De Meester 2007, Chase 2010). Such effects have been demonstrated in model (Atkinson & Shorrocks 1981, Law & Morton 1996), small-scale experimental (Drake 1991, Fukami & Morin 2003, Warren *et al.* 2003), and field systems (Weslien *et al.* 2011, Dickie *et al.* 2012). Assembly order effects can be profound: arriving at a patch even marginally before another may transform an inferior competitor into a superior one (Shorrocks & Bingley 1994), allowing a species to persist where it might otherwise be excluded. Moreover, because species may be competitively excluded based on the order in which they arrive in a habitat, assembly order could also play an important role in the survival of species at a local or regional scale (Shorrocks & Bingley 1994, Chase 2010).

Recent work has started to look at how environmental factors, including disturbance (Jiang & Patel 2008) and productivity (Chase 2010), may alter the role of community assembly processes. It has been suggested that assembly order effects are most likely to be important when the species pool is large and the habitat is both productive, and stable (Chase 2003). So far, however, despite the acknowledged importance of temperature effects on biological processes, and the importance of understanding the consequences of environmental warming, the interaction between assembly order and temperature has received little attention.

In this study we investigated the interaction between temperature and assembly order using a laboratory experiment where temperature and the assembly order of a three-species protist community were manipulated factorially to assess: (i) how temperature alters the advantage of initially colonising a habitat, (ii) whether colonising a habitat early has a long term advantage for a species, and (iii) whether the order in which species invade a habitat can modify the strength, and direction, of the effect of temperature on species abundances.

6.3 Methods

We performed a two-way factorial manipulation of assembly order and temperature in microcosm communities assembled with three species of bactiverous ciliate protozoa: *Blepharisma japonicum*, *Paramecium caudatum*, and *Loxocephalus sp.* (subsequently denoted by the letters B, P, and L). These three species were chosen because they compete for similar resources, can co-occur in natural environments, and they are morphologically very distinct, facilitating accurate sampling. One species, *Blepharisma japonicum*, is known to be able to form enlarged predatory morphs, however over the course of the experiment none of these morphs were observed, and previous experiments have shown that predatory morphs form most frequently when nutrients were low (half the concentration used in this experiment) and populations persisted for an elongated period of time (Clements pers. obs.). Therefore we feel justified in considering the communities presented here as communities of competitors only.

Microcosms consisted of petri dishes (diameter 100mm, height 25mm) containing 50ml of medium, composed of Chalkley's solution (Thompson *et al.* 1988) and 0.2g/l crushed protist pellets (Carolina Biological Supply, USA) autoclaved together. Medium was batch inoculated with the bacteria *Serratia marcescens* and *Bacillus cereus*, and incubated for 7 days at 18.5 °C, to allow bacterial populations to develop. Medium was then mixed and split among the microcosms (experimental day 0) when a single wheat seed was added to each to provide an additional source of nutrients.

Protists were added sequentially at 7-day intervals (on days 0, 7 and 14). On each day a sample of high density stock culture containing ~30 individuals of each species was added to each microcosm. Assembly orders covered all seven possible combinations of species invasions: BPL (i.e. B on day 0, then P on day 7, then L on day 14), BLP, PBL, PLB, LBP, LPB, and a control group, ALL, where all three species were added at day 0. Each assembly order was replicated three times at each of six temperatures (11, 14, 17, 20, 23 and 26 °C) in six individual incubators, giving a total of 126 microcosms. The microcosms were randomly assigned a position on a shelf within each incubator. As the incubator facility was shared, with other experiments being run concurrently with this one, we were unable to switch treatments between incubators during the experiment to guard against possible incubator effects, though we have no reason to suspect such effects were likely to be present. The abundances of all species present in each microcosm were sampled on days 7, 14, 21, 42 and 70. A setup error in all three replicates of BPL at 23 °C meant that this treatment had to be excluded.

Sampling to estimate species abundances was based on that of Lawler and Morin (1993). Microcosms were mixed thoroughly, and then known volumes (between 0.2 and 0.5ml) were sampled using a Gilson pipette. Individuals of each species present in these subsamples were counted under a stereoscopic microscope. If no individuals of a species were observed, the microcosm was re-sampled up to three times. For rare species the entire microcosm was placed under the microscope and searched, with a species being recorded as extinct if no individuals were observed after 5 minutes of searching. All sampled medium was returned to the microcosm. Evaporative loss was checked on a weekly basis, and microcosms were topped up to 50ml with distilled water as required. No additional nutrients were added to the microcosms, and no replacement of medium (save for evaporative loss) occurred.

Count data recorded during the experiment were highly skewed, with some species (especially *Loxocephalus*) having high numbers of extinctions (i.e. zero densities) whilst also having some populations at extremely high densities (>11,000 in a microcosm). Consequently, generalized linear models (GLMs), with Gaussian or quasi-Poisson distribution families were used to model abundances of *Blepharisma* and *Paramecium*. A GLM with zero-inflated negative binomial distribution family (henceforth ZNBR) was used to model the abundance of *Loxocephalus* due to the high proportion of zero counts and overdispersion of the observed data (Ridout *et al.* 2001). Analyses were repeated on data from days 42 and 70, the last two days at which microcosms were sampled. This allowed us to investigate long-term community structure, and how the relative strength of factors influencing species abundance changed over time.

We calculated the strength of any advantage of colonising a habitat 1st, 2nd, or 3rd as the difference in abundance between treatments where the species were added sequentially, and the mean abundance in the control treatment where all the species were added simultaneously (i.e. with no assembly order effects). This gave six values (one from each of the three replicates of the two treatments where a species was added 1st, 2nd, or 3rd); we then calculated the mean and standard error of these.

All statistical analyses were carried out using R (R Development Core Team 2013).

6.4 Results

6.4.1 Analysis of abundance patterns at days 42 and 70

ABUNDANCES of *Paramecium* were significantly negatively, and *Blepharisma* significantly positively, correlated with temperature (Fig. 6.1; Table 6.1, 6.2). This general pattern held for both day 42 and day 70, although the strength of the effect of temperature on species abundance tended to be higher at day 70 than at day 42 (Fig. 6.1; Table 6.1, 6.2).

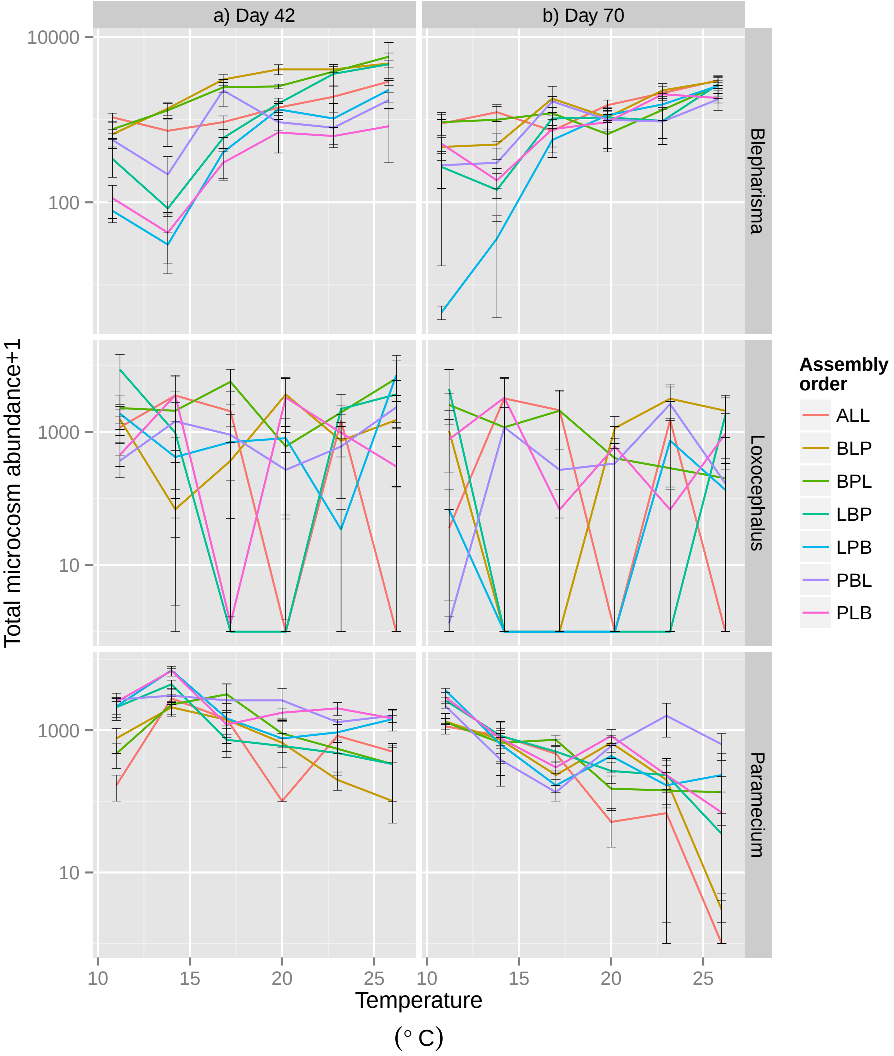

Figure 6.1: Mean abundances of the three species at day 42 and day 70 for each assembly order and temperature treatment, highlighting the individual species responses to temperature. Line colour indicates assembly order. Bars represent +/- 1 standard error.

Assembly order also altered the abundances of both *Paramecium* and *Blepharisma* (Fig. 6.1; Table 6.1, 6.2), although the strength of this effect decreased from day 42 to day 70, and accounted for less of the variation in abundance than the effect of temperature (Table 6.1, 6.2). *Blepharisma* at day 42 showed particularly clear differences in abundance between assembly orders (Fig. 6.1a), with some assembly orders having consistently lower or higher abundances than others (e.g. PLB, BLP, Fig. 6.1a).

In addition to directly altering *Paramecium* and *Blepharisma* abundances, assembly order could also alter the relationship between a species's abundance and temperature (Fig. 6.1; Table 6.1, 6.2). This interaction could either

Table 6.1: Analysis of deviance of generalized linear models fitted to the abundance of *Paramecium* at days 42 and 70. Statistically significant interaction coefficients of generalized linear models presented as Temp ∼ the relevant assembly order. 95% significance is highlighted in **bold**. Error structures are: "G" = Gaussian, "q-P" = quasi-Poisson.

Term	Day 42				Day 70			
	Error	Df	F-value	p-value	Error	Df	F-value	p-value
Temp	**G**	**1, 120**	**24.95**	**<0.001**	**q-P**	**1, 120**	**138.22**	**<0.001**
Ass. Or.	**G**	**6, 114**	**5.41**	**<0.001**	**q-P**	**6, 114**	**2.42**	**<0.05**
Interaction	G	6, 108	1.71	>0.05	q-P	6, 108	3.45	<0.01
	Error	Estimate	t-value	p-value	Error	Estimate	t-value	p-value
Temp∼BPL	**G**	**-0.17**	**2.12**	**<0.05**	q-P	0.08	0.94	>0.05
Temp∼LBP	**G**	**-0.17**	**2.30**	**<0.05**	q-P	-0.01	0.08	>0.05
Temp∼PBL	G	-0.04	0.50	>0.05	**G**	**0.19**	**2.58**	**<0.05**

Table 6.2: Analysis of deviance of generalized linear models fitted to the abundance of *Blepharisma* at days 42 and 70. Statistically significant interaction coefficients of generalized linear models presented as Temp ∼ the relevant assembly order. 95% significance is highlighted in **bold**. Error structures are: "G" = Gaussian, "q-P" = quasi-Poisson.

Term	Day 42				Day 70			
	Error	Df	F-value	p-value	Error	Df	F-value	p-value
Temp	**q-P**	**1, 120**	**149.06**	**<0.001**	**G**	**1, 120**	**152.10**	**<0.001**
Ass. Or.	**q-P**	**6, 114**	**21.70**	**<0.001**	**G**	**6, 114**	**4.87**	**<0.001**
Interaction	**q-P**	**6, 108**	**3.44**	**<0.01**	**G**	**6, 108**	**2.36**	**<0.05**
	Error	Estimate	t-value	p-value	Error	Estimate	t-value	p-value
Temp∼LBP	**q-P**	**0.13**	**3.08**	**<0.01**	G	0.64	1.03	>0.05
Temp∼LPB	**q-P**	**0.11**	**2.16**	**<0.05**	**q-P**	**1.68**	**2.71**	**<0.01**

increase or decrease the strength of the effect of temperature (Fig. 6.1), and in some cases this interaction accounted for a two order of magnitude difference in the abundance of *Blepharisma* (e.g. the assembly order LPB at day 70, Fig. 6.1b) and *Paramecium* (e.g. the assembly order PBL at day 70, Fig. 6.1b). These interactive effects are particularly clear, but less frequent, at day 70 (Fig. 6.1b).

Variation in abundance among the different assembly orders correlated with temperature, with the direction and strength of this correlation dependant on both species identity and the time since community assembly (Fig. 6.2). *Blepharisma* showed a strong positive correlation between temperature and variance in abundances at day 42 (i.e. large differences between assembly orders, especially at higher temperatures), and a still positive, but weaker, relationship at day 70. *Paramecium* meanwhile showed exactly the opposite relationship, with temperature negatively correlating with variation in abundance between assembly orders, however the strength of this relationship again decreased from day 42 to 70 (Fig. 6.2).

The abundances of *Loxocephalus* in each treatment exhibited little evidence of systematic trends at either day 42 or day 70, and abundances were

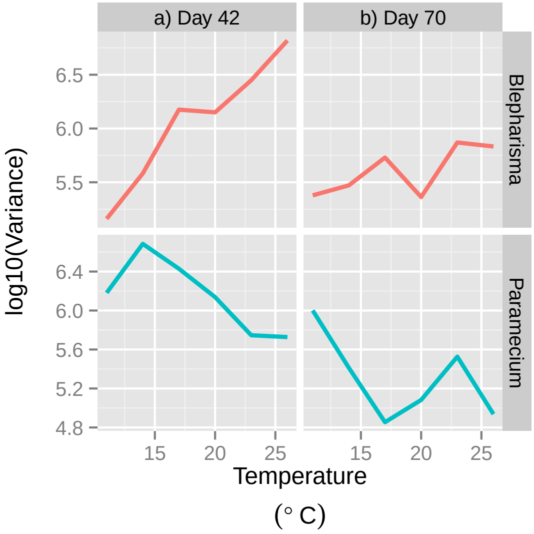

Figure 6.2: Variation in abundance between assembly orders as a function of temperature.

not significantly affected by temperature, assembly order, or any interaction between the two. There was, however, a significant increase in zero counts (i.e. extinctions) with increasing temperature, and the assembly order BLP at day 70 (Appendix 8.2).

6.4.2 Effect of sequential invasion on species abundances at days 42 and 70

WHILST there appeared to be some advantage, in terms of increased abundance, of colonising a habitat sequentially over colonising simultaneously there was not necessarily an advantage of colonising earlier and the magnitude of any advantage could also be modified by temperature (Fig. 6.3). Higher temperatures did not necessarily lead to a larger long-term advantage of colonising a habitat early, rather species-specific responses to temperature often drove the magnitude and direction of assembly order effects at each temperature treatment (Fig. 6.3): *Blepharisma* was more abundant at higher temperatures, and *Paramecium* was less abundant.

Of the four assembly orders where *Blepharisma* and *Paramecium* were added before any other species (i.e. added 1st; BLP, BPL and PBL, PLB) the initial colonisers tended to have higher, but not significantly higher, abundances (Fig. 6.3). At day 70 only one assembly order showed significantly higher abundances of the initial coloniser: PBL (Fig. 6.3b; Table 6.1).

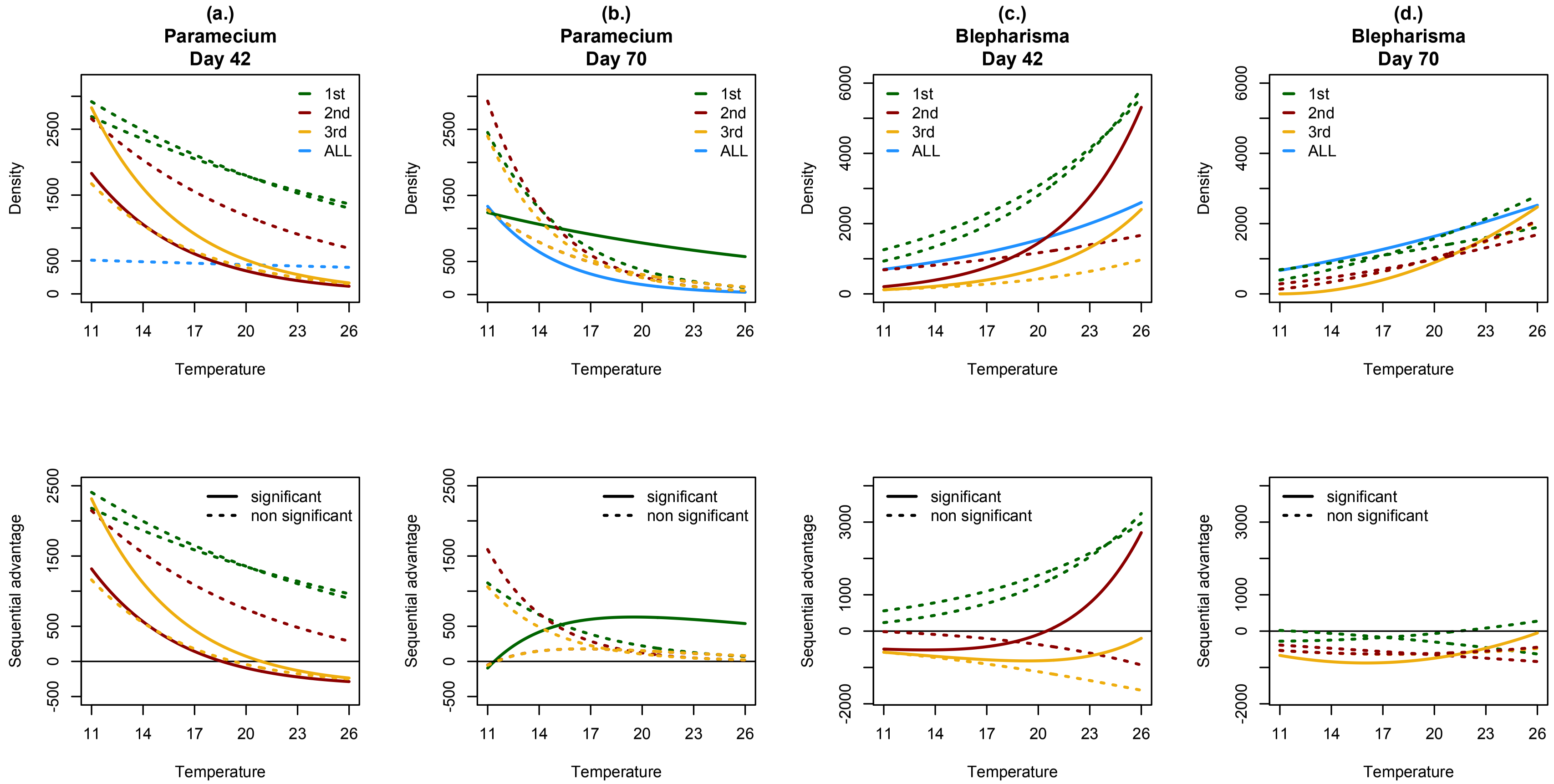

Figure 6.3: The abundances of *Blepharisma* and *Paramecium* were estimated at days 42 and 70, across the temperature range and assembly orders, from the coefficients of the fitted (GLM) models (upper "density" plots). The advantage of colonising a habitat 1st, 2nd, or 3rd, as opposed to simultaneously with other species (i.e. the difference in abundance between sequential treatments and the treatment ALL) is plotted below ("sequential advantage" plots). For each species there are two assembly orders, and consequently two lines, where that species was added 1st, 2nd, or 3rd (e.g. *Loxocephalus* is added 1st in the assembly orders LBP and LPB, 2nd in the orders BLP and PLB, and 3rd in the orders BPL and PBL). "Significant" indicates a difference in abundance between an assembly order where a species has been added sequentially and the treatment ALL (where they have gone in simultaneously) that has a p-value <0.05. Assembly orders with significantly higher abundances all also exhibited significant interaction between temperature and assembly order (Appendix 8.2).

Loxocephalus abundances appeared to be almost randomly distributed across temperatures and treatments, and so were excluded from the analysis of early colonisation advantage at days 42 and 70.

Arriving at a habitat after initial colonisation by another species (i.e. arriving 2nd or 3rd) could alter abundance (when compared to the treatment ALL), but the direction of this effect was species and temperature specific (Fig. 6.3). At day 42 *Paramecium* showed an advantage of being added to a microcosm 2nd or 3rd (Fig. 6.3a) but by day 70 these effects had disappeared (Fig. 6.3b; Table 6.1). For *Blepharisma*, however, there appeared to be some disadvantage of colonising a habitat late (after the two other species): populations had significantly lower abundances at days 42 and 70 when added to a community 3rd, although this was to a large extent negated by higher temperatures (Fig. 6.3a, b; Table 6.2).

6.5 Discussion

ALTHOUGH both temperature and assembly order are known to be important drivers of community composition (Shorrocks & Bingley 1994, Jiang & Morin 2004, Kleinteich *et al.* 2012) there has been little investigation of the potential interaction between these two factors. The experimental evidence presented here suggests that the effect of temperature on species abundances, and therefore community composition, can be contingent on the order of assembly of that community. This does not appear to be driven by an advantage of colonising early, as we only occasionally found a significantly higher abundance of initial colonisers at days 42 and 70, however those species that colonised later were often at a disadvantage. Furthermore, we showed that the strength of the interaction, and of the main effects of temperature and assembly sequence, are a function of both time and species identity. These findings have important implications for modelling the potential effects of future climate change on community structure and species distributions.

In line with previous findings, our experimental work shows that species-specific responses to temperature are a major determinant of abundance, and thus community composition (Fig. 6.1). Over the period of this experiment (~100 protist generations for these species at 20 °C (Clements *et al.* 2013)) the strength of this temperature effect increased, possibly because there has been a greater period of time for inferior competitors to be excluded (Fig. 6.1, Table. 6.1, 6.2). In contrast, whilst there were assembly order effects (Table 6.1, 6.2), the strength of these are species-specific and transient; the size of the assembly order effect decreased from day 42 to 70, a finding supported by a decrease in the variance between assembly orders over the same period (Fig. 6.2). In addition, by day 70 the effect of assembly order was small when compared to the dominant effect of temperature (Table 6.2). Our results indicate that intermediate levels of environmental change may have the potential to mask assembly order effects, leading to multiple similar community types regardless of assembly history. However, greater levels of change may, occasionally, promote the prevalence of such effects as, within the 15 °C temperature range of our experiment, assembly order effects were most evident where it was either hottest or coldest.

Whilst interactions between temperature and assembly order appear to be rare, where they do occur they can significantly alter the long-term structure of a community (Fig. 6.1). Although the magnitude of this interactive effect is small when compared to the effect of temperature alone (Table 6.1, 6.2), and whilst it is only present in two of the seven assembly orders at day 70, the impact on the abundance of a species can be dramatic (Fig. 6.1b, *Blepharisma* and *Paramecium*). Clearly there is the potential for such significant increases or decreases in a species's abundance to have a substantial effect on a community, especially if the species affected is a key pollinator (Memmott *et al.* 2004) or an invasive alien (Lowe *et al.* 2000).

Accurately predicting the potential impacts of future climate change on global diversity requires knowledge of the effects temperature can have at a population, community, and ecosystem level (Cramer *et al.* 2001, Brown *et al.* 2004, Jiang & Morin 2004). Earlier work has identified the role of temperature and other abiotic factors in shaping a species's fundamental niche (Hutchinson 1957), and such fundamental niches provide the underpinnings for "climate envelope" approaches to estimating future species distributions in relation to climatic change (Davis *et al.* 1998). However, this approach has been criticised, as species exist within a realised niche that is defined not only by the abiotic conditions but also interactions between species (Davis *et al.* 1998), as well as stochastic processes such as dispersal (Mitikka *et al.* 2007). If the interactions between species, that shape the realised niche, are also altered by climatic change, then climate envelopes, and other models that fail to take into account temperature dependent interspecific interactions, may provide misleading estimates of future species distributions or community composition (Davis *et al.* 1998). Such concerns seem well founded, as previously small shifts in temperature have been shown to interact with a species's specific thermal tolerance to reverse competition in model systems (Jiang & Morin 2004). Our results add to this body of knowledge by highlighting the interaction between a stochastic driver of community composition and environmental change. However, further work is required to understand the mechanistic underpinnings of the interactions between temperature and assembly order presented here if we are to improve predictive frameworks.

6.6 Conclusions

In conclusion, the results presented here suggest that our ability to understand how communities may react to climate change is complicated by species-specific responses to temperature, ephemeral effects of assembly order, and, occasionally, complex interactions between the order in which species invade a habitat and their competitive ability, as well as the time frame over which this occurs. Incorporating such interactions, in addition to stochastic and deterministic drivers of community composition, in future modelling is essential if one aims to encompass the full range of potential climate driven future community states. Whilst this may sound daunting, some heart should be taken from the fact that long-term dynamics are generally driven by abiotic conditions, and the potential complexity added by strong priority effects, at least in this system, appears in general to be short lived. Thus, understanding general patterns of diversity under climatic change may be feasible, but identifying when and where temperature and assembly order will interact to alter community composition is likely to remain challenging.

6.7 Acknowledgements

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7 Discussion

In this thesis I have demonstrated the utility of using small-scale experimental communities to address large-scale ecological and conservation problems, especially those issues surrounding the loss of a population or species where studying the loss of a wild population is often time consuming, expensive, or impractical.

In Chapter 2 I demonstrated that the intrinsic properties of the microcosm system (its replicability, controllability, and small scale) could be used to produce data suitable for testing methods to infer extinction events from records of historic sightings. This work suggested that underlying population dynamics (such as the speed at which a population declines to extinction), as well as historic search efforts, could alter how accurately extinction events could be inferred.

In Chapter 3 I presented a new package for the statistical software R that brings together six different methods that have been proposed as ways to infer extinction from sets of historic sighting events.

Chapter 4 utilised the framework pioneered in Chapter 2, in combination with real-world extirpation data and simulated population extirpations, to test the six methods for inferring extinction included in the R package detailed in Chapter 3, with a specific focus on testing these methods for robustness against known drivers of error which could affect our ability to infer extinction events accurately. This work highlighted the importance of selecting a method that is robust to multiple different drivers of predictive error.

Whilst accurately inferring the extinction status of a species is important if we are to gauge current rates of biodiversity loss, arguably more important is our ability to understand how future environmental change may alter rates of species loss. In Chapter 5 I showed that it is possible to use simple models to predict how directional environmental change can alter population dynamics, and ultimately the timing of extinction events in an experimental system.

The work in Chapter 5 showed that temperature change could, through alterations to an individual's metabolic rate, strongly affect population dynamics. This lead on to the experiments in Chapter 6 in which I investigated how environmental change might interact with ever-present community assembly processes to alter a species's chance of persistence, or extinction.

The implications of each of these chapters are detailed in their individual conclusions, however below I provide an overview of how the work presented in this thesis has contributed to our understanding of both conservation biology and ecology, and how the techniques detailed here have demonstrated that using small-scale experimental communities can help answer applied, real-world, issues.

7.1 Understanding the timing of extinction events

CURRENT rates of biodiversity loss are thought to be significantly higher than at any period in the recent past, a function of both a burgeoning, industrialised, human population (Fig. 1.2, Thomas, Singer & Boughton 1996; Thomas *et al.* 2004; Travis 2003; Turvey *et al.* 2007; Wake & Vredenburg 2008), and a level of species-diversity higher than perhaps any other point in the earth's history (Fig. 1.1, Sepkoski 1993). Whilst this general trend of biodiversity loss is reasonably well understood, on an individual level determining whether a species is extant or extinct remains fundamentally challenging. Simply put, it is impossible to survey a habitat with a 100% probability of observing a species should it be extant. One approach to countering this issue is to repeatedly survey of as much of a species's habitat as possible, over several years, thus reducing the probability of failing to see it should it be extant (Turvey *et al.* 2007; BirdLife International 2010). However, such an approach requires significant input of time and money. Consequently, being able to accurately infer whether a species is extant or extinct, using historic data (and therefore eliminating any need to conduct further surveys) is of considerable value (Clements *et al.* 2013), leading to many methods being proposed to tackle this problem (e.g. Solow 1993, 2005; McCarthy 1998; Gotelli *et al.* 2011). Rigorously testing such methods requires data where the date of extinction of the species is accurately known, and this restriction has limited previous studies to rely, in the main, on data from *in silico* simulations of population extinction (e.g. Rivadeneira, Hunt & Roy 2009).

In Chapter 2 of this thesis I proposed a novel approach for producing data with extinction dates that are accurately known, allowing methods such as that proposed by Roberts & Solow (2003) to be tested. This analysis concentrated on testing Optimal Linear Estimation as it had previously been suggested that this method could be used to inform a proposed IUCN category of "Critically endangered - possibly extinct" (Butchart, Stattersfield & Brooks 2006; Collen, Purvis & Mace 2010). Using this microcosm-based approach I showed that historic search effort (the amount of the habitat searched to produce a sighting event), and the population dynamics of the species going extinct could significantly alter the accuracy of inferred dates of extinction produced by Optimal Linear Estimation. Estimates of extinction were particularly poor when search efforts had historically declined, were irregular, or when species persisted for extended periods of time at very low abundances (one or two individuals). The ability for species to persist at low abundances for extended periods of time was dependent on the temperature at which the microcosms were incubated, as higher temperatures increased an individual's metabolic rate, and consequently the rate of senescence and need for resources, reducing the probability that one or two individuals survived (Fig. 2.6). This work therefore suggested that historic changes to a population's abiotic environment may alter the accuracy of inferred extinction events (investigated in Chapter 4).

This was the first work to approach the problem of testing methods for inferring extinction with experimental data, and it set the groundwork for Chapter 4. However, whilst the use of small-scale systems has distinct advantages over large-scale field manipulations or observational studies, there are still some limitations. The ability to quantify, with a high degree of certainty, that a species has been lost is a feature of the microcosm system which lends itself to the testing of extinction theory, but gauging exactly when a species is lost depends upon the frequency at which sampling of the microcosm occurs. In the experiment in Chapter 2 sampling occurred three times a week, and so the date of extinction could (with a high degree of certainty) be narrowed down to within two days of the actual date of extinction. Ideally sampling of the microcosms for populations abundances would occur far more frequently, but more frequent sampling would limit the number of replicates and treatments feasible. Recently, developments in video monitoring have allowed multiple individuals of multiple species to be tracked through time (Owen Petchey, pers. comm.). Utilising such a monitoring system would produce precise times of extinction, population dynamics (estimated from subsamples of the total microcosm in Chapter 2), and spatial dynamics, at the resolution of minutes or seconds. This would allow more realistic search regimes to be simulated that could include, for example, non-random spatial distributions of a population (in Chapter 2 a random distribution of individuals was modelled). However the data presented in Chapter 2 still have significant advantages over limited real-world extirpation data (see Chapter 4) and represent the current best option for testing such sighting based methods for inferring extinction.

7.2 Selecting robust methods to inform conservation planning

LIMITED conservation funding may be rapidly allocated to save a species thought to be extant based on a single sighting event (Wilcove 2005; Fitzpatrick *et al.* 2005). Whilst this rapid deployment of funds may in some circumstances be necessary save a species on the brink of extinction, care must be taken to learn from recent mistakes which have seen millions of dollars invested to save a species that was likely to have been extinct for a quarter of a century (Wilcove 2005; Gotelli *et al.* 2011). Generally, and perhaps obviously, this means that a careful assessment of the probability of a species still persisting should be made prior to any investment of time and money to save it. To do this, however, methods for inferring extinction must be selected that are robust to a range of potential drivers of error. In Chapter 2 I demonstrated that search effort, search regularity, and intrinsic factors such as population dynamics, could alter the accuracy of inferred dates of extinction. I used the framework piloted in Chapter 2, and the R package detailed in Chapter 3, as the basis for the work presented in Chapter 4, with a particular focus on how environmental change could alter extinction dynamics (Chapter 5) and in turn alter the accuracy of inferred dates of extinction produced by the different methods in the “sExtinct” R package (Chapter 3).

I demonstrated in Chapter 4 that most methods are prone to either overestimating or underestimating the date of extinction of a species, although this may depend on extrinsic (e.g. historic search efforts) and intrinsic (e.g. rate of population decline) factors. Overall, I showed that, of the six methods tested, Optimal Linear Estimation was the most robust to all drivers of error, and, of particular interest to conservation practitioners (and contrary to what had previously been suggested by Solow (2005)), that Optimal Linear Estimation produced the most accurate results when more than ten historic sighting events were used to calculate the probable date of extinction. These are novel insights, which will hopefully shape how such techniques are applied in the future, and potentially have an impact on how and when such methods are used to inform real-world conservation decision-making.

As with the data described in Chapter 2, the data presented in Chapter 4 have their limitations (see above). Additionally, whilst the number of methods tested in Chapter 4 is significant, and on a par with the number tested previously by Rivadeneira, Hunt and Roy (2009), it is hardly exhaustive. Testing all the proposed methods for inferring extinction (both sighting only (e.g. Roberts & Solow 2003), or otherwise (e.g. Thompson *et al.* 2013)) in a single publication, or even series of publications, is unfeasible. Consequently, this work, along with that previously done by Rivadeneira, Hunt and Roy (2009), forms the groundwork upon which future methods may be tested - through a combination of experimental and modelled population data. Ideally, this framework would be made available for newly proposed methods to be tested within, a task which the R package “sExtinct” (Chapter 3) begins to address. Currently the R package does not include example data within it, however it would be useful to do so (perhaps a subset of the data presented in Chapter 2), along with the ability to generate modelled population declines, and simulate a range of sampling regimes to generate sighting data. This would more easily allow for new methods to be directly compared to those previously proposed, and eventually could produce a database of methods with their individual advantages and disadvantages clearly identified, work that could be tied into recent advances that have sought to produce a quantitative framework for selecting what method to use when (Vogel *et al.* 2009, see below).

7.3 Predicting the effects of future climate change on species persistence

THE dramatic loss of species over the last century, driven in the main by anthropogenic alterations to the global biome (Thomas *et al.* 2004; Barnosky *et al.* 2011; Walters, Blanckenhorn & Berger 2012), seems likely to be overshadowed only by the imminent future loss of an even greater amount of biodiversity (Brook, Sodhi & Bradshaw 2008; Cahill *et al.* 2013).

Global temperatures are set to rise considerably over the next 90 years (IPCC 2007), although by how much depends on both geographic location and future greenhouse gas emissions. Consequently, interest has focused on building predictive frameworks to model the effects of temperature change on populations and assemblages of species. Central to any predictive framework is likely to be the metabolic theory of ecology, where the effects of temperature change on an individual's metabolic rate - and associated functions - can, in general, be predicted using a simple law (Brown *et al.* 2004; although see White, Cassey & Blackburn 2007). Understanding the responses of complex, multi-species, multi-trophic level communities to environmental change first requires one to attempt to predict the response of simple communities. An understanding of how and when model predictions fail under these simple circumstances allows data to be collected to circumvent such failures in more complex models.

The findings in Chapter 5 showed that the effects of different rates of environmental change on population dynamics could be predicted by a simple four parameter phenomenological model that incorporated metabolic theory. In addition, I showed that the temperature component of metabolic theory drives more of the model accuracy than the body-size component, but that when body-size and temperature are both taken into account the model produces the most accurate predictions. This work also formed the basis of Chapter 4, where I investigated how rates of environmental change (shown in Chapter 5 to alter extinction dynamics) might also alter our ability to infer extinction status correctly.

The modelling presented in Chapter 5 provides a general framework for predicting the effects of environmental change on population dynamics (a function of the simplistic nature of the phenomenological model employed, and the general, 3/4 power, scaling of metabolic rate with body size (Brown *et al. 2004*)). It should be noted, however, that this 3/4 power scaling law (know as Kleiber's law (Kleiber 1932)) is not universally applicable, and the actual relationship between metabolic rate and body size depends upon the evolutionary history of the organism (DeLong *et al.* 2010). Care should thus be taken to choose appropriate scaling relationships when using such a framework to predict the effects of environmental warming on complex, multi-species systems.

Whilst the system in which this work is carried out in is clearly a simplification of many real-world communities (e.g. there is no immigration or emigration, no influx of resources, and the model predicts the mean response of several populations to environmental change), these results do lay the theoretical groundwork to develop more complex modelling approaches to predict the potential effects of environmental change on more species-rich communities.

7.4 Linking environmental change to stochastic drivers of community composition

FUTURE temperature increases seem likely to drive continued species extinctions, as well as dramatic range shifts (Chen *et al.* 2011). At a fundamental level temperature can alter the functions of an individual that are associated with its metabolic rate (e.g. growth rate, carrying capacity, interspecific interaction strength, and senescence (Van Voorhies & Ward 1999; Brown *et al.* 2004)), and consequently a species's chance of persisting, or not, at any given point in time (see Chapter 5). However, species do not reside in isolation, but within complex networks of interacting organisms, and such interspecific interactions can affect the probability of a species persisting in a community (Shorrocks & Bingley 1994; Jiang & Morin 2004). The strength of these interactions can be modified not only by environmental conditions such as temperature (e.g. Jiang & Morin 2004), but also by stochastic processes such as the order in which species invade a habitat (Shorrocks & Bingley 1994; Chase 2003; Warren, Law & Weatherby 2003; Fukami *et al.* 2010; Weslien *et al.* 2011).

Past work had started to look at the interaction between abiotic environmental conditions (such as disturbance (Jiang & Patel 2008)) and community assembly processes in shaping community composition. However, the potential for an interaction between temperature and assembly order to alter community composition had not been studied. This was a significant gap in our understanding, especially in the light of predicted future climatic change (IPCC 2007) and the dramatic effects that environmental change can have on extinction dynamics (Chapter 5).

I demonstrated in Chapter 6, for the first time, that interactions between community assembly processes and temperature could significantly alter the abundance, and persistence, of a species in a simple community. In the majority of cases a species's abundance was predicted by the temperature of the habitat, however there were occasional interactions between temperature and community assembly order, and where this interaction occurred the effect could be dramatic: in some cases species survived where they would otherwise have been outcompeted, and in others the interaction drove a species to dramatically lower abundances (two orders of magnitude) than would otherwise have been expected. These results suggested that climate envelope modelling, a stalwart for predicting distributions under climate change scenarios, may in some circumstances provide dramatically wrong predictions of where species may be found, and at what densities. Unpicking the mechanistic drivers of the temperature-assembly order interaction should be an avenue for future research, as without a mechanistic understanding we are unlikely to be able to identify when and where these interactions will occur.

As with Chapter 5, the findings detailed in Chapter 6 are limited, necessarily, in scope, and consequently form the basis for future work, rather than the completion of this line of investigation. A major constraint of the work is the low species diversity (three competitors), unrealistically simplistic when compared to the majority of real-world systems (although instances of simple communities do exist, for example *Hirudo verbena*, the medicinal leech, has been shown to have a natural community of only two bacteria inhabiting its crop (Kikuchi & Graf 2007)). This low species diversity was necessitated by the experimental design (fully factorial over a large range of temperatures), and consequently the time required to sample all of the microcosms. It is interesting to note, however, that even in such a simple system, with a single trophic level, the interaction between assembly order and temperature can alter community composition drastically, and consequently future work should look to see whether such interactions persist in more complex scenarios (see below).

7.5 Critique of the microcosm system

DATA generated from microcosm experiments has featured heavily within this thesis, because advantages of the system lend themselves particularly to the questions addressed (specifically their small size, which allows extinction events to be confirmed easily, communities to be replicated, and temperature treatments administered). However some issues with the use of this system have also been highlighted (see also above).

Chapter 4 tested multiple methods for inferring extinction events, using a combination of microcosm, real-world extirpation, and simulated data. The purpose of this work was to assess not only how robust the different methods were, but also how intrinsic factors (such as population dynamics) could alter our ability to infer extinction status correctly. Data from the microcosm experiment detailed in Chapter 5 (where the rate of temperature change through time was manipulated) were used, as this experiment had produced significantly different extinction dynamics based on environmental conditions. For microcosm data, as predicted by earlier work (Chapter 2), populations that declined more rapidly had lower error associated with predicted dates of extinction than those that declined more slowly. The wild population extirpation data, however, showed the opposite pattern, with species that declined more slowly to extinction having lower error associated with predicted dates of extinction. Subsequent work, that used modelled population declines (Appendix 8.1.5), suggested that the size of the population may interact with the rate at which that population declined to extinction to alter the accuracy of inferred dates of extinction. This has important implications for the design of future microcosm experiments, namely that whilst the tractability of these systems can be very useful, more care should be taken to produce populations analogous in size to those of the wild populations one is interested in studying, a fact that may have been missed had Chapter 4 not combined data from several different sources.

7.6 Future research

7.6.1 Further testing of methods for inferring extinction

IDENTIFYING when, or if, a species has gone extinct remains a key goal for conservation biology, and methods to achieve this aim are still being developed (e.g. Thompson *et al.* 2013). The most recent methods seek to explicitly incorporate potential drivers of predictive error, such as survey effort (e.g. Thompson *et al.* 2013) or uncertain sighting events (e.g. Solow *et al.* 2011). The continued development of these methods must be accompanied by robust testing of their practicality, accuracy, and performance in relation to previously proposed metrics; simply put, *do new methods perform better than those previously tested*?

Key to advancing the practicality of such methods is to clearly identify when certain methods should be used, where each method performs well, and where its weaknesses lie. Recent work by Vogel *et al.* (2009) has suggested a framework, using L-moments, to identify how well the assumptions of a proposed method are met by the distribution of the sighting data to be used to infer extinction; in effect, how appropriate it is to use a particular method given the temporal distribution of a set of sightings. Because the distribution of historic sighting events can be based on search effort, as well as the rate at which a population declines, microcosm experiments provide the perfect system in which to test, *ex silico*, whether the L-moments approach correctly identifies what method to use in a given situation, work that would form a logical development to that presented in Chapter 4.

Predicting when a taxon has been lost is not only useful for current conservation decision making, but also forms an important part of the paleontological literature, as people seek to link extinctions in deep time to, for example, the shift in the distribution of human populations (Solow, Roberts & Robbirt 2006) or climatic change (Lister & Stuart 2008). The problem of correctly inferring when extinction has occurred is in many ways analogous to that of identifying modern extinction events from the distribution of historic sightings, as the fossil record provides points in time at which a species is known to have existed (in effect, a sighting event). The similarity of paleontological methods to those tested within this thesis raises the possibility of using small-scale experimental systems to compare and contrast the various paleontological methods (e.g. Marshall 1997; Weiss & Marshall 1999; Solow, Roberts & Robbirt 2006), work which, to the best of my knowledge, has not yet been done.

7.6.2 Predicting the effects of the rate of temperature change on community composition

WHILST it is well known that global temperatures will change dramatically over the coming century, the exact rate of warming remains uncertain (IPCC 2007). Consequently, being able to predict how populations and communities will respond to varying rates of environmental change is fundamental to our understanding of the future rates of species loss. The work presented in Chapter 5 demonstrates that, in a very simple system, the effects of directional environmental change on population dynamics can be predicted with a reasonable degree of accuracy. However, this work concentrated on single-species populations, whereas real-world species reside in complex matrices of interacting species. Using the experimental framework set out in Chapter 5, but expanding the work to multi-species communities, and then to multiple trophic levels, are interesting next logical steps. Comparing how well simple (phenomenological) and more complex (mechanistic) models predict observed experimental dynamics, and assessing the trade-off between the information required to parameterise such models and the accuracy of predictions would also be important.

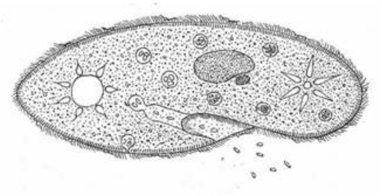
7.6.3 Modelling the interactions between temperature and assembly order

The work presented in Chapter 6 has raised interesting questions regarding the potential interactions between assembly order and temperature, especially in the light of predicted future climatic change. However, the results presented are purely observational, and more work is required to unpick the mechanisms underpinning this potentially important interaction between abiotic and biotic factors that govern community composition. Without such a mechanistic understanding we cannot hope to predict where and when such an interaction will occur, and how important it will be for the survival of a species.

Key to a mechanistic understanding of this interaction is the detailed quantification of the community dynamics early on during the assembly process. Once a detailed understanding of the transient dynamics of a community is known, it may then be possible to identify the drivers of the occasional interactions between temperature and assembly order, and to begin to suggest where such interactions may occur in more complex systems. This process would be facilitated by the fitting of mechanistic models that incorporate, for example, attack rates and the temperature dependence of parameters, a task only possible with a detailed knowledge of the population dynamics of the different species. Such a mechanistic model would then form the basis for a predictive framework to identify when and where such interactions may occur in more complex systems. Key future questions include *how would further trophic levels affect the frequency of temperature-assembly order interactions*, and *when will such interactions manifest in more species-rich competitive systems*?

7.7 Final conclusions

This thesis has sought to use small-scale experimental systems, in conjunction with mathematical modelling and data from real-world populations, to address issues surrounding the quantification of current biodiversity loss, and build new foundations to begin to understand how future climatic change may alter the probability of species persisting. In doing so I have shown that small-scale experimental systems can have a central role in developing our understanding of biodiversity issues, and should be utilised more widely, especially to test conservation theory where manipulating real-world communities is often undesirable. The results presented here move beyond the small-scale nature of the systems employed by providing not only a foundation for important future work on the effects of climatic change on population persistence and community structure, but also a basis for making more informed decisions in the field of conservation, by demonstrating the strengths and weaknesses of methods for inferring the extinction status of species.



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8 | Appendices

8.1 Appendices for Chapter 4

8.1.1 Experimental set up and sampling

Microcosms were lidded petri dishes (diameter 100mm, height 25mm) containing 50ml of medium. The medium consisted of 0.05g/L of crushed protist pellets (Carolina Biological Supply, Burlington, NC), providing organic nutrients, and Chalkley's solution (Thompson et al. 1988), containing essential salts. On day -14 the medium was inoculated with the bacteria *Bacillus cereus* and *Serratia marcescens* and incubated at 20 °C. On day -10, a volume of high-density stock culture containing ∼200 individuals of the ciliate bacterivore *Loxocephalus sp.* was added to each litre of medium. This culture was sampled every two days, and the experiment started (day 0) when the density of *Loxocephalus* had reached approximately the carrying capacity of the medium (i.e. exponential growth had stopped). On day zero the medium was homogenised, and 50ml added to each of 27 petri dishes (three replicate populations of nine temperature treatments).

The temperature treatments comprised: one treatment kept at a constant 20 °C for the duration of the experiment (treatment C), four treatments that were heated at different rates, and four treatments that were cooled at different rates (Fig. 4.1a). Cooling and heating were achieved by moving replicate populations between nine incubators set at 1.5 °C increments from 14 °C to 26 °C. The four heated treatments were: i) increasing 0.5 °C/week (I0.5), ii) increasing 0.75 °C/week (I0.75), iii) increasing 1.5 °C/week (I1.5) and iv) increasing 3 °C/week (I3). The four treatments that decreased in temperature (D) mirrored the heated, and were thus D0.5, D0.75, D1.5 and D3.

Microcosms were sampled to estimate population abundances twice per week for 10 weeks. Sampling was based on the protocol of Lawler and Morin (1993). Microcosms were homogenised by repeat pipetting of the medium,

Figure 8.1: Mean error of predictions made by each method under the different simulated search regimes. Error is the difference between the predicted date of extinction and the observed date of extinction. Bars represent +/- 1 s.e. “con” = constant, “dec” = decreasing, “inc” = increasing, “irr” = irregular, “reg” = regular.

and then a known volume (0.1-0.3ml) extracted using an adjustable-volume pipette. The individuals within this known volume were then counted under a stereoscopic microscope (7.5-30x magnification), and the total population in the microcosm estimated. When densities became very low the whole microcosm was placed under the microscope and the individuals counted. A species was recorded as extinct if, on two consecutive sampling days, no individuals were observed after 5 minutes of searching. This method has been shown to reliably identify when a species has gone extinct (Clements *et al.* 2013), and no populations that were initially recorded as extinct were re-observed at the next sampling occasion. All medium was replaced after counting, and any evaporative loss (checked with a balance) was replaced with distilled water.

8.1.2 Effect of search regularity on the accuracy of extinction estimates

When sampling was irregular, in most cases the error associated with estimates was higher than with regular sampling (Fig. 8.1).

Table 8.1: The six methods included within the sExtinct package, the assumptions made, and data required for each.

Method	Main Assumptions	Data	Reference
Burgman	Chance of observing a species does not change over time.	Counts	Burgman *et al.* (1995)
OLE	Search effort never falls to zero, but does not have to be constant, and the most recent sightings have a Weibull extreme value distribution.	Presence	Roberts & Solow (2003), Solow (2005)
Robson	Chance of observing a species does not change over time.	Presence/absence	Robson & Whitlock (1964)
Solow1993.eq2	Chance of observing a species does not change over time.	Counts	Solow (1993)
Solow2005.eq7	Declining pre-extinction sighting rate.	Counts	Solow (2005)
Strauss	Chance of observing a species does not change over time.	Presence/absence	Strauss & Sadler (1989)

8.1.3 Brief summaries of each of the methods tested

Briefly summarised are the assumptions made by the six methods tested, the data required by each, the abbreviation used in the chapter, and the original publications proposing the methods (Table 8.1).

Burgman

Estimates the probability of getting a run of times where a species has not been observed, given the maximum time between previously recorded sighting events. Assumes that the sightings are independent and randomly distributed through time. Sightings are recorded as presence, absence, or multiple sightings at each time point.

OLE

Assumes that the most recent sighting events have the form of a Weibull extreme value distribution, and estimates the shape parameter of this distribution from the temporal spread of the sighting events. The beginning and end of the observation period need not be specified, but the method assumes (unrealistically) that search effort never falls to 0. Sightings, or multiple sightings, are recorded as presence data (i.e. the species is extant at a given time point) and are not assumed to be randomly distributed through time.

Robson

Estimates the the time of extinction based on the rate of sightings at the end of the observation period, reflected by the interval between the two most recent sighting events. This estimation is unbiased when the chance of observing a species is uniformly distributed over time. Sightings are recorded as binary data: a species is either present or absent.

Solow1993.eq2

Estimates the probability that a species is extinct when it has not been observed as extant for a given period of time after the last sighting event, and the observations are independent and randomly distributed through time. So, as the period of observation increases, and there are no new sighting events, the probability that a species is extinct also increases. Sightings are recorded as presence, absence, or multiple sightings at each time point.

Solow2005.eq7

Estimates the probability of extinction over an observation period, but unlike Burgman and Solow1993.eq2 the sightings are not assumed to be randomly distributed over time, but that as a species becomes rarer the frequency of observation events decreases. Sightings are recorded as presence, absence, or multiple sightings at each time point.

Strauss

Estimates the time at which a species has gone extinct, using the mean gap between sightings, and assuming that sightings are uniformly distributed through time. I.e. how large does the gap have to be after the last sighting event for a species to be unlikely to be observed again. Sightings are recorded as binary data: a species is either present or absent.

8.1.4 Probability of extinction on a method-by-method basis

The probability that a species is extinct at any given time point varies from method to method, depending upon each method's underlying mathematical assumptions (Fig. 8.2). Some methods, noticeably Solow1993.eq2, have sharp declines in the probability that a species persists, and are therefore more likely

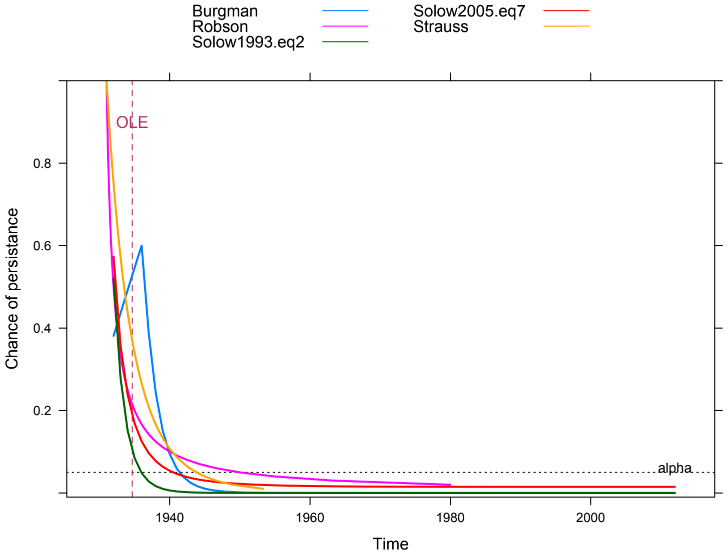

Figure 8.2: Diagnostic plot showing the predicted probability of persistence given the sighting record in the example.data data frame across all methods in the sExtinct package. OLE produces a point estimate of extinction only (represented as the vertical dashed line), and the alpha level set is marked with the dotted black line.

to underestimate the date of extinction. Others, such as Robson, initially predict a rapid decline in the probability of persistence with time, but subsequently an elongated tail where a species has a low possibility of persisting. This may lead to a bias toward overestimating the date of extinction.

8.1.5 Interaction between small population size and rate of decline on the accuracy of estimates

Ten different rates of population decline were simulated by creating populations with various starting abundances (from 5 to 95 individuals), and making them decline to extinction over a 20 time step period (Figure 8.3). Abundances at each time step had some random fluctuations added (with a magnitude relative to the population's rate of decline) and were rounded to whole numbers. This produced 10 different rates of population decline, from -0.28 individuals per time step to -5.21 individuals per time step. This data was then analysed in exactly the same way as the wild extirpation data, and showed a somewhat similar pattern: error produced by OLE, Solow1993.eq2, Solow2005.eq7, and Strauss tended to increase as the rate of population decline increased, although this pattern is less obvious than when the extirpation data were used (Figure 8.4). As with the wild extirpation data, the error associated with estimates

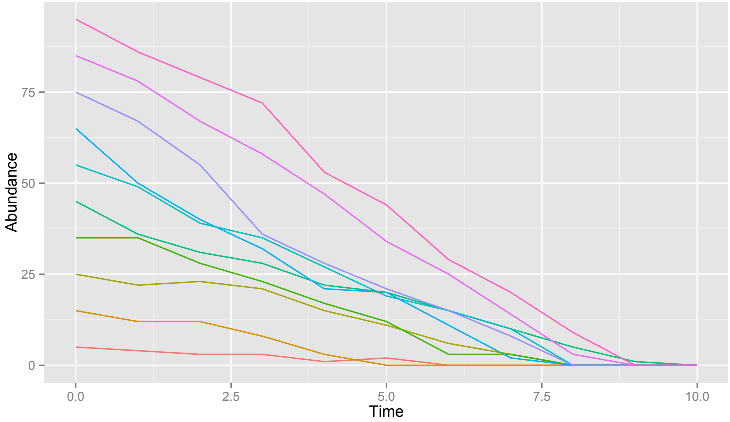

Figure 8.3: Simulated declines of model populations.

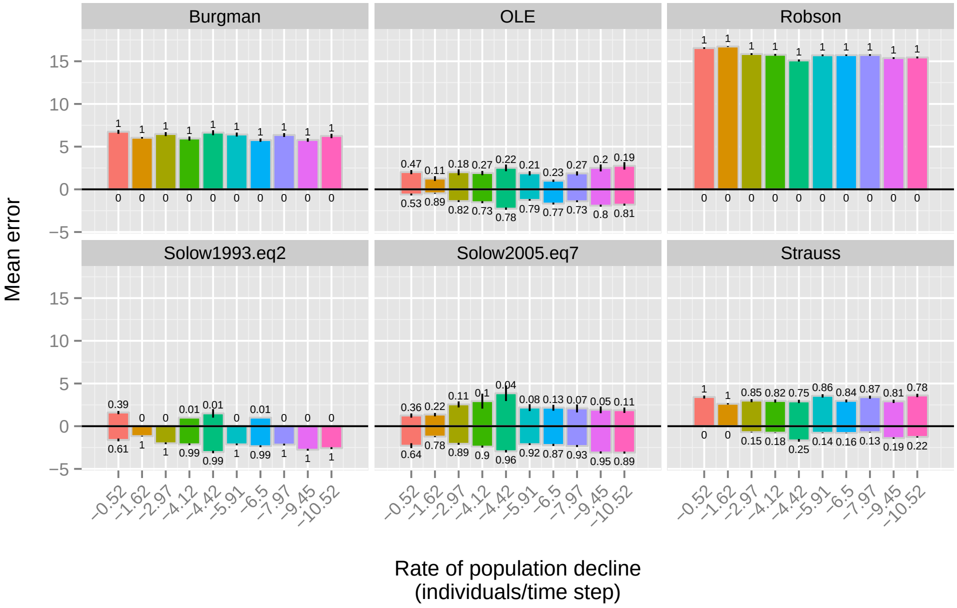

Figure 8.4: Magnitude of error produced by the six different techniques, across the 10 different rates of population decline.

made using Robson decreased as the rates of population decline increased (Figure 8.4).

8.1.6 Overall accuracy of methods using wild population data

As with the experimental data, one of the main drivers of error was the method chosen to produce the predictions (Fig. 8.5). OLE, Solow1993.eq2, Solow2005.eq7, and Strauss all had much lower error in predictive accuracy than Burgman or Robson. The majority of the methods predicted that extinc-

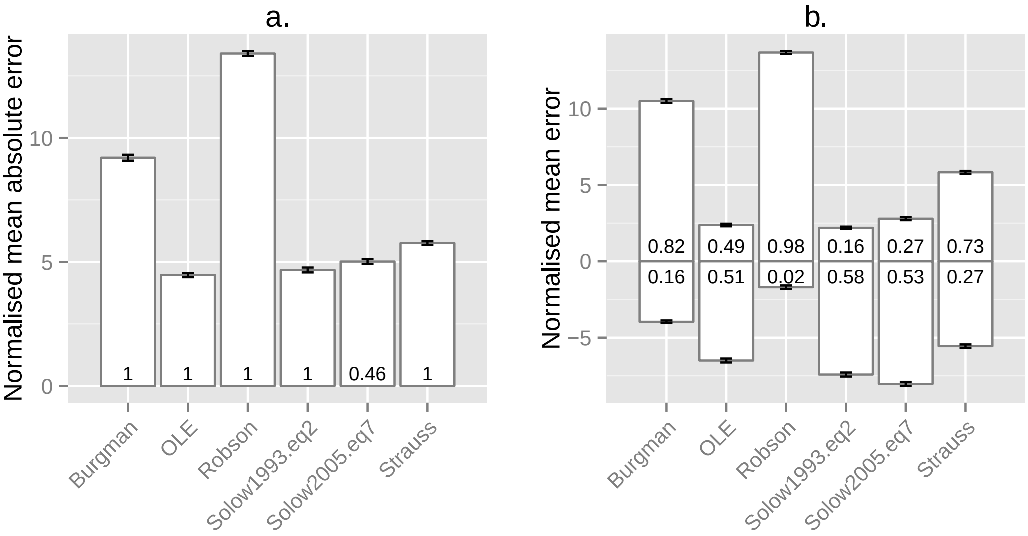

Figure 8.5: a) Normalised mean absolute error, printed in each bar is the fraction of the simulations for which each method produced an extinction estimate that fell before the year 2012, and b) normalised mean relative error, with the proportion of estimates that were after the observed date of extinction, or before the observed date of extinction. Proportions do not always sum to 1 as estimates that fall on the recorded year of extinction are neither positive or negative. Error is the difference between the predicted date of extinction and the actual date of extinction, and is normalised across the different species by dividing by the minimum time between recorded abundances. Mean is calculated from a random sample of 500 data points from each search regime, for each method. Bars show +/- 1 s.e.

tion had occurred before the year 2300 in a high proportion of the simulations (note that the latest recorded extinction, that of the Hawaiian Crow, was 2003, Fig. 4.2), with the exception of Solow2005.eq7 (Fig. 8.5).

When the error is split into overestimation and underestimation of the date of extinction (Fig. 8.5b) the distribution of error amongst the methods is almost identical to that seen with the experimental data (Fig. 4.6b). The notable exceptions to this are Robson (where effectively all of the predictions are now large overestimates of the date of extinction), and Solow2005.eq7 and (where overestimates and underestimates are roughly as frequent as one another, whereas with the experimental data >93% of estimates were underestimates of the date of extinction).

8.2 Appendices for Chapter 6

Presented are the full results of the Generalized Linear Models and Zero-Inflated Negative Binomial Regression fitted to the abundance data of *Blepharisma japonicum*, *Paramecium caudatum*, and *Loxocephalus sp.* at days 42 and 70. This data is presented as supplementary information in the manuscript published in Ecology and Evolution which is based on this chapter.

Table 8.2: Coefficients of zero-inflated negative binomial regression models fitted to the abundances of *Loxocephalus* at days 42 and 70. Zero-inflated model is the effect of the factors on the number of zeros (i.e. extinctions) recorded in the data. “nb” refers to the negative binomial error structure.

	Day 42					Day 70				
	Error	Std. Er	Coef.	z-value	p-value	Error	Std. Er.	Coef.	z-value	p-value
Count model										
Temp	nb	0.13	0.03	0.23	>0.05	nb	0.14	0.03	0.24	>0.05
BLP	nb	2.49	-1.39	0.56	>0.05	nb	3.06	0.12	0.04	>0.05
BPL	nb	2.57	-0.52	0.2	>0.05	nb	2.92	2.29	0.79	>0.05
LBP	nb	2.45	1.2	0.49	>0.05	nb	2.91	1.4	0.48	>0.05
LPB	nb	2.44	-1.71	0.7	>0.05	nb	3.46	-3.46	1	>0.05
PBL	nb	2.58	-1.8	0.69	>0.05	nb	3.18	-1.16	0.37	>0.05
PLB	nb	2.87	0.47	0.16	>0.05	nb	2.88	1.7	0.59	>0.05
T∼BLP	nb	0.15	0.03	0.22	>0.05	nb	0.17	-0.02	0.1	>0.05
T∼BPL	nb	0.16	0.05	0.3	>0.05	nb	0.17	-0.16	0.93	>0.05
T∼LBP	nb	0.15	-0.05	0.33	>0.05	nb	0.17	-0.05	0.29	>0.05
T∼LPB	nb	0.15	0.08	0.53	>0.05	nb	0.19	0.09	0.5	>0.05
T∼PBL	nb	0.16	0.05	0.35	>0.05	nb	0.18	0.02	0.14	>0.05
T∼PLB	nb	0.17	-0.05	0.27	>0.05	nb	0.16	-0.11	0.67	>0.05
Zero-inflation model										
Temp	nb	0.25	0.13	1.99	<0.05	nb	0.12	0.1	1.14	>0.05
BLP	nb	-1.22	4.36	0.28	>0.05	nb	5.73	3.03	1.89	>0.05
BPL	nb	1.78	3.24	0.55	>0.05	nb	0.31	2.71	0.12	>0.05
LBP	nb	2.14	3.03	0.71	>0.05	nb	0.97	3.03	0.32	>0.05
LPB	nb	1.45	3.13	0.46	>0.05	nb	4.41	3.23	1.37	>0.05
PBL	nb	2.3	3.09	0.75	>0.05	nb	2.36	2.69	0.88	>0.05
PLB	nb	2.6	3.03	0.86	>0.05	nb	2.18	2.68	0.81	>0.05
T∼BLP	nb	-0.06	0.21	0.28	>0.05	nb	-0.32	0.16	2.03	<0.05
T∼BPL	nb	-0.17	0.17	0.97	>0.05	nb	-0.05	0.15	0.31	>0.05
T∼LBP	nb	-0.15	0.16	0.9	>0.05	nb	0	0.17	0.01	>0.05
T∼LPB	nb	-0.12	0.16	0.75	>0.05	nb	-0.19	0.17	1.15	>0.05
T∼PBL	nb	-0.18	0.16	1.08	>0.05	nb	-0.14	0.14	0.94	>0.05
T∼PLB	nb	-0.18	0.16	1.14	>0.05	nb	-0.12	0.14	0.84	>0.05

Table 8.3: Full coefficients of generalized linear models fitted to the abundances of *Paramecium* at days 42 and 70. Error structures are as follows: “G” = Gaussian, “q-P” = quasi-Poisson.

	Day 42					Day 70				
	Error	Std. Er	Coef.	t-value	p-value	Error	Std. Er.	Coef.	t-value	p-value
Temp	G	-0.01	0.02	0.3	>0.05	q-P	-0.24	0.07	3.66	<0.001
BLP	G	1.2	0.63	1.9	>0.05	q-P	-0.95	1.2	0.79	>0.05
BPL	G	1.36	0.64	2.11	<0.05	q-P	-0.94	1.24	0.75	>0.05
LBP	G	1.57	0.63	2.48	<0.05	q-P	0.66	1.17	0.57	>0.05
LPB	G	1.07	0.63	1.69	>0.05	q-P	1.03	1.14	0.9	>0.05
PBL	G	0.86	0.63	1.36	>0.05	q-P	-2.18	1.1	1.98	<0.05
PLB	G	0.94	0.63	1.48	>0.05	q-P	0.25	1.12	0.22	>0.05
T∼BLP	G	-0.06	0.63	1.89	>0.05	q-P	0.08	0.08	1	>0.05
T∼BPL	G	-0.07	0.03	2.12	<0.05	q-P	0.08	0.09	0.94	>0.05
T∼LBP	G	-0.08	0.03	2.28	<0.05	q-P	-0.01	0.08	0.08	>0.05
T∼LPB	G	-0.03	0.03	0.97	>0.05	q-P	-0.02	0.08	0.27	>0.05
T∼PBL	G	-0.01	0.03	0.38	>0.05	q-P	0.19	0.07	2.58	<0.05
T∼PLB	G	-0.02	0.03	0.5	>0.05	q-P	0.03	0.08	0.41	>0.05

Table 8.4: Full coefficients of generalized linear models fitted to the abundances of *Blepharisma* at days 42 and 70. Error structures are as follows: “G” = Gaussian, “q-P” = quasi-Poisson.

	Day 42					Day 70				
	Error	Std. Er	Coef.	t-value	p-value	Error	Std. Er.	Coef.	t-value	p-value
Temp	q-P	0.09	0.03	3.22	<0.01	G	1.63	0.44	3.71	<0.001
BLP	q-P	0.46	0.71	0.64	>0.05	G	-12.44	11.89	1.04	>0.05
BPL	q-P	-0.09	0.74	0.13	>0.05	G	5.57	12.12	0.46	>0.05
LBP	q-P	-2.65	0.95	2.8	<0.01	G	-21.18	11.89	1.78	>0.05
LPB	q-P	-3.01	1.19	2.54	<0.05	G	-44.19	11.89	3.71	<0.001
PBL	q-P	0.29	0.85	0.34	>0.05	G	-8.85	11.95	0.74	>0.05
PLB	q-P	-2.33	1.35	1.72	>0.05	G	-20.86	11.89	1.76	>0.05
T∼BLP	q-P	0.01	0.03	0.36	>0.05	G	0.58	0.62	0.94	>0.05
T∼BPL	q-P	0.03	0.03	1	>0.05	G	-0.47	0.65	0.73	>0.05
T∼LBP	q-P	0.13	0.4	3.08	<0.01	G	0.64	0.62	1.03	>0.05
T∼LPB	q-P	0.11	0.05	2.16	<0.05	G	1.68	0.62	2.71	<0.01
T∼PBL	q-P	-0.03	0.04	0.69	>0.05	G	-0.01	0.63	0.02	>0.05
T∼PLB	q-P	0.05	0.06	0.85	>0.05	G	0.61	0.62	0.99	>0.05